eternalyoung

Nourish the skin
for longer-lasting youth

Monteloeder
Digital Nutraceuticals for you

What is eternalyoung?

Is a proprietary botanical blend of different fruit and herbal extracts that has been specially designed to delay the normal skin aging process at the cellular level, targeting the origin of youth: the cell telomeres and cell senescence.

eternalyoung promotes a slower aging process and longer skin longevity, leading to a more youthful, radiant, and better-looking appearance.

CISTANCHE EXT.

CENTELLA ASIATICA EXT

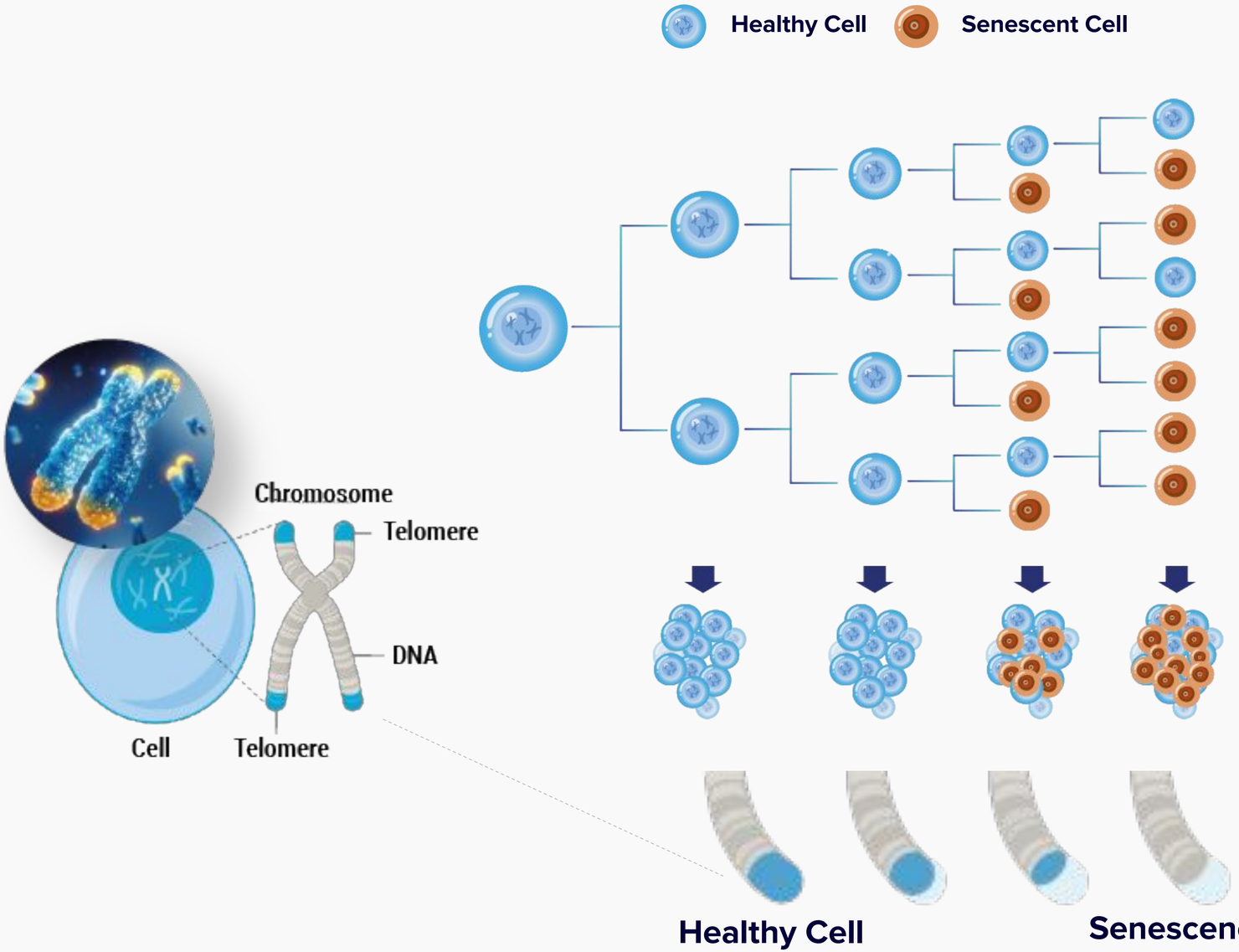
POMEGRANATE FRUIT EXT.

SWEET ORANGE EXT

Telomeres and aging

Our skin ages because, individually, each cell of the body ages and enters the senescence phase, according to a chronology defined by a cellular biological clock: the telomere.

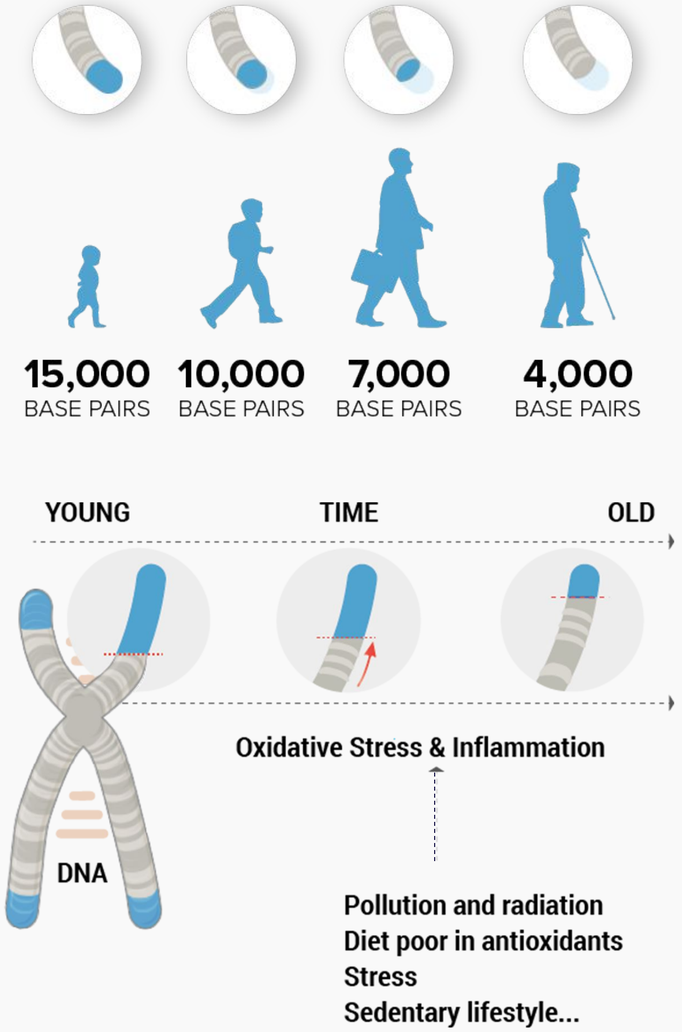
Telomeres are repetitive DNA base units that protect the chromosomes “caps” during cell division. As cells divide over time, telomeres shorten and when telomere length becomes really short, cells stop their division/replication and enter senescence.



Telomere and aging

Telomere length decreases over time and may predict lifespan. In humans, average telomere length declines from 15 kilobases at birth to less than 4 kilobases in old age

Telomere shortening has negative effects on health conditions and has been linked to cellular senescence, and many health issues including aging and cancer.
Skin is a self-renewing tissue that is required to go through extensive proliferation throughout the lifespan of an organism. So, telomeres in skin cells are particularly susceptible to accelerated shortening because of both proliferation and external DNA-damaging agents such as sun irradiation, environmental pollution or reactive oxygen species.

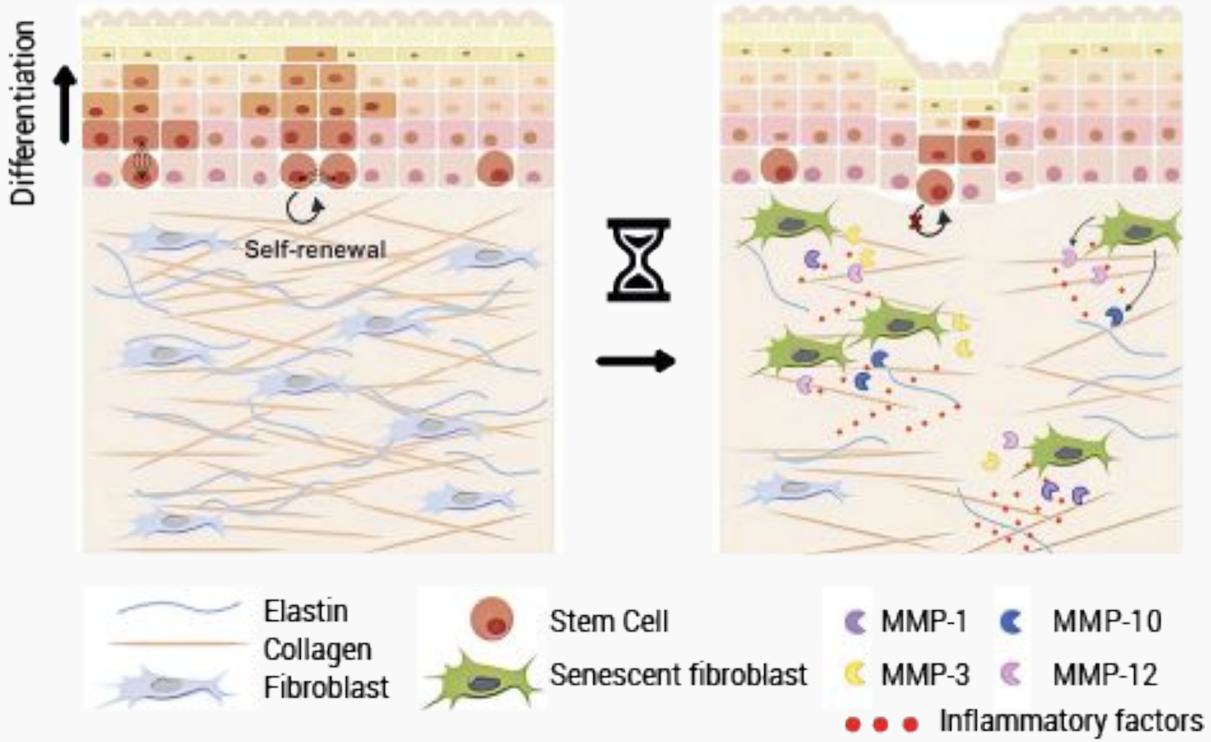


TELOMERE GET SHORTER AS WE AGE

TELOMERE GET SHORTER DUE TO EXTERNAL FACTORS

In response to telomere shortening skin cells become senescent and accelerates aging

Senescent cells accumulate with age in the skin, leading to a decline in various aspect of skin function.
Senescent cells are deleterious because they develop into a senescence-associated secretory phenotype (SASP), which is believed to be one of the major causes of aging. SASP skin cells communicate with nearby cells using proinflammatory cytokines, which include catabolic modulators such as Matrix metalloproteinases (MMPs) and release reactive oxygen species. This, in turn, causes a decline in both the function and appearance of the skin leading to a decrease in skin's natural self-regenerative potential and a reduction in the collagen and elastin fibers.



Efficacy studies

In vitro efficacy testing

Antiaging studies in Normal Human Dermal Fibroblasts (NHDF)

- Analysis of the effects on cell proliferation and in aged fibroblast
- Analysis of accumulation of free radicals (ROS) in aged fibroblast
- Analysis of the protective effects of a prolonged treatment on telomere shortening, after senescence induced by cell doubling with and without oxidative stressor.
- Analysis of Telomerase activity in presence of eternalyoung
- Protective effect on induced protein glycation (Glycation End-products (AGEs))

Anti-melanogenic activity in Normal human melanocytes

Human clinical study

Revert signs of aging

60 subjects (from 35-65 years old)
3 months
225 mg Zeropollution

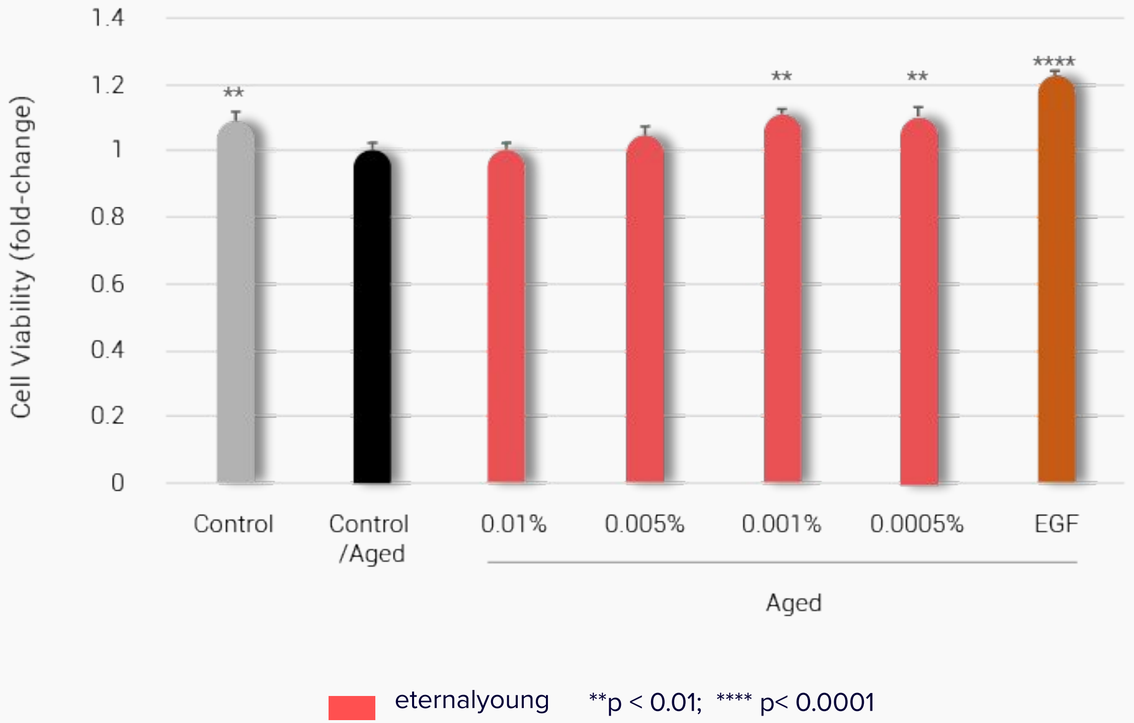
eternalyoung prevents the loss of proliferative potential in pro-aging conditioned medium protecting them from cellular senescence

METHODOLOGY

For cell proliferation assay, Fibroblast were exposed for 3 h to aging-inducing conditioned medium containing H_2O_2. Then, medium was replaced by fresh medium containing **eternalyoung** at different concentrations for 96 hours. Epidermal Growth Factor (EGF) was used as a positive control and cells without H_2O_2 pre-treatment as a non-aging control (Control). Cell viability was then quantified using MTT assay.

RESULTS

Fibroblast pre-treated with H_2O_2 (Control Aged) significantly decreased cell viability (-9.2%), compared to the Control cells. Cells supplemented with **eternalyoung** recovered their proliferation ability and a significant increase in the cell viability was observed at 0.001% (+11.3%) and at 0.0005% (+10.8%) raising growth rate levels similar to dose no aged fibroblast, and somewhat lower than the control cells incubated with the EGF (+ 22.9%).



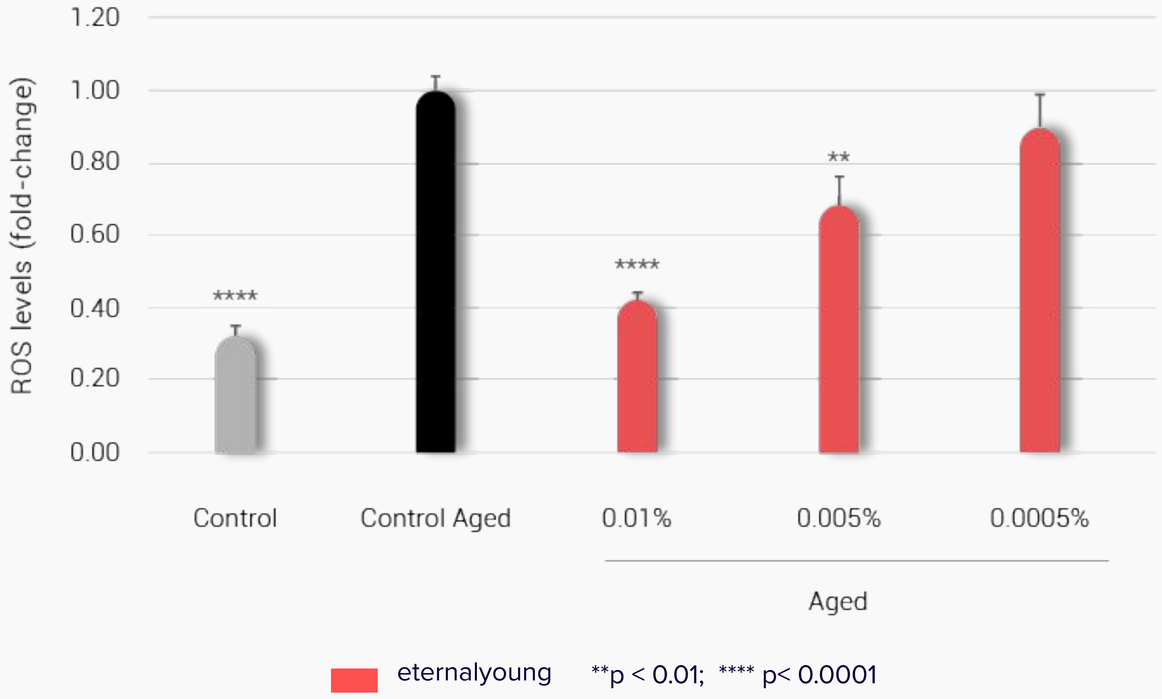
eternalyoung prevent ROS generation in pro-aging conditioned medium

METHODOLOGY

Fibroblast were exposed for 3 h to aging-inducing conditioned medium containing H_2O_2. Then, medium was replaced by fresh medium containing **eternalyoung** at different concentrations for 24 hours. ROS production was then measured by fluorimetry. ROS generation measurements were calculated versus untreated cells conditioned with H_2O_2 (Control Aged)

RESULTS

Senescence-inducing dose of H_2O_2 displayed a significantly higher level of intracellular ROS over untreated cells (+60%), a hallmark of skin aging. But when cells were supplemented with **eternalyoung** for 24 h, ROS level decreased. Treatment with **eternalyoung** at 0.005% and 0.01% decreased ROS levels by 32%, and 57.7%, respectively.



eternalyoung increases the telomesase activity in skin fibroblasts

The telomerase enzyme slows or even reverses telomere shortening that occurs as we age. Telomerase activators enhance the efficiency of the DNA repair process and protect cells from stress and DNA-damaging conditions.

Thus, activating telomerase can:

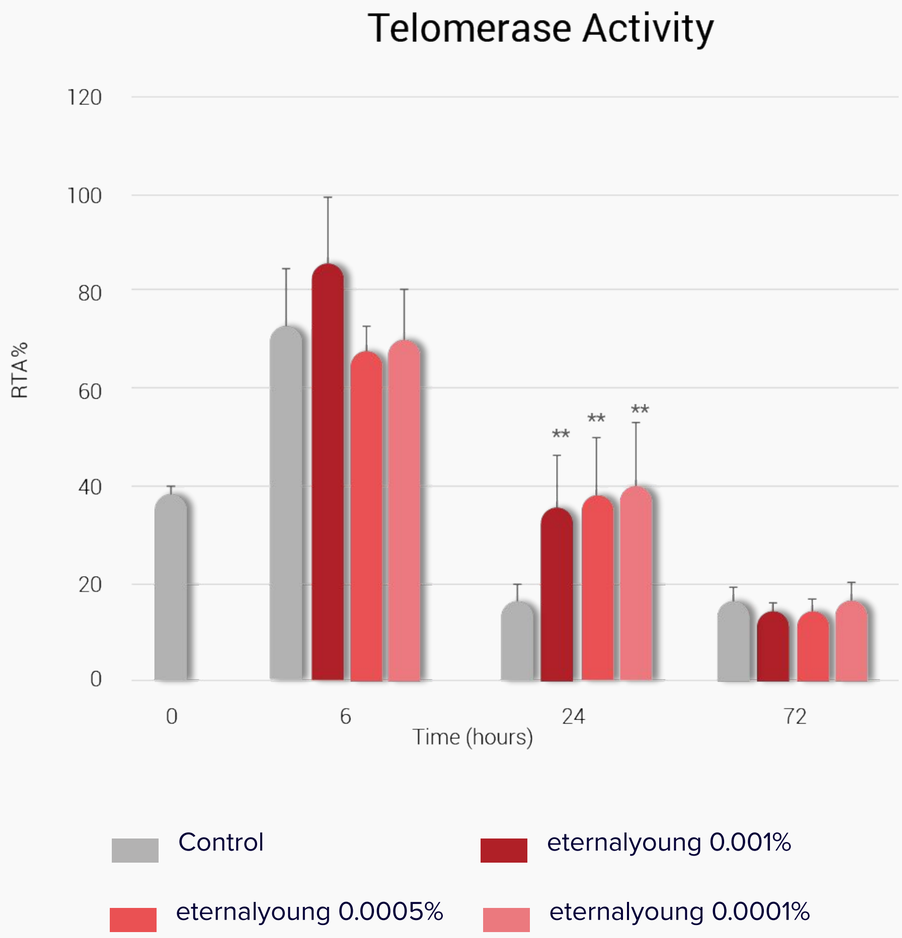
- Address telomere shortening and cell aging
- Help cells live longer and continue to function properly
- Make old cells function as they did when they were younger (by changing gene expression to a younger phenotype)

METHODOLOGY

Telomerase activity is determined by Q-TRAP in primary cultures of adult human fibroblast after 6, 24 and 72 hours of treatment with **eternalyoung** at different concentrations

RESULTS

At 24h a significantly increase in telomerase activity was observed in the cells treated with **eternalyoung** compared with untreated control cells.



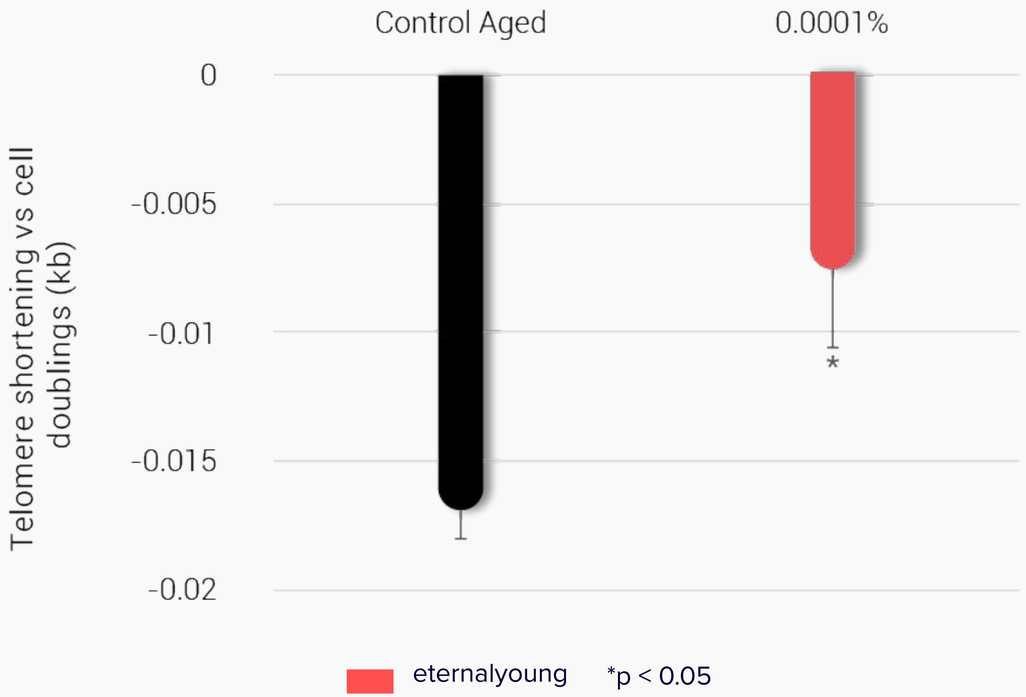
eternalyoung displays antiaging and senescence-induced protective effects, through protection telomere shortening

METHODOLOGY

Fibroblast were cultured and grown during 24-29 cell doublings, inducing ageing and telomere shortening due to cell passage. The cells were treated or not with **eternalyoung** changing fresh product every 3 days. After the incubation period, cells were collected, gDNA was extracted and telomere length through RT-qPCR was quantified. gDNA was also extracted from cells at each passage. The number of cell doublings was calculated through cell counting.

RESULTS

Fibroblasts aged for 24-29 doublings without treatment showed significantly decreased telomere length, compared with unaged control. Conversely, treatment with a concentration as low as 0.0001% of **eternalyoung** significantly increased telomere length compared with untreated aged cells. Telomere shortening per cell division was 0.010 kb/division slower. This implied a telomere shortening rate about 57.7% slower than untreated Control.

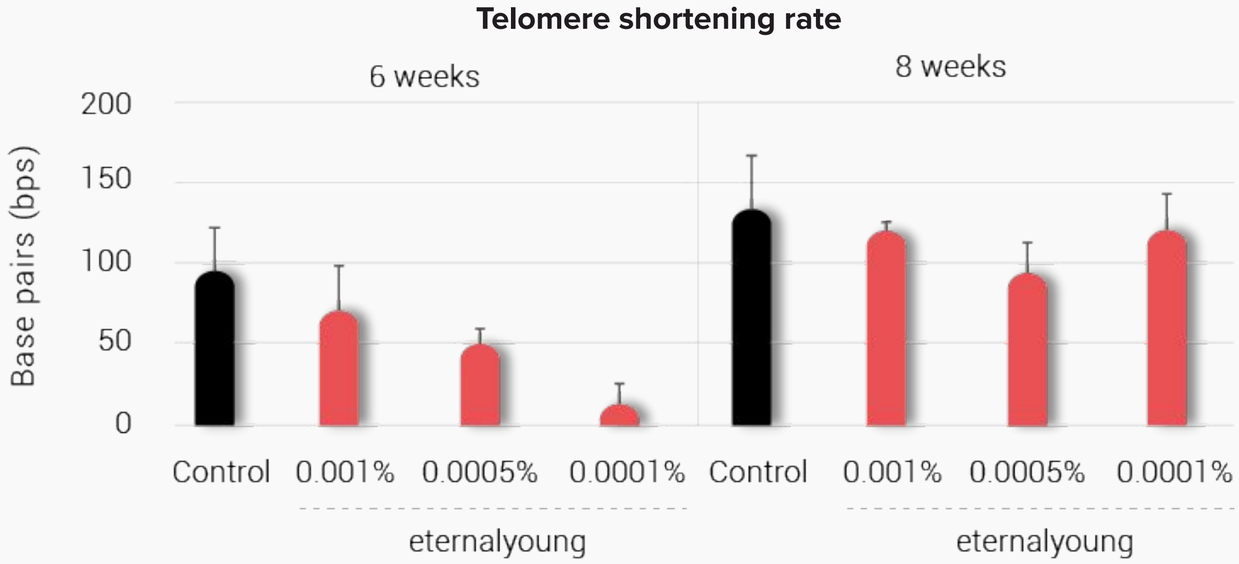


eternalyoung protects telomeres from oxidative stress, the major contributor of telomere shortening

Fibroblasts aged for 6 and 8 weeks and under oxidative stress with H2O2) showed decreased telomere length as well as higher percentage of small telomeres compared with unaged control. Conversely, fibroblasts treated with **eternalyoung** presented a higher median telomere length and 20th percentile values, as well as a lower percentage of very small telomeres (<3 kb), compared to the untreated control group, during week 6 and week 8 (table).

After normalizing the data by the population doubling, a lower telomere shortening rate was also observed in the fibroblast treated with **eternalyoung** suggesting a protective effect of the telomeres under oxidative stress and aging conditions (graph).

		MEDIAN LENGTH (bp)	20TH PERCENTILE LENGTH (bp)	TELOMERES <3 KBP (%)
Start (Week 0)	Un-aged Control	9955	6704	3,9
Week 6	control	8292	5356	5.8
	eternalyoung 0.001%	8854	5819	4.6
	eternalyoung 0.0005%	9356	6385	3.6
	eternalyoung 0.0001%	9523	6530	3.5
Week 8	control	6813	3621	16.4
	eternalyoung 0.001%	7088	3765	16.0
	eternalyoung 0.0005%	8013	4627	11.6
	eternalyoung 0.0001%	7626	4718	9



eternalyoung inhibits the accumulation of AGES in fibroblasts

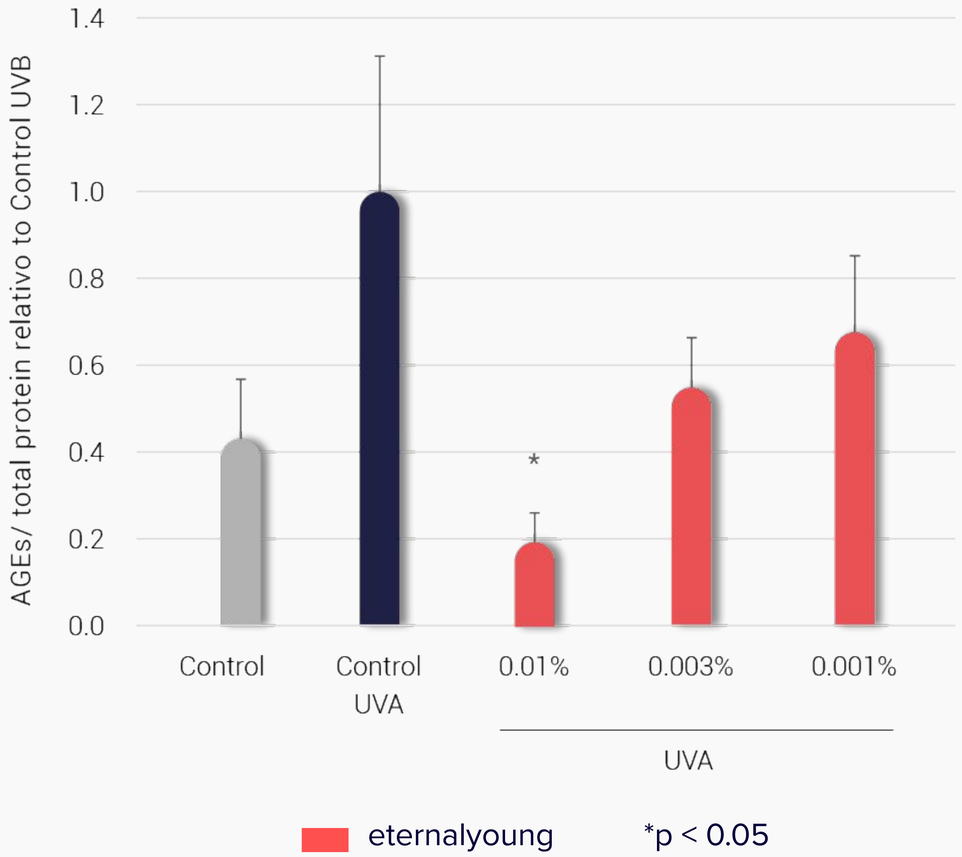
Glycation is one of the main processes responsible for skin aging through the formation of advanced glycation end products (AGEs). AGEs alter skin physiology by impairing deposition, organization and physicochemical properties of dermal extracellular matrix component. Glycated fibers become rigid, less elastic and have reduced regenerative ability, which lead to damage such as laxity, cracking and thinning skin. Counteracting glycation is considered an important anti-aging approach in the maintenance of healthy skin texture.

METHODOLOGY

Fibroblast were treated with different concentrations of **eternalyoung.** After 24 h of incubation, cells were exposed to UVA irradiation for 36 s. 4 h after irradiation samples were processed for AGE quantification by ELISA.

RESULTS

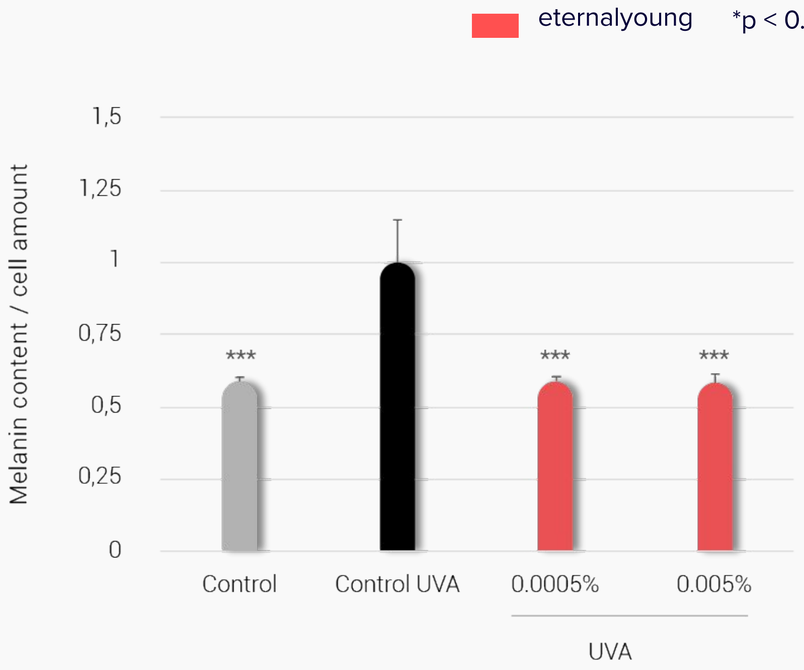
eternalyoung at 0.01% significantly reduced the levels of AGEs by 81% compared to UVA-irradiated untreated cells. And at 0.003% and 0.001% respectively reduced the relative AGE levels by 45% and 32%, but in a non-statistically significant manner.



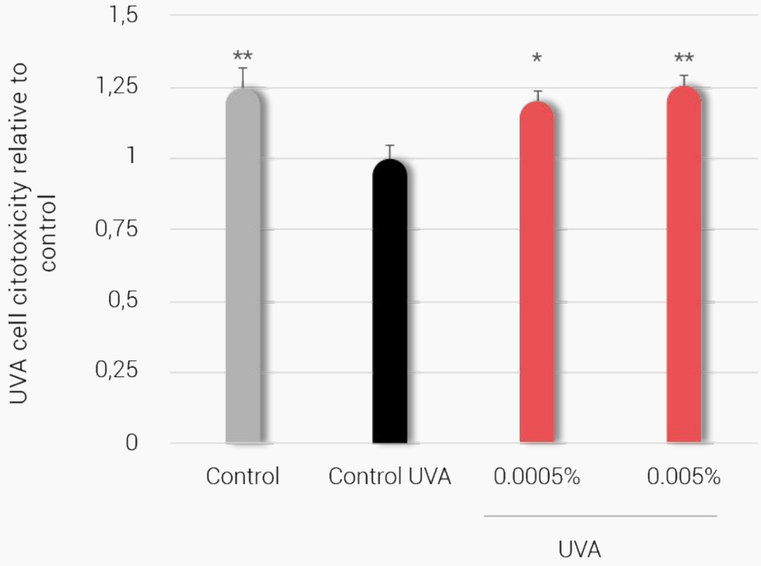
eternalyoung protects cells from UVA damage and can display whitening effects through melanin synthesis inhibition

METHODOLOGY

Normal Human Epidermal Melanocytes were cultured during 72 h with **eternalyoung.** During the incubation period, cells were irradiated with UVA twice a day . After the last irradiation, samples were incubated for extra 96 hours before melanin and cell viability quantification.



UVA radiation increases melanin levels about 41%, compared to non irradiated control. **eternalyoung** at 0.005% and 0.005% significantly decreases melanin levels by 40 and 41%, respectively.



UVA radiation decreases melanocyte viability by about 25%, compared to non irradiated control. **Eternalyoung** at 0.005% and 0.005% significantly improve cell survival and reduce UVA damage by 20% and 25%, respectively.

Human clinical trial

PLACEBO-CONTROLLED CLINICAL EVALUATION OF THE EFFICACY OF ETERNALYOUNG. ANTI-AGING BENEFITS

60 females showing clinical sign of aging

225 mg/day Placebo

Study: 3 months
Checks: 4, 8 and/or 12 weeks

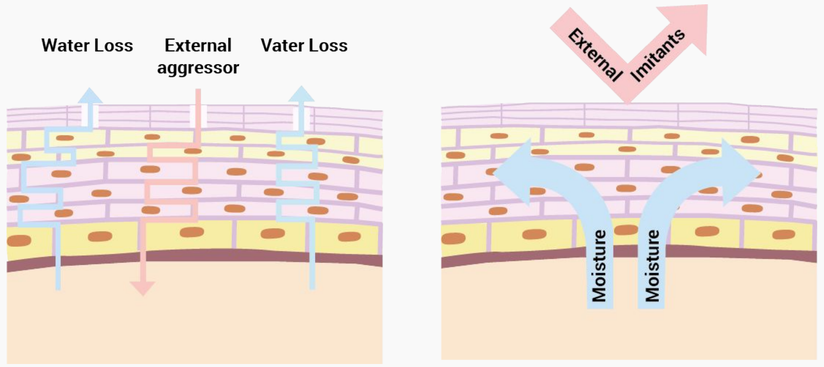
Outcomes

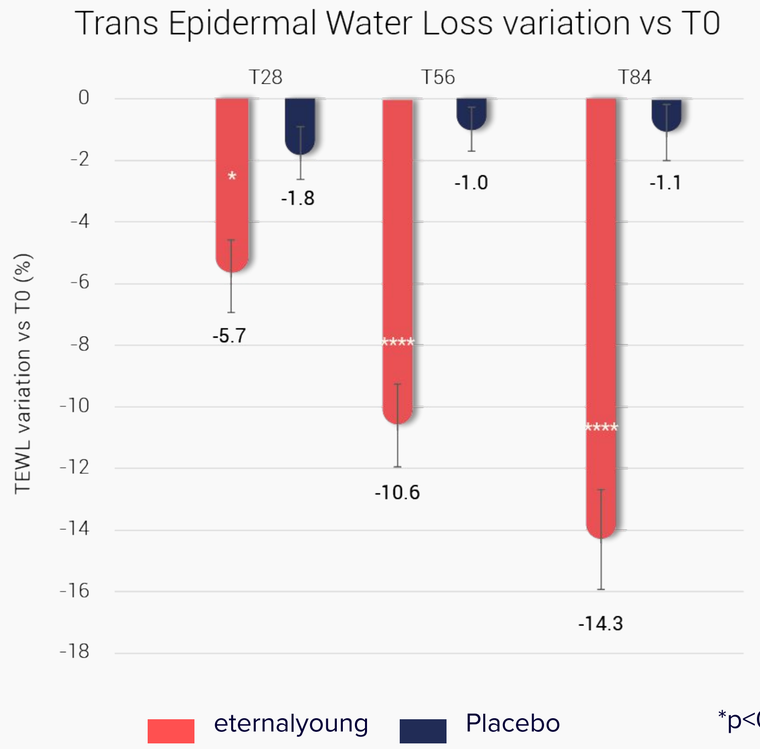
- Skin profilometry (wrinkle depth on crow's feet and smoothness) by 3D microtopography
- Skin biomechanical properties by Cutometer
- R0 (skin firmness/tone)
- R2 (overall skin elasticity)
- R5 (net-elasticity)
- R3 and R9 (tiring effects of the skin)
- Skin moisture by Corneometer®
- Trans Epidermal Water Loss (TEWL) by Tewameter®
- Dark spots intensity and skin radiance by Spectrophotometry
- Skin thickness by ultrasounds

Other: Self-assessment questionnaire

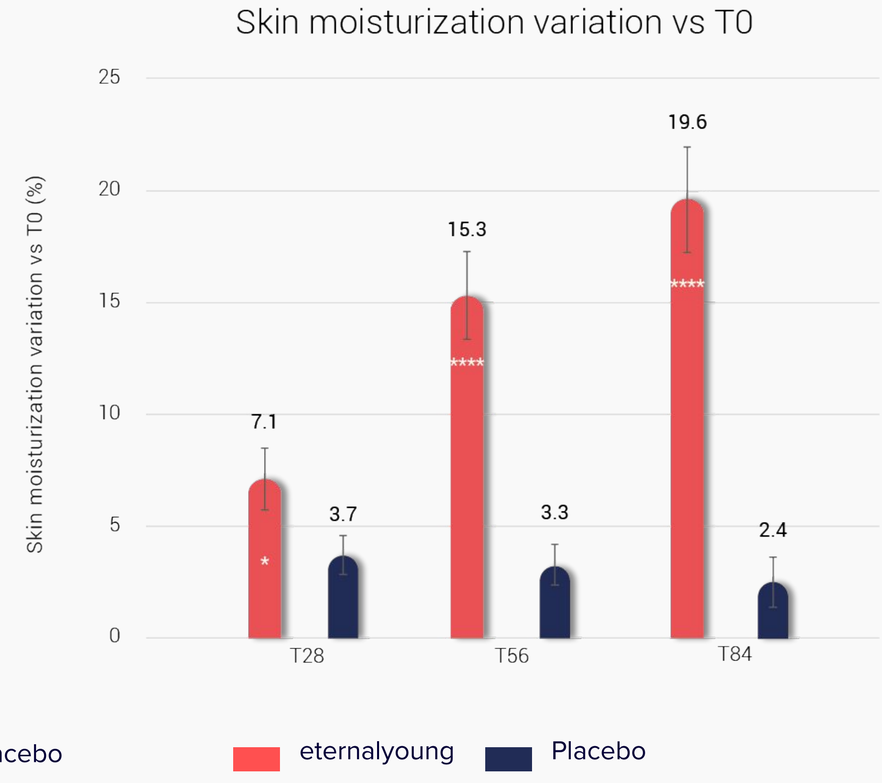
Creates a cumulative hydrating effect and decreases skin permeability

· Improves the barrier function condition since it decreases TEWL
· Produces a progressive increase in skin hydration





*p<0.05, **p <0.01 ***p <0.001 ****p <0.0001 vs placebo



Improves the mechanical properties of the skin

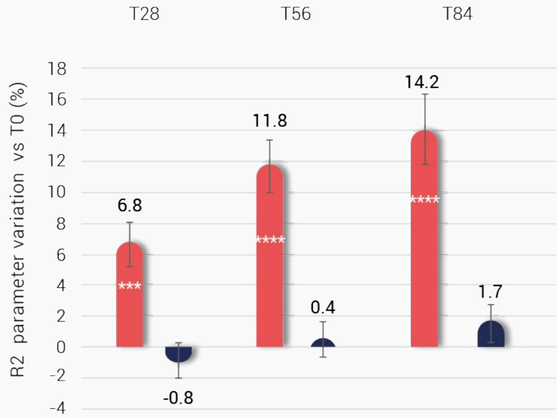
Improves skin elasticity

eternalyoung
Placebo

*p<0.05, **p <0.01 ***p <0.001
****p <0.0001 vs placebo

Net elasticity vs T0

Gross elasticity parameter vs T0



Increases skin firmness

eternalyoung
Placebo

*p<0.05, **p <0.01 ***p <0.001
****p <0.0001 vs placebo

Skin Firmness vs T0

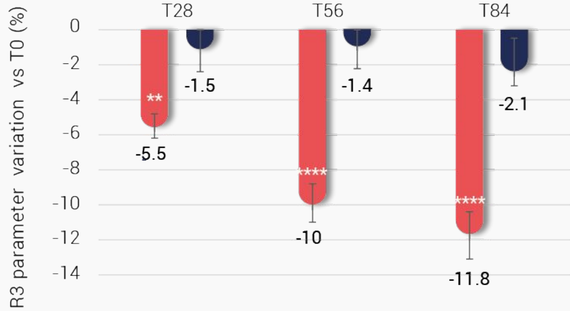
A decrease of R0 parameter can be expressed in absolute values as an increase of skin firmness

Reduces Skin fatigue

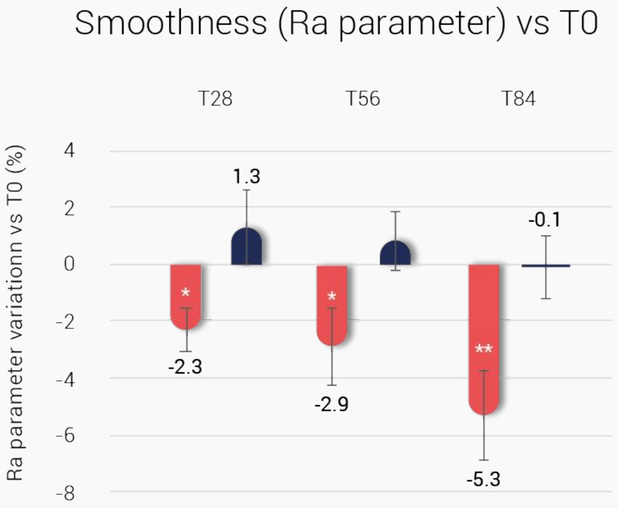
eternalyoung
Placebo

*p<0.05, **p <0.01 ***p <0.001
****p <0.0001 vs placebo

Skin Fatigue vs T0



Reduces wrinkles depth and smoothens the skin surface



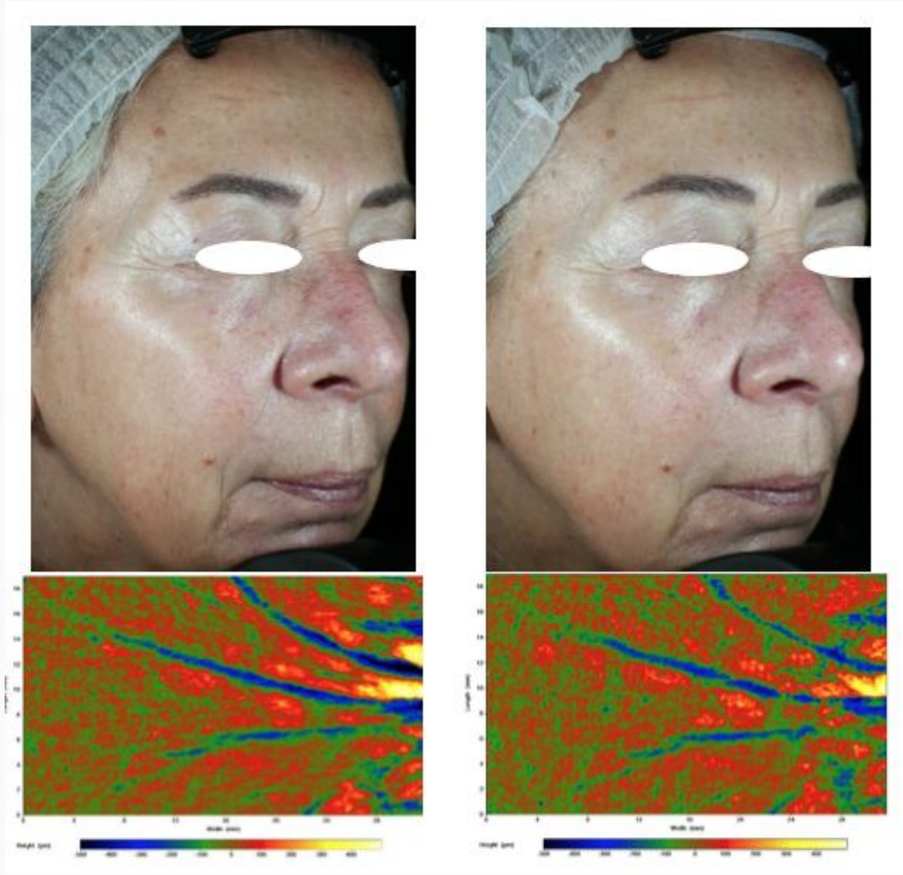
eternalyoung

Placebo

*p<0.05, **p <0.01 ***p <0.001
****p <0.0001 vs placebo

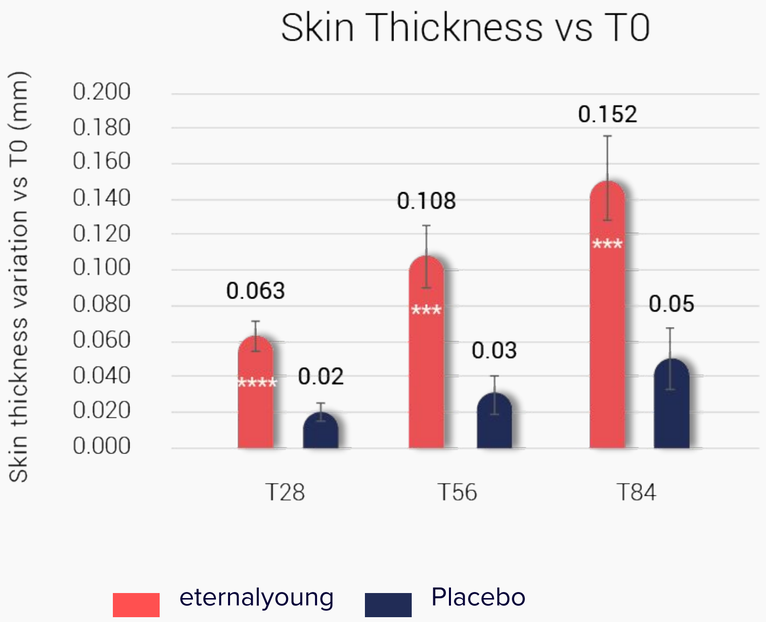
A decrease of Ra parameter can be expressed in absolute values as an **increase** of skin smoothness

BEFORE AFTER



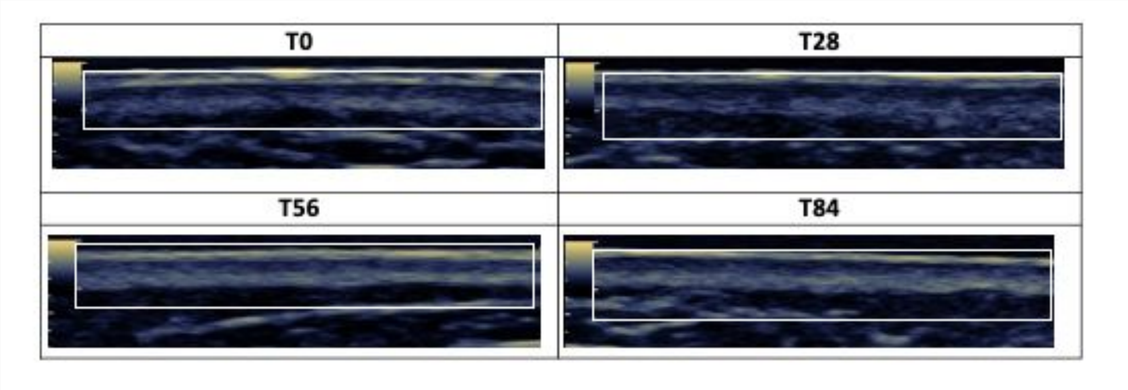
In the after picture it is possible to notice a decrease of periocular wrinkles visibility. Blue area is less evident and intense and the colour moves from the blue to the green which is index of a less wrinkle depth.

Increases skin thickness and redensify the skin



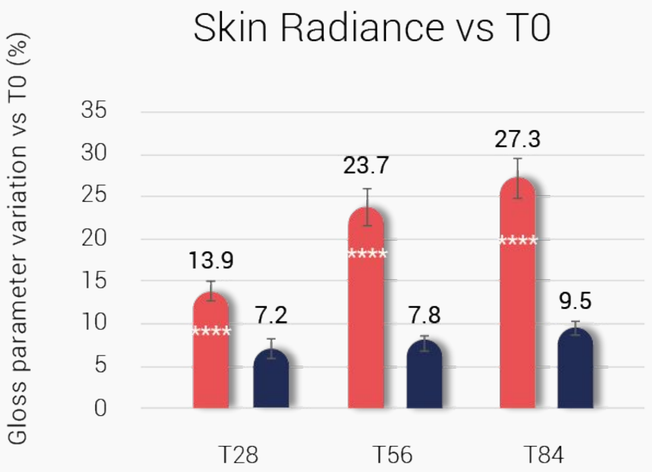
eternalyoung statistically significant increase of skin thickness at each monitored check, compared to placebo group.

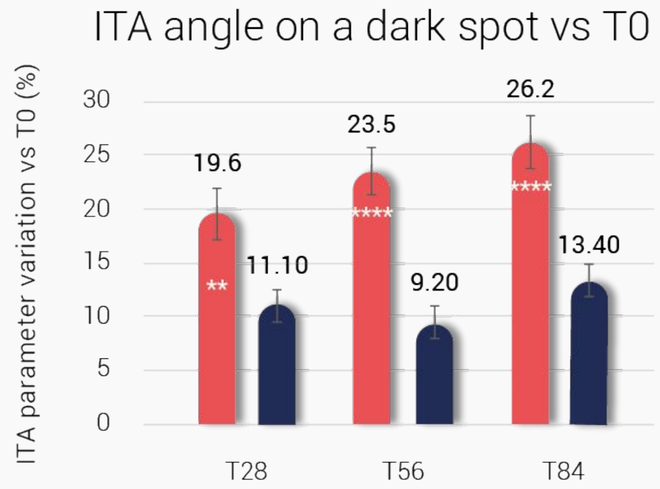
Ultrasond image of the skin



In the analyzed skin region (epidermis + dermis) skin thickness is measured in five points.
An evident increase in the echogenicity and density of the dermis can be seen in the subject taking **eternalyoung**. (image of one volunteer)

Achieved a significant reduction of brown spots pigmentation and provides a growing skin radiance over time



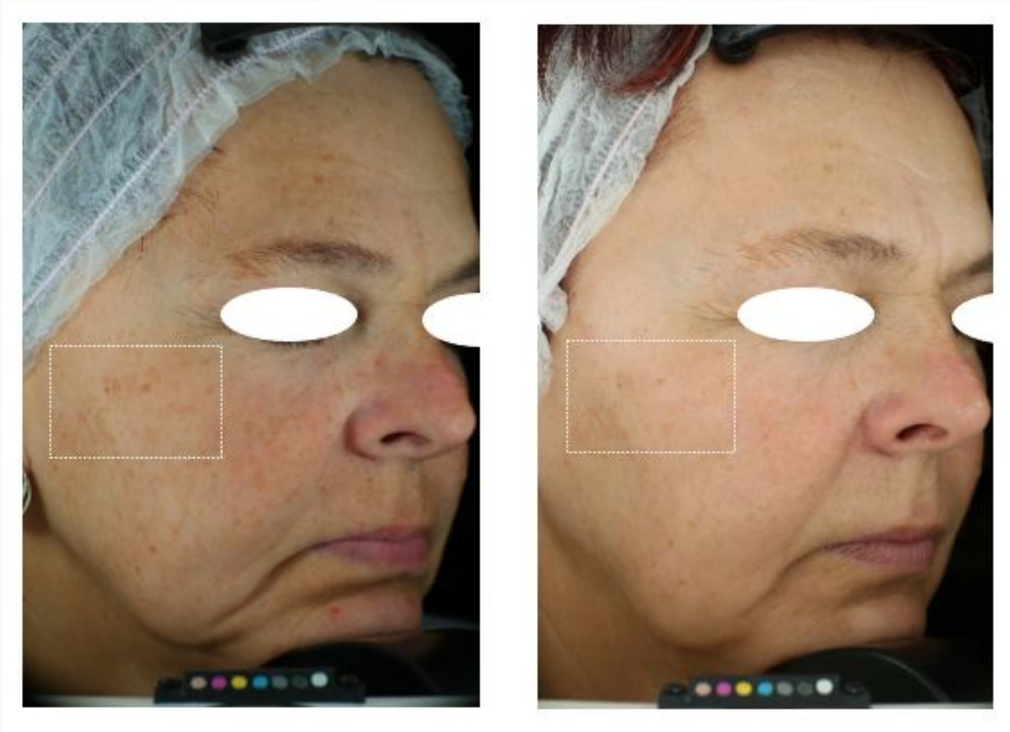


eternalyoung

Placebo

**p <0.01 ***p <0.001
****p <0.0001 vs placebo

A low ITA° indicates a brown pigmentation, while a high ITA° value indicates light pigmentation



BEFORE

AFTER

What participants taking eternalyoung felt?

71% My skin looks younger. Product reduces signs of stress and fatigue.

75% My fine lines and wrinkles are less visible, my skin looks brighter and my complexion looks healthier.

79% Product improves the overall aspect of skin. It looks more rested, more homogenous and with a more even tone.

82% My skin looks more elastic and denser.

86% My skin looks more moisturized.

89% My skin is firmer.

Subjects notice an overall improvement in their skin after only 4 weeks of treatment.
Being the perception higher with the time of consumption.
Product also helps to reduce the stress and fatigue increasing the sense of well-being

Summary of proven efficacy

In vivo

eternalyoung after only 4 weeks:

- Reduces wrinkles and skin roughness
- Improves skin elasticity, firmness and reduces skin fatigue.
- Increases skin thickness and redensify the skin
- Moistures the skin and improves the barrier function
- Reduces dark spots pigmentation and provides a growing skin radiance over time
- Subjects notice an overall improvement in their skin.
- Helps to reduce the stress and fatigue increasing the sense of well-being

In vitro

eternalyoung prevents and reduces the signs of skin aging by:

- Delaying the fibroblast telomere shortening. This, in turn, prolongs cellular and tissue life-span, allowing cells to escape the typical cell senescence pathway.
- Protecting skin cells from oxidation damage that occurs with aging.
- Reducing the AGE formation in the skin fibroblasts that contributes to the preservation of the skin’s biomechanical properties.
- Contributing to fibroblast proliferation that helps strengthen the skin structure.
- Inhibiting the melanin production that contributes to reducing or preventing hyperpigmentation.

Monteloeder
Digital Nutraceuticals for you

Nuria Caturla, Ph.D
Chief R&D Officer

T. +34 965 68 52 75 · Ext.133
nuriacaturla@monteloeder.com

HEADQUARTERS
Miguel Servet 16, nave 17
Elche Parque Industrial · Apdo. 580
03203 Elche · Alicante · España
T. +34 965 68 52 75 · F. +34 965 68 52 76
www.monteloeder.com
info@monteloeder.com

ASIA PACIFIC
Level35-02 (East Wing), QSentral
2A, Jalan Stesen Sentral 2, KL Sentral
Kuala Lumpur 50470, MALAYSIA

313ho,30, Teheran-ro 6-gil,
Gangnam-gu, Seoul, REPUBLIC OF KOREA

MONTELOEDER USA INC
8950 SW 74th Ct., Suite #1406
Miami , Florida 33156 USA
Phone (305) 285 85 61
Fax 954 206 6880
www.monteloederusa.com
info@monteloederUSA.com

RESULT. CLINICAL PARAMETERS

ETERNALYOUNG® positively influences all the clinical monitored parameters, both compared to baseline and placebo group, except for R9 parameter at T28 (obtained results are statistically significant compared to baseline but are not significant compared to placebo treated group).

	Eternalyoung			Placebo		
PARAMETERS	**28 d**	**56 d**	**84 d**	**28 d**	**56 d**	**84 d**
% variation vs. start (T0)						
• Skin moisturization	+7.1%^{S*}	+15,3%^{S*}	+19,6%S*	+3.7%^{S}	+3.3%^{nS}	+2.4%^{nS}
• Trans epidermal water loss	-5.7%^{S*}	-10.6%^{S*}	-14.3%^{S*}	-1.8%^{S}	-1.0%^{nS}	-1.1%^{nS}
• Wrinkle depth	-10.2%^{S*}	-11.2%^{S*}	-14.4%^{S*}	+3.1%^{S}	-2.2%^{nS}	-3.7%^{S}
• Smoothness Ra parameter (1)	-2.3%^{S*}	-2.9%^{S*}	-5.3%^{S*}	+1.3%^{NS}	+0.8%^{NS}	-0.1%^{NS}
• Skin gross elasticity – R2 parameter	+6.8%^{S*}	+11.8%^{S*}	+14.2%^{S*}	-0.8%^{nS}	+0.4%^{nS}	+1.7%^{nS}
• Skin firmness – R0 parameter (2)	-5.5%^{S*}	-9.9%^{S*}	-11.7%^{S*}	-1.3%^{nS}	-1.1%^{nS}	-1.9%^{nS}
• Net-elasticity - R5 parameter (3)	+5.8%^{S*}	+14.1%^{S*}	+17.3%^{S*}	-1.2%^{nS}	+1.1%^{nS}	+1.5%^{nS}
• Tiring effects of the skin – R9 (4)	-5.1%^{S}	-9.6%^{S*}	-11.7%^{S*}	-2.0%^{nS}	-4.0%^{S}	-4.7%^{S}
• Skin colour (ITA angle) (5)	+19.6%^{S*}	+23.5%^{S*}	+26.2%^{S*}	+11.1%^{S}	+9.2%^{S}	+13.4%^{S}
• Skin brightness (gloss parameter)	+13.9%^{S*}	+23.7%^{S*}	+27.3%^{S*}	+7.2%^{S}	+7.8%^{S}	+9.5%^{S}
variation vs. start (T0)						
• Skin thickness (mm)	+0,06^{S*}	+0.11%^{S*}	+0.15%^{S*}	+0.02^{S}	+0.03^{S}	+0.05^{S}

(1) A decrease of Ra parameter can be expressed in absolute values as an increase of skin smoothness. | (2) A decrease of R0 parameter can be expressed in absolute values as an increase of skin firmness. | (3) An improvement of R5 parameter indicates an improvement of skin elastic recovery after deformation. | (4) The closer this value is to 0, the lower are the tiring effects. Its decrease indicates an improvement./ (5) A low ITA° indicates a brown pigmentation, while a high ITA° value indicates light pigmentation.
S: statistically significant vs. start of the study (T0) | *: statistically significant vs. Placebo | NS: not statistically significant vs. start of the study (T0)

What participants taking eternalyoung felt?

SELF-ASSESSMENT QUESTIONNAIRE	Positive Answers T 28	Positive Answers T 56	Positive Answers T 84
My skin looks more moisturized	64%	86%	86%
My skin is firmer	68%	82%	89%
My skin looks more elastic and denser	68%	71%	82%
My Fine lines and Wrinkles are less visible	54%	68%	75%
My skin looks younger	57%	64%	71%
Product improves the overall aspect of skin	61%	71%	79%
My skin is more homogenous and has a more even tone	68%	71%	79%
My skin looks brighter	71%	71%	75%
My skin looks more rested	68%	71%	79%
My complexion looks healthier	68%	71%	75%
Product reduces signs of stress and fatigue	64%	71%	71%
Product enhances my sense of well-being	61%	71%	75%

- Subjects notice an overall improvement in their skin after only 4 weeks of treatment. Being the perception higher with the time of consumption.

- Product also helps to reduce the stress and fatigue increasing the sense of well-being