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INTRODUCTION

The term senescence is derived from the Latin "senectus" and it is a synonym for aging. Applied to skin, cellular senescence is the main process of erosion and loss of fibroblasts.

As we age, senescent fibroblasts tend to prevail over young fibroblasts, and as a result skin loses some of the functional characteristics of young skin.

VITASOURCE™ is an innovative purified active product which delays cellular senescence and recovers characteristics that skin cells had 10 years earlier.

DESCRIPTION

Over time, cells show a decrease in their replicative and metabolic capacity, resulting in a reduction in their number of duplications. This phenomenon of loss of replicative capacity is known as **replicative or cellular senescence**. The existence of a molecular clock is suggested, marking the moment when the capacity of cell replication stops and senescence starts. This marker would be placed on the DNA molecules located at the ends of chromosomes, called **telomeres**, term which comes from the Greek word "telos" (end) and "meros" (part). Each time a cell divides, its telomeres are shortened, thus reducing their subsequent cellular replicative capacity. In the 90s it was established that the start of replicative senescence is controlled by a shortening of the telomere.

CELLULAR SENESCENCE IN FIBROBLASTS AND SKIN AGING

Skin is formed by an external layer of epithelial cells in continual renewal called epidermis and an internal layer of connective tissue called dermis. The major cell type in the dermis is the fibroblast, a cell that synthesizes protein products, such as the extracellular matrix, and connective tissue fibers, such as collagen, elastin and reticulin. These proteins are responsible for the proper integrity and elasticity of skin as an organ.

Senescence of fibroblasts is the main process of loss of fibroblasts in the skin and is closely related to aging. During their lifetime, fibroblasts undergo a slowing down process of their cell duplication cycle until, after a certain number of cell divisions, they enter a state of cellular senescence until they die. Senescent cells suffer important metabolic and morphological changes, that have a clear negative effect on skin tissue, causing skin aging.

It has been demonstrated that the number of cell duplications of fibroblasts depends on age. Fetal fibroblasts can duplicate approximately 50 times on average before entering senescence, while from 30 years of age onwards, the number of duplications is reduced by approximately 10% every decade, as shown in figure 1 (Schneider, El. et al., 1976).

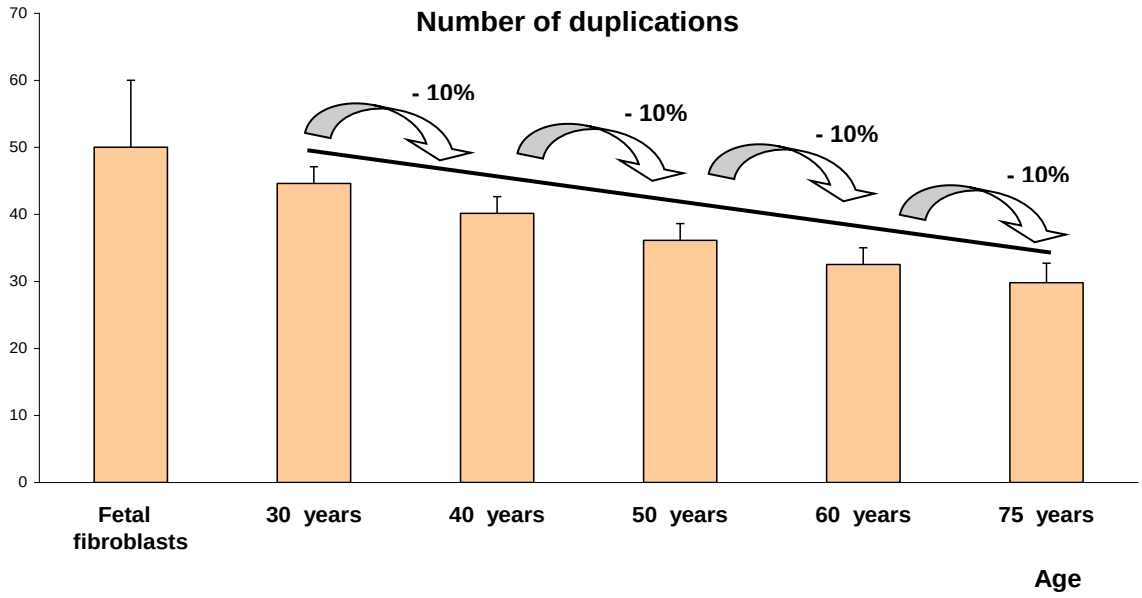


Figure 1. Link between the number of cell duplications of fibroblasts and age.

TELOMERES AND CELLULAR SENESCENCE

The molecular mechanisms responsible for cellular senescence have been widely studied in recent years (Stewart, SA., Weinberg, RA., 2002) and, as discussed above, one of the main causes of the transition from the proliferative cellular state to senescence is the shortening of the **telomeres** (figure 2).

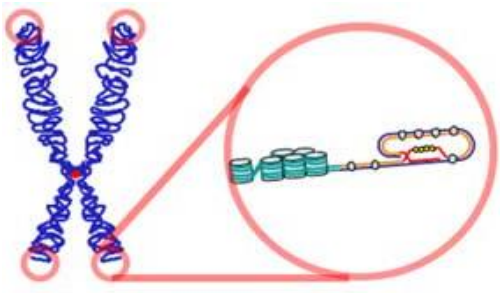


Figure 2. Location of telomeres.

The telomere is a region of non-coding DNA that is located at the ends of chromosomes. Telomeres are involved in many cellular functions related to the stability of chromosomes and cell division.

The role of telomeres is twofold. On the one hand, they avoid the double DNA strand to be recognized by the cell itself as an anomaly that the cell tries to repair; on the other hand, they enable the replication of the ends of the chromosomes in each cell division cycle.

Although the exact mechanism by which telomeres are associated with the state of cellular senescence is not fully defined, experimental studies show that their progressive shortening during the division process of adult cells results in lower rates of cell proliferation and triggers the senescent process.

Cells exhibit the senescent phenotype when one or more of their telomeres reaches a critical length This critical length can compromise chromosome integrity and certain DNA damage can also cause cellular senescence.

Adult cells shorten their telomeres at each cell division, except embryonic stem cells that keep the length of their telomeres unchanged, as this allows them to acquire an unlimited capacity for cell proliferation. This phenomenon is due to the activity of a ribonucleoprotein complex called **telomerase**, whose expression is restricted to stem cells. Therefore, most adult cells, including fibroblasts, do not express it.

TELOMERASE: STRUCTURE, FUNCTION AND REGULATION OF EXPRESSION

Telomerase (figure 3) is a ribonucleoprotein enzyme responsible for maintaining the integrity of the terminal telomeric fragments of linear eukaryotic chromosomes.

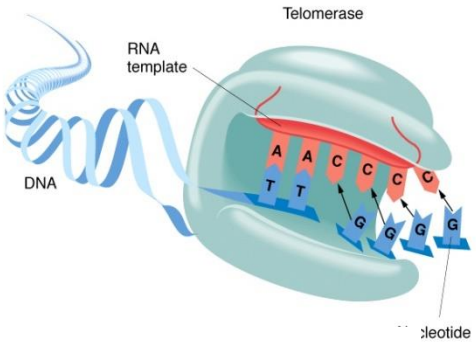


Figure 3. Structure of telomerase

It is an RNA-dependent DNA polymerase that synthesizes telomeric DNA sequences, consisting of two basic units. *In vitro* studies have showed that these two components are essential for telomerase activity; hTR (*human Telomerase RNA*) is an RNA molecule that provides the template for the synthesis of telomeric repeats, and hTERT (*human Telomerase Reverse Transcriptase*) is the catalytic subunit with reverse transcriptase activity.
This enzyme maintains the length of telomeres through successive cell replications.

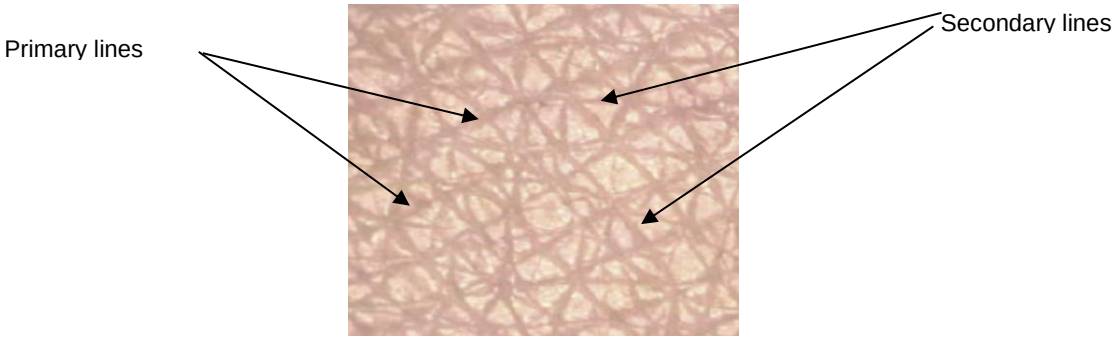
PHYSIOLOGY OF MATURITY

Skin reflects the passage of time, but some of these changes can only be seen from very close and are strongly linked to **skin microrelief** (figure 4).

The skin surface is furrowed by lines like a topographical map full of valleys and plateaus, which can be classified into 4 different types depending on their orientation and depth:

- Primary lines: they are the deepest ones (20-200 µm) and are influenced by the organization of the superficial dermis: they form geometrical shapes, mainly triangles and quadrilaterals.
- Secondary lines: they are less deep than primary lines and their disposition is determined by the granulose layer of the epidermis. They cross the geometric shapes formed by primary lines.
- Tertiary and quaternary lines: they are the thinnest ones and are caused by the organization of the stratum corneum (Vöros, E. et al., 1990; Quatresooz, P. et al., 2006).

Figure 4. Image of skin microrelief



The skin of a 20-25 year old person has a smooth texture with fine and thin lines, projected in various directions, and the surface of polygons is rather small (figure 6). However, from the age of 30-40 onwards, skin texture becomes irregular and its lines are thicker, deeper and in one direction, becoming more visible. The surface area of polygons gradually increases, and when they are viewed under the microscope, their number decreases by study area (Robert, C. et al., 2003).

The characteristics of the microrelief primary lines depend on the structure of the papillary dermis. Their orientation and depth depend on their location and change with aging and under the influence of certain environmental factors. With age, the polygonal structure becomes more anisotropic (disordered) and the so-called irregularity index increases. These changes are associated to a progressive loss and atrophy of the reticular dermis, decreasing its flexibility, which precedes the appearance of the characteristic wrinkles of old age (Piérard, GE. et al., 2004). It has been demonstrated that around 30 years of age, elastic fibers in the papillary dermis gradually disappear, and this fact is directly linked to changes in the microrelief, which loses the appearance of a continuous mesh.

Therefore, skin microrelief is a reliable indicator of the degree of skin aging.

BOTANY AND CHEMISTRY

VITASOURCE™ is a purified fraction obtained from roots of *Scutellaria baicalensis Georgi* standardized in baicalin (figure 5). This active product has been obtained as a result of research conducted from a screening with more than 40 plant extracts, which has enabled the identification of the fraction responsible for the cosmetic activity.

The *Scutellaria* genus includes more than 350 species distributed worldwide from Siberia to South America and North America, as well as Japan and most of Europe and Asia.

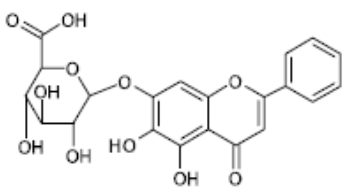


Figure 5. Chemical structure of baicalin

This genus has been used by indigenous peoples to treat a wide variety of diseases, including neurological disorders, chronic diseases, hepatitis, cirrhosis, jaundice and anxiety.

The species of the *Scutellaria* genus are the source of a wide range of active compounds and, therefore, have been used in traditional medicine in their countries of origin. *Scutellaria baicalensis Georgi*, commonly known as *Haung-qin* (= *Haungqqin*) or *wogon*, is the most prescribed plant in traditional Chinese medicine and it is also widely used in Japanese Kampo medicine. Nowadays, it is officially registered in the JPXIII Japanese Pharmacopoeia and in the Chinese Pharmacopoeia.

Scutellaria baicalensis Georgi (=*Scutellaria macrantha Fisch.ex Rchb; Scutellaria adamsii A. Ham.*; *Scutellaria lanceolaria Miq.*; *Scutellaria speciosa Fisch. ex Turcz.; Scutellaria grandiflora MF*) belongs to the Lamiaceae family and it is known as Asian scutellaria, Chinese skullcap or baical skullcap.

Figure 6. *Scutellaria baicalensis*

This biennial perennial plant can reach up to 1.2 m in height. It has a branched and fleshy root 2 cm in diameter and quadrangular stems. It is found mainly in China, Korea, Mongolia and Russia.

Its leaves are opposite and heart-shaped at the base, but as they climb they become lanceolate with 2 mm long petioles. The flowers are bluish purple in color and are grouped in terminal clusters with basal bracts similar to the leaves on the stem (figure 6). Flowering is from May to August. The English name *"skullcap"* describes the shape of the flower calyx, which resembles the helmets that were used in the Middle Ages.The fruits appear in late August and have the appearance of small ovoid nuts with a brown color.

The chemical composition of the roots of Asian scutellaria is characterized by its high content in flavonoids. Out of a total of 26 identified flavonoids, five of them are C-glycosides, twelve are O-glycosides and the remaining nine are aglycones (Han, J. et al., 2007). The characteristic components of this root are baicalin (the glucuronic derivative of the flavone family (baicalein), 6-9%), wogonin-7-O-glucuronide (wogonoside) (2-8%), baicalein (0.1-1.6%) and wogonin (0.01-0.3%).

The presence of other flavonoids in lower concentrations has also been described, such as oroxylin A, visidulin, skullcapflavone II, neobaicalein, acteoside, chrysin and their corresponding glucosides.

Traditionally, *Huangqin* (*Radix Scutellariae*, a preparation widely used in traditional Chinese medicine obtained from the roots of *Scutellaria baicalensis Georgi*) has been used for the alleviation and detoxification of fever episodes.

IN VITRO INNOVATIVE MECHANISM

To evaluate the activity of **VITASOURCE™** on cellular senescence, Provital developed an innovative *in vitro* technology in collaboration with the *Unitat de Biologia Cel.lular I Molecular – IMIM* (Hospital del Mar, Barcelona) and the results have been presented at 25th IFSCC Congress (October 2008):

New In Vitro Methodology To Measure Telomerase (hTERT) Expression And Its Application For The Search Of Novel Activities That Delay Dermal Fibroblast Senescence

The objective of this study is to find vegetal origin active ingredients that inhibit cell senescence through the induction of the gene expression of human telomerase (TERT). As part of this study, Provital developed a technology which allows the screening of groups of natural products in order to identify their active principles.

The main conclusions of the study were:

- ✓ **VITASOURCE™ activates the promoter of telomerase (TERT) in primary human fibroblasts.**
- ✓ **VITASOURCE™ induces the cellular expression of telomerase in primary human fibroblasts.**

IN VITRO EFFICACY RESULTS

VITASOURCE™ HAS A REAL EFFECT ON TELOMERASE IN THE FIBROBLASTS.

To study whether **VITASOURCE™** has any effect on cellular senescence, IMR-90 cultures were continually treated with this active element at the non-toxic concentration of 3 µM, starting with cultures that were in an exponential growth stage until they stopped growing when they reached senescence.

The number of duplications of the cell population throughout the experiment was calculated and this showed that the treated cells underwent **5 additional cell divisions** from the time that untreated cells entered senescence (figure 7).

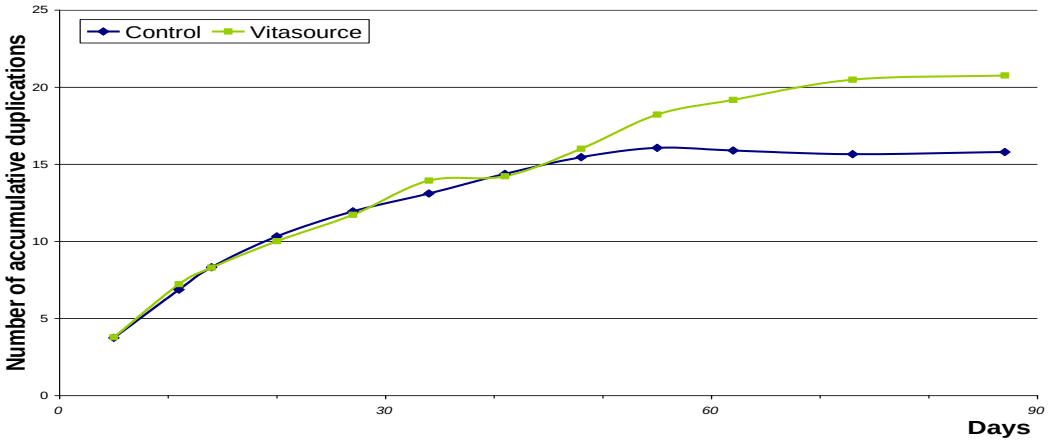


Figure 7. Accumulated number of duplications for the control treatment and **VITASOURCE™**

Taking into account that the total number of replications of a fibroblast is 50, if they are able to duplicate 5 times more, it means an increase of 10%.

If we apply this factor to real human skin, we could state that, as it is the same percentage that is lost each decade from 30 years of age onwards, a 10%, **VITASOURCE™** delays cellular senescence and rejuvenates skin to a condition comparable to that of one decade younger (Schneider, EL. et al., 1976).

IN VIVO EFFICACY

The objective of the assay is to assess the *in vivo* efficacy of **VITASOURCE™** as a cosmetic active product for skin rejuvenation. To this effect, skin microrelief and skin elasticity were objectively evaluated using instrumental measures (fringe projection and cutometry, respectively).

The test was conducted on the forearm, with 1 group of 20 volunteers between 35 and 45 years of age. The volunteers applied the active formulation of 1.5% of **VITASOURCE™** on one forearm and the placebo formulation on the other forearm. The products were applied twice a day during 56 days. Measurements were taken at the beginning of the treatment (D0) and after the last application (D56).

	Active product formulation	Placebo formulation
Aqua (Water)	c.s.p. 100%	c.s.p. 100%
Disodium EDTA	0.09	0.09
Alcohol Denat.	6.21	6.21
Isopropyl Alcohol	0.79	0.79
VITASOURCE™	**1.50**	---
Glycerin	3	3
Macadamia Ternifolia Seed Oil	2	2
BHT	0.05	0.05
Preservative	0.73	0.73
Parfum (Fragrance)	0.30	0.30
Polyacrylate-13	1.91	1.91
Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Squalane, Polysorbate 60	0.28	0.28

Table 1. Formulations used in the *in vivo* study

SKIN MICRORELIEF EVALUATION (FRINGE PROJECTION)

The evaluation of skin microrelief was performed using a 3D imaging technique called fringe projection applied to silicone replicas of the skin.

Analysis of the results is performed by the Toposurf system and using the collected data anisotropy values are obtained (coefficient of skin roughness uniformity in various directions; it measures the destructuring of skin). An increase of this parameter is linked to the destructuring of the skin relief.

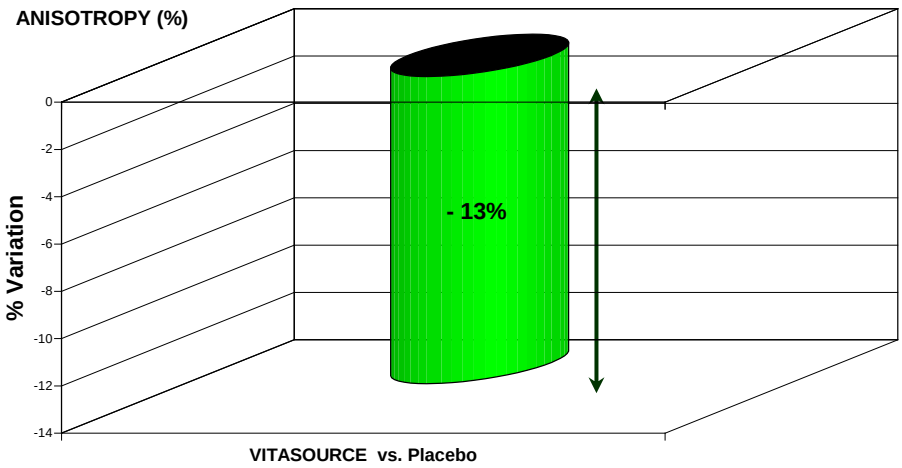


Figure 8. Percentage of anisotropy variation on the skin of VITASOURCE™ versus the placebo treatment.

As shown in the graph (figure 8), if the difference between the value of the placebo treatment at T0 and T56 is calculated and compared to the value of the difference between the value of VITASOURCE™ at T0 and T56, a decrease in anisotropy of **13% is observed**

This leads to an increase in skin tissue organisation, and indicates that a restructuring of skin relief is initiated after applying VITASOURCE™, unlike the result obtained with the placebo.

It can also be stated that the continuous application of VITASOURCE™ substantially improves skin relief, as the typology of skin lines is kept, as well as the models and sizes of superficial polygons, as can be seen in the following images (figure 9).

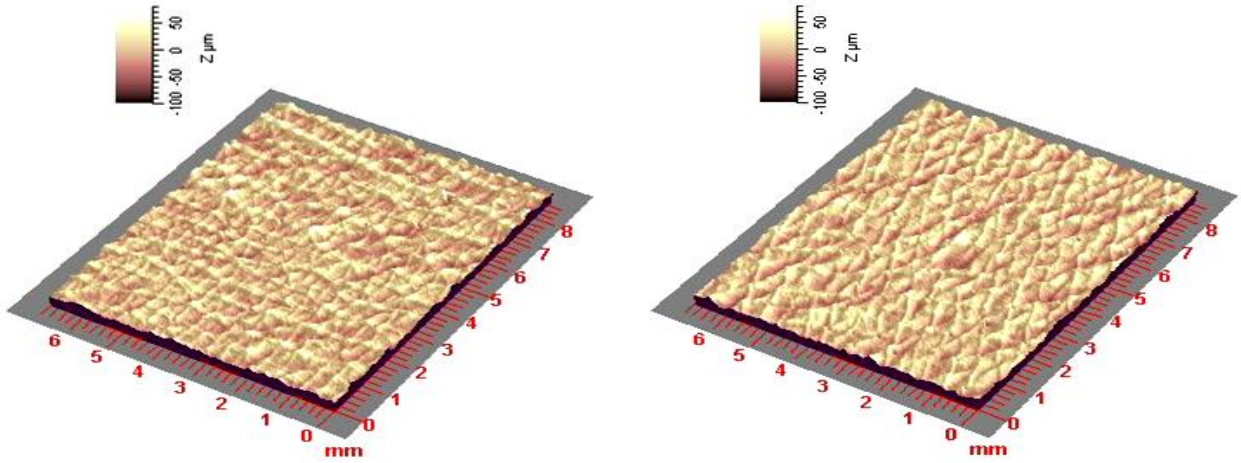


Figure 9. Appearance of skin relief at T0 and T56 respectively.

SKIN ELASTICITY

The evaluation of skin elasticity was performed with the Cutometer 575® (Courage & Khazaka). The principle of this technique is based on the creation of a negative pressure of 300 mbar on the skin surface and the measurement of the vertical deformation that is originated.

The resistance of skin to absorption due to negative pressure (Uf, Uv) and its ability to return to its original position (Ur, Ue) is shown in the form of curves at the end of each measurement, allowing us to obtain highly interesting parameters (figure 10).

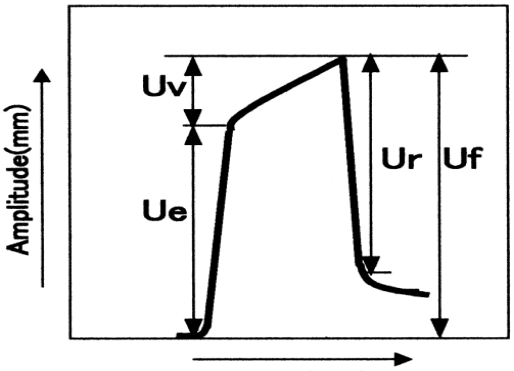


Figure 10. Parameters obtained with Cutometer 575®

<u>Firmness</u>

Uf: total maximum skin extensibility (mm)

Uv: viscoelastic extensibility and delayed extensibility (mm)

<u>Elasticity</u>

Ue: immediate extensibility (mm)

Ur: recovery of immediate elasticity (mm)

Figures 11 and 12 illustrate the results obtained after applying negative pressure on the skin for a limited time and the response of skin after a 56-day treatment with **VITASOURCE™**. The results show the percentage difference between the treatment with **VITASOURCE™** and with the placebo, once their individual differences at T0 and T56 have been calculated.

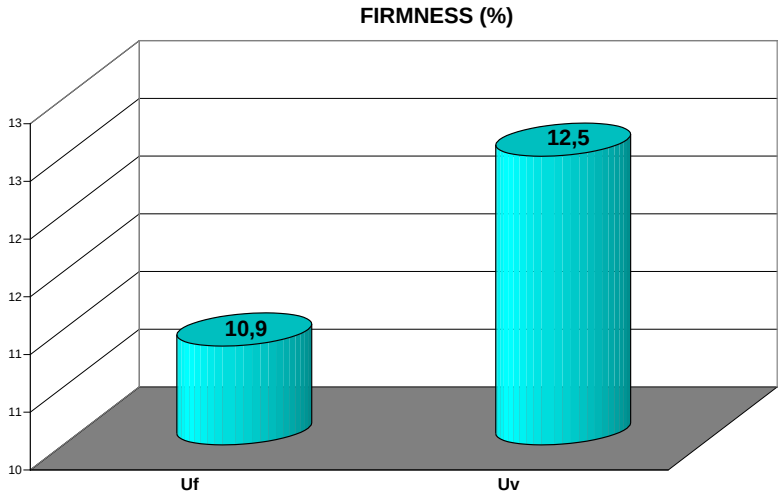


Figure 11. Firmness results, variation between **VITASOURCE™** and placebo considering T0 and T56.

The parameters of firmness Uf and Uv showed improvements of 10.9% and 12.5% respectively, and the increases in elasticity Ue and Ur were 10.4% and 12.4% respectively. Therefore, all skin biomechanical indicators show positive values over 10% compared to placebo.

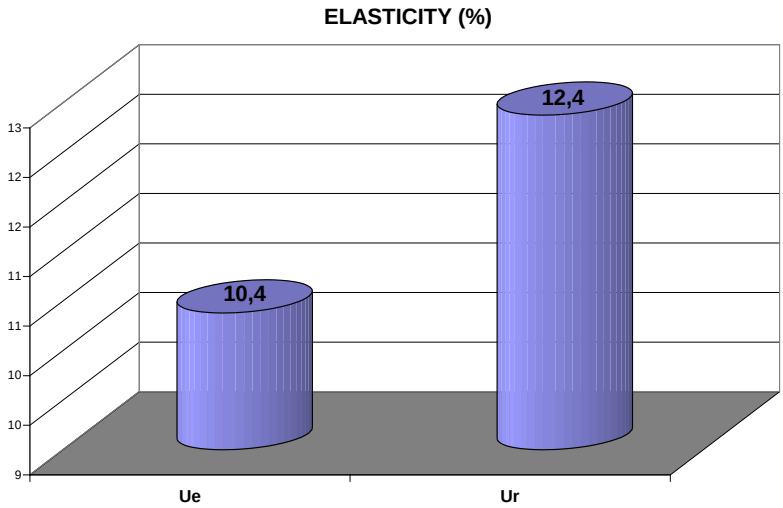


Figure 12. Elasticity results, variation between **VITASOURCE™** and placebo considering T0 and T56.

It is reported that over time skin loses its capacity to temporarily stretch and also its ability to recover. Results clearly show that VITASOURCE™ **increases the values of skin elasticity and firmness and, therefore, maintains the characteristics of a young skin, maximum extensibility and quick recovery capacity. The treatment with VITASOURCE™ maintains skin's young look and quality, as it improves biomechanical indicators.**

COSMETIC PROPERTIES AND APPLICATIONS

To summarize, table 2 shows the cosmetic properties of VITASOURCE™ based on the results of the efficacy assays that have been carried out.

PROPERTY	*EFFICACY*	*COSMETIC APPLICATIONS*
Cell rejuvenator 10 years	It delays fibroblast senescence, +10% proliferative stage	**Cosmetic products for skin rejuvenation**
Skin microrelief	Anisotropy: -13%	**Cellular reactivator for skin**
Firmness	Uf: +10.9% Uv: +12.5%	**Anti-aging preventive cosmetics**
Elasticity	Ur: +12.4% Ue: +10.4%	**Cellular regenerator** **Intensive facial treatments**

Table 2. Cosmetic properties of VITASOURCE™.

RECOMMENDED DOSE

The recommended dosage of VITASOURCE™ is 1-5%.

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