論文 EyesOn

01

Dermohacking Senescence with a New AI-proven & Biotech Ingredient

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Modern science has opened a variety of new ways to improve survival and quality of life. Meanwhile, the recent movement towards preventative health among the ever-increasing mature segment of the population, has boosted the emerged success of new healthcare concepts such as biohacking. This one-health movement goes hand in hand with unprecedented scientific advances appearing in the anti-aging field, particularly for cellular senescence.

As these new advances on cellular senescence take ground in the scientific community, the beauty industry can better tap into the opportunities that this longevity era can offer.

In this regard, Provital is taking the lead in a new type of cosmetics that will leverage technology, science, natural and holistic preferences to push the boundaries on efficacy and selectivity, while still supporting an environmentally friendly brand positioning: Dermohacking cosmetics.

Altheostem™ is Provital's first dermohacker. An *Althaea rosea* stem cell active that has proven its ability to selectively eliminate cellular senescence.

This lab-grown active ingredient leverages biotechnology for a selective biological action on aged skin. In this article, Provital describes how the positive effects of its senolytic activity are thoroughly tested *in vitro* and *in vivo*, using both instrumental and Artificial Intelligence analysis, thus unveiling its well-aging power.

Welcome to DERMOHACKING COSMETICS

Humans have long harboured an obsession with living forever, but not at any price. Ever since some of humanity's oldest tales, one of its deepest desires has been to attain everlasting life [1)]. Now, modern science has opened up a variety of new ways to improve survival and quality of life, and members of the technology-driven ultra-rich are adopting these new approaches in an attempt to extend and enjoy their own lives for longer.

That longstanding appetite for life intensifies along with a worldwide population ageing phenomenon. As this is a large, growing and relatively affluent demographic, older consumers offer a great chance for innovation to cater to their special demands, where health takes a

central stage and the approach to well-aging is holistic, seeking balance in body and mind with wider lifestyle choices that include supplementation, exercise, diet, beauty products and therapy. In response to the increased interest in this type of "healthy fix" for ageing among older consumers, manufacturers across consumer health have taken different approaches to leverage preventative health.

With this recent trend in preventative health, new terms and movements are leading innovations in healthcare. Biohacking, for example, is a term that is getting more and more popular amongst healthcare. It basically refers to the reasoning behind the health benefits one can achieve by employing certain changes in daily habits. It details how these little "hacks" to our own physiology can affect our aging process, and how technology can make them feasible.

The emerged success of the biohacking concept in healthcare goes hand in hand with the unprecedented scientific advances that are occurring in the anti-ageing field, particularly with the discovery that the rate of ageing is controlled by genetic pathways and biochemical processes conserved in evolution – such as cellular senescence [2)]. As this relation between ageing and cellular senescence becomes established in the scientific community, the beauty industry can better tap into the opportunities that the longevity era and the innovation-eager consumer offer.

In this regard, Provital is taking the lead in a new type of cosmetics that will leverage technology, science and natural preferences to push the boundaries on efficacy and personalisation, while still supporting an environmentally friendly brand positioning: Welcome to ***Dermohacking*** cosmetics.

· Dermo-like Science ·

When further analysing the current proactive approach to health, we see that the consumer is looking for efficacy and results. This aligns with the notable growth that dermocosmetics segment has experienced in the last years, where brands leverage science-backed claims, which subsequently gives them a greater perception of safety, efficacy and transparency. Now that the dermocosmetic user tends to be younger, digitally-savvy and have more natural preferences; clean beauty, plant-based, traditional medicine-based, and doctor-founded brands can overlap with dermocosmetic consumers' demands by combining the perceived safety of their natural origin with new powerful science-backed claims.

· Technological Hacking ·

At present, personalisation is only about using technologies like AI, AR, and VR to guide consumers to personalised advice or product matching. However, it is through selective mechanisms of action that a beauty product will be able to address the specific needs of each person's skin by selectively 'hacking' the damaged cells. Plus, through a specifically designed biotechnological obtention, an ingredient can tackle both sustainability and technological ad-

vances to achieve the desired specific effect.

Altheostem™ (*Althaea rosea* stem cells active) appears as a new kind of well-aging ingredient that blends both dermocosmetic and biohacking concepts: A new plant stem cell-based active that selectively eliminates skin cellular senescence.

1. Sustainable lab-grown ingredient: *Althaea rosea* petal stem cells

As introduced above, the new ingredient launched by Provital is sustainably obtained from lab-grown stem cells derived from the flower petals of *Althaea rosea*. This eco-responsible biotechnological obtention method allows the preservation of the flower's environment in nature, a full traceability of the raw material, and a production process that consumes less water; thus, providing this active ingredient with superlative ecological standards such as Vegan-compliance, COSMOS-Approved and 100% Natural Origin (ISO16128).

2. Selective mechanism of action: Leading-edge senolytic activity

Cellular senescence is a stress response to damaging inputs such as genotoxic or oxidative stress, telomere shortening, DNA injury or mitochondrial dysfunction, which results in irreversible resistance to apoptosis. Although it is a normal and healthy cellular response in young tissues, the accumulation of senescent cells over time has deleterious consequences in some critical physiological processes [3) 4)]. In fact, senescence is considered one of the most important hallmarks of ageing, and one of the reasons why human skin develops certain age-related alterations in elastic fibre morphology, facial wrinkles, and perceived age [5) 6)]. So, senescent cells are occupying a spot while not participating positively in the maintenance of the healthy skin tissue; they are a hindrance for a young skin tissue.

It is not surprising then that cell senescence, and the inflammatory factors that follow it – known as the senescence-associated secretory phenotype (SASP) – are widely studied in pharmacology as treatment targets. In the anti-ageing field, the strategies followed when attempting to block the negative effects of senescent cells can be classified as *Senomorphic* (when the objective is to supress the SASP phenotype) and *Senolytic* (when the target is the selective elimination of senescent cells).

Although senolysis is an emerging anti-ageing pharmacological strategy, its use in the cosmetic field is still very limited; yet the skin was one of the first organs in which senescent cells were identified [8)]. In fact, it may contain up to 55% of senescent fibroblasts [9)], whose specific type of SASP has shown unique features related to various skin ageing and homeostatic processes other than the common features such as pro-inflammatory and matrix-degradation phenotypes [10)].

Provital saw the scientific opportunity that this represented and embarked upon the development of a new biotechnological plant ingredi-

ent that displayed senolytic activity in dermal fibroblasts to ultimately provide a cosmetic formula with an effective way of prolonging the skin's youthful and healthy state.

2.1. *In vitro* methods

To evaluate the leading-edge senolytic activity of the ingredient, Provital performed various assays that demonstrate how the active selectively reduces the viability and the number of senescent Human Dermal Fibroblasts (HDFs) by inducing their apoptosis, and how such activity is transferred into certain biological pathways of the skin.

These experiments allowed the quantification and double-substantiation of the senolytic activity thanks to the use of two different HDF senescent models.

- First, HDFs were induced with H_2O_2 to cause extrinsic cell senescence. Then, these chemically induced senescent cells were treated with different concentrations of Altheostem™. Their viability was then analysed by quantifying the ATP levels. On the other hand, the number of senescent cells was quantified by the proportion of β-galactosidase-positive cells.
- Furthermore, a second type of senescent model was used to quantify the selective induction of apoptosis on senescent HDFs. In this case, the replicative model of cellular senescence was used, where HDFs from a young donor were continuously subcultured, until they lost their division capacity and showed a previously defined senescent marker [9]. Then, these naturally induced senescent cells were treated with different concentrations of Altheostem™ and two different apoptotic markers were quantified.

2.2. *In vitro* results

All the measurements of Senescent HDFs were compared to Normal HDFs, which were the same cell lines that had not been induced for any kind of senescence.

The resulting *in vitro* analysis is featured in the poster *"Senolysis, a cutting-edge strategy for healthy skin ageing, is activated by Althaea rosea stem cells"* [5], which was ranked among the Top 10 best posters at the IFSCC Congress 2020 in Yokohama (out of a total of 367 exhibited); and could be summarized as:

2.2.1. Dose-dependent senolytic activity:

β-galactosidase is a known and specific biomarker for senescent cells [8]. So, it is no surprise to see how senescent HDFs show a dramatic increase in the proportion of β-galactosidase-positive cells (Figure 1). The interesting part of this graph is that this proportion decreases as the concentration of the active increases, thus indicating a dose-dependent reduction in the number of senescent cells.

2.2.2. Selective elimination of senescent cells:

The other viability evaluation performed with the *Althaea rosea* stem cells active was the quantification of the ATP levels of the senescent and normal HDFs. To this end, Figure 2 shows how it significantly reduces the viability

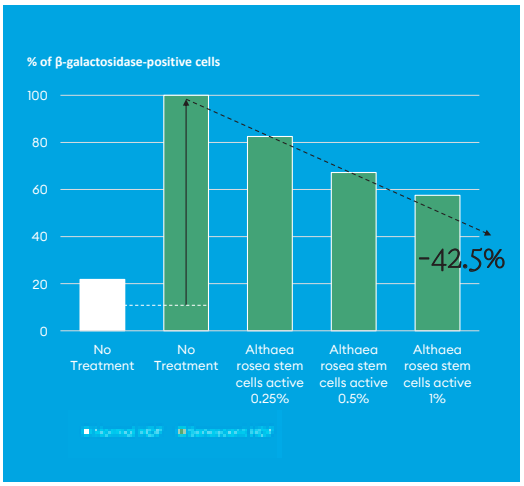


Fig. 1 The proportion of *β*-galactosidase-positive senescent cells diminishes proportionally to the increasing dose (%) of *Althaea rosea* stem cells active.

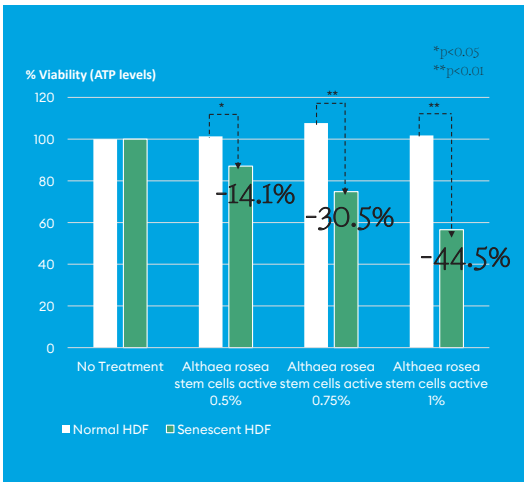


Fig. 2 The viability of senescent cells is significantly and selectively reduced by treatment with the *Althaea rosea* stem cells active.

of senescent cells at any of the assayed concentrations (ranging from 0.5 to 1%); and, even more importantly, the differences in the reduction detected in the viability of senescent and normal HDFs were statistically significant at all the assayed concentrations and go down to 44.5% when treating with the active at a dose of 1%. This not only proves that such effect is selective to senescent cells, but also shows a dose-dependent activity.

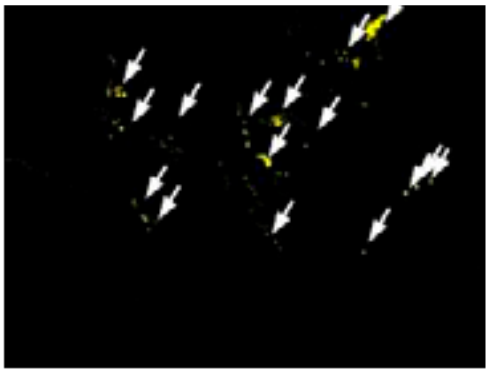
2.2.3. Selective apoptotic effect on senescent cells:

One of the identifying phenotypes of senescent cells is their resistance to apoptosis. In fact, a proposed mechanism to evaluate the activity of the senolytic compounds is their capacity to induce the apoptosis of senescent cells, often by upregulating pro-apoptotic molecular pathways.

In the case of the analysed active, the analysis of the apoptosis was first performed through the quantification of the levels of phosphatidylserine exposure (a biomarker of apoptotic early events) on the surface of the senescent HDFs treated with different concentrations of the active. The results were compared with those obtained in normal HDFs and showed that, while normal HDFs remained the same, the treatment of senescent HDFs with the active increased the apoptosis levels by 36% at highest concentrations, thus indicating a selective induction of apoptosis for those formerly resistant to it.

Such selectivity was also observable thanks to the evaluation of a second marker of apoptosis, the activation of Caspase-3/7 analysed by high-throughput automated imaging acquisi-

Normal HDF
(+ *Althaea rosea* stem cells active at 0.5%)



Senescent HDF
(+ *Althaea rosea* stem cells active at 0.5%)

Fig. 3 Images showing Caspase-3/7 activation events (Apoptosis biomarker) thus proving that induction of apoptosis is only observable and quantifiable on formerly resistant-to-apoptosis senescent HDFs.

tion and high-content screening (Figure 3).

2.2.4. Positive anti-ageing outcomes resulting from Senolytic activity on the skin

To further evaluate the biological relevance of the recently discovered senolytic activity of the active in the context of its potential anti-ageing application [7], a gene expression analysis of relevant genes involved in Extracellular Matrix (ECM) remodelling was performed. For this purpose, the expression levels of certain genes on natural aged HDFs (from an old donor) treated or not treated with the active were compared.

The results in Figure 4 show a clear induction of the genes involved in the formation of the extracellular matrix, including COL1A2, ADAMTS2 (both involved in collagen formation), HAS3 and FBN2 (involved in hyaluronic acid and fibrillin biosynthesis, respectively). Conversely, genes involved in extracellular matrix degradation such as the metalloproteinases MMP7 and MMP9 and the pro-inflammatory factor GM-CFS are strongly repressed [11].
Altogether, these *in vitro* results strongly suggest the positive outcomes that the senolytic activity of the *Althaea rosea* stem cells active exerts on ageing skin cells.

3. Quantifiable well-aging power: Clinical study strengthened by Artificial Intelligence

3.1. Clinical methods

- Instrumental analysis:

  A panel of 70 healthy female subjects, aged between 45 and 65 years were studied in a double-blind *in vivo* study. A formulation with 2% Altheostem™ was applied by 35 volunteers, and 35 applied the placebo, (according to a previously defined randomisation list) and efficacy tests were performed after 28 and 56 days of treatment.

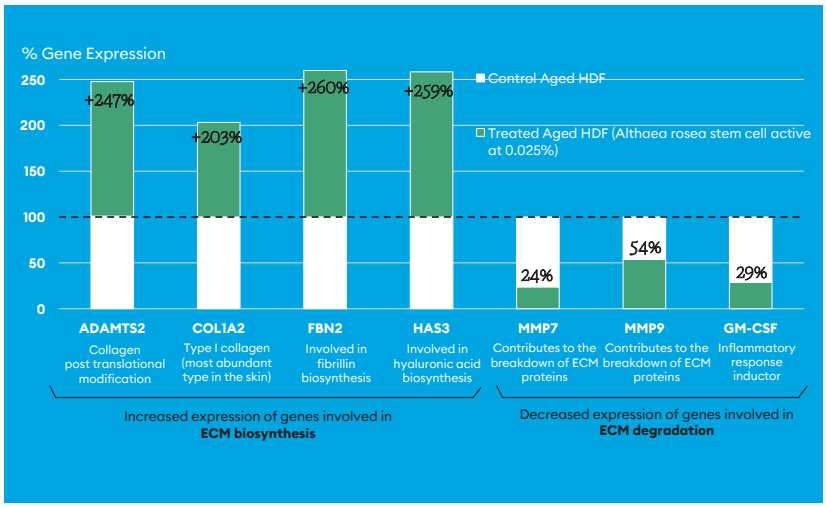


Fig. 4 The activation of senolysis promotes the right modulation of the expression of genes involved in the ECM remodelling of senescent HDFs

The methods and instruments used for the different parameters studied in this part of the study were:

- Spectrophotometer/Colorimeter CM-700D
- Cutometer®
- Ultrasound analysis
- Skin Profilometry (Primos 3D analysis)

- Artificial Intelligence analysis:

The Visual Apparent Age of the same panel of 70 healthy female subjects was thoroughly studied *in vivo* thanks to a cutting-edge and highly reliable system based on Artificial Intelligence. This age estimation module consists of a Machine Learning system that, based on Image Data, predicts the age of subjects in a controlled environment. To create an efficient system for apparent age estimation, an ensemble of different convolutional neural networks (CNNs) was used. The latter works together to extract information from each of the analysed pictures. The age detection model was initially trained using 55,134 images from 13,617 subjects with ages ranging from 16 to 77 years old. The source data in Provital's study consists of 207 videos showing the evolution of the subjects during different stages of treatment (D0-D28-D56). These videos were recorded in Full HD at 30 frames per second (FPS) and have an average duration of 36 seconds, with a total of 223,560 images analysed. These CNNs firstly isolate and crop the subject's face from the image to eliminate possible background noise with a face detector, and then that portion of the image containing the subject's face is fed to 3 different models that estimate the age of the subject (Figure 5).

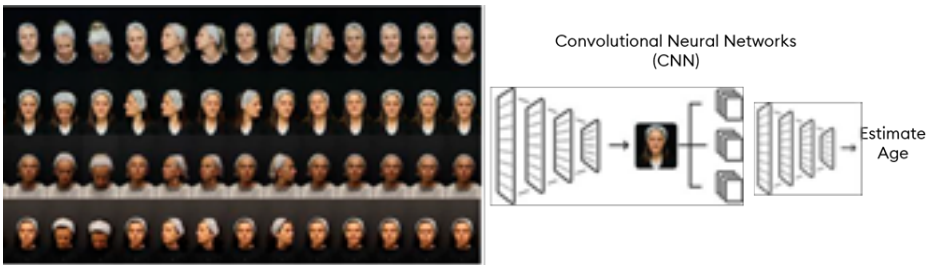


Fig. 5 Age estimation module used for the AI-test. The images in this representation are real examples of some volunteer's cropped faces (extracted and isolated from the videos). Then, each portion of the image containing the subject's face is fed to 3 different models that estimate the age of the subject.

3.2. Clinical results & discussion

3.2.1. Instrumental Analysis:

The results at day 56 of the various instrumental tests performed prove that the active significantly promotes:

- +8.5%* Healthy glow
  The active provides 7.4% more skin radiance than the placebo after only 28 days, a difference that keeps on increasing until the last day of this clinical assay, providing the significant improvement expressed above in the glowing appearance compared to the placebo.
- +16.8%* Elastic recovery (ability to recover the skin's original position after deformation)
- -18.6%* Skin sagging (uplifting and moisturising properties)
- +17.9%* Biological elasticity (balanced composition of the skin's elastin fibre network)
  The active showed a statistically significant effect in all three elasticity parameters after 56 days of treatment and compared to the placebo. However, it already provided a better elastic recovery (12.19%), a significant lifting effect (18.87%*) and improved skin elastic composition (7.98%) after only 28 days of treatment.
- +7.8%*** Skin redensification
  The resulting increase in total skin thickness was impressive: after only 28 days, the ingredient increased dermal and epidermal thickness by 6.4%, which represents a statistically significant difference of 4.8%*** vs placebo in that same period. Nonetheless, these numbers almost doubled after 56 days, suggesting that the active reverses the loss of skin thickness associated with age in both the dermis and the epidermis in a significant and cumulative fashion.
- -13.8%*** Wrinkle depth
  The active significantly decreases wrinkle depth both after 28 and 56 days of treatment, by -8.01% and -13.86%, respectively.
- -6.7%* Wrinkle volume
  It also markedly decreases wrinkle volume down to -2.55% at day 28 and -6.76%

*[*p<0.05; ***p<0.001; all results expressed as a difference vs the variation of each parameter in the placebo.]*

- -5.7 years less in the periocular area
  This "X-year less" effect is evaluated by fitting the periocular wrinkle depth data obtained in this study into a reference curve constructed from a large database that links the biological age of female volunteers with wrinkle depth. This estimation shows how the active decreases the average estimated age of the volunteers by 2.8 years and 5.7 years after 28 and 56 days of treatment, respectively, and as always, compared with the placebo.

This remarkable and cumulative anti-wrinkle effect is also visible in the 3D Primos images of the volunteers' crow's-feet area, as can be observed in Figure 6.

3.2.2. Visual apparent age based on Artificial Intelligence:

The results obtained from this AI-analysis show how the apparent age decreased by approximately 3.26 years in the group treated with the active at the end of treatment (56 days) vs the group treated with placebo. In fact, the difference was already of 1.04 years less in the treated group than in the placebo group after only 28 days.

It is thanks to this last calculation that Provital could estimate the change in the Visual Apparent Age of all volunteers, thus proving the well-aging power of the *Althaea rosea* stem cells active through Artificial Intelligence.

4. Dermohacking senescence through the eternal power of plant stem cells

In summary, Provital anticipated the effect of the current fervour for life in the ever-increas-

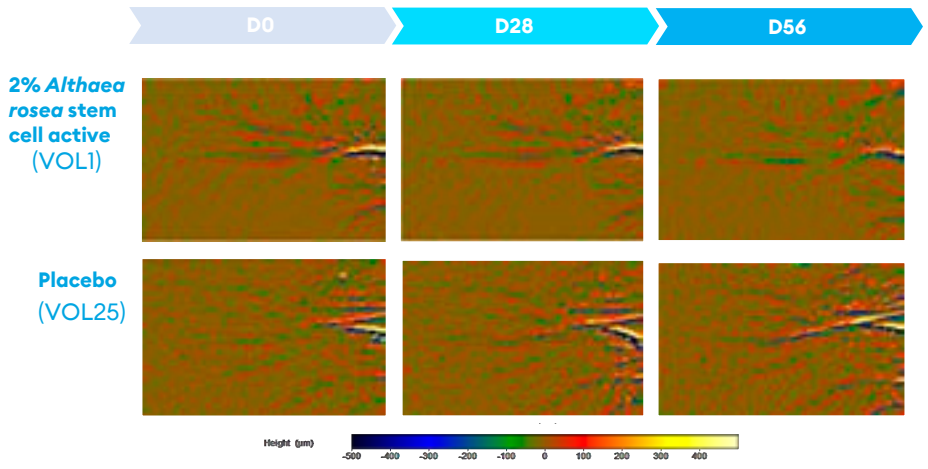


Fig. 6 The active's anti-wrinkle effect is significant and visible (Primos 3D images of two volunteers' periocular area).

ing mature segment of the population, and combined nature and science to capture the essence of immortality in a brand-new approach to well-aging: Dermohacking Cosmetics.

Altheostem™ (Provital's *Althaea rosea* stem cells active) appears as the first of its kind, blending both dermocosmetic and biohacking concepts in this new plant stem cell-based active that selectively eliminates skin cellular senescence, thus providing the next-level efficacy, selectivity, and sustainability aspects that the global well-aging market demands.

As demonstrated in this article, this active ingredient displays senolytic activity on senescent HDF. This is demonstrated in different cellular senescent HDF models, as well as and using various molecular and cellular techniques (including cell viability, β-galactosidase staining and apoptosis quantification). Such senolytic effect on dermal fibroblasts leads to a series of positive biological consequences for ageing skin.

So, it is by selectively triggering this senolytic mechanism, that Altheostem™ appears as an undeniable 'dermohacker', with such a significant improvement on ageing skin that the apparent age − ultimately calculated for all volunteers using an AI-system − decreases over 3 years on average vs placebo.

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