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## ***Anti-Aging Efficacy of a Botanical-Based Skincare Formulated with Syringa vulgaris Extract***

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### **1. Introduction**

Reduced regenerative capacity, driven by impaired cell cycle regulation and declining epidermal turnover, is a hallmark of skin aging, leading to thinning, dermal breakdown, and visible aging signs. To address these challenges, we developed an anti-aging skincare product enriched with a botanical extract from *Syringa vulgaris*, designed to promote cell proliferation and counteract key aging factors. The efficacy of the anti-aging cream was demonstrated through complementary *ex vivo* and *in vivo* studies.

### **2. Materials and Methods**

**Characterization of cell regeneration on RHE:** Reconstructed Human Epidermis (RHE) were treated for 5 days with an anti-aging cream formulated with *Syringa vulgaris* extract or KGF, as positive control. At the end of the treatment, RHE were detached from the synthetic membrane by enzymatic action and fixed before immunostaining of Ki67 proliferation marker. The entire RHE basal layer was observed from bottom, using epifluorescence microscopy. Ki67-positive cells, representing proliferative basal keratinocytes, were characterized and quantified by image analysis.

***In vivo assessment of epidermal cell renewal:*** Cell renewal was evaluated by measurement of fluorescence on the forearm of 22 volunteers after 10 days of application of the anti-aging cream.

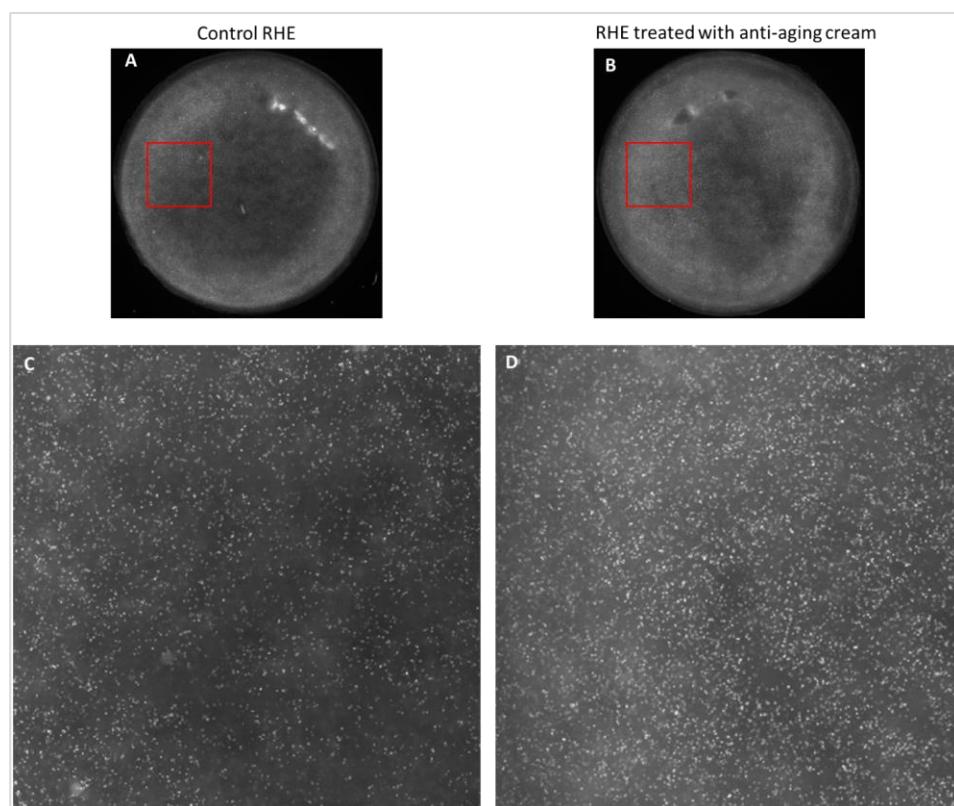
***In vivo anti-ageing efficacy:*** Volunteers applied the cream 2 times a day for 28 days. Skin ageing parameters were assessed before and after treatment using cutometry, echography, fringes projection on replica, clinical scoring and self-scoring.

### 3. Results

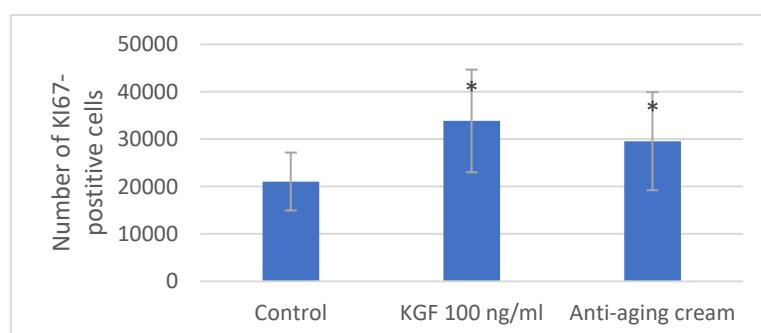
#### 3.1. Characterization of cell regeneration on RHE

We developed and patented a novel methodology to characterize epidermal cell regeneration through microscopic analysis of KI67-positive cells in the basal layer of reconstructed epidermis.

The extract of *Syringa vulgaris* was incorporated into an anti-aging cream. When evaluated using the developed model, the anti-aging cream demonstrated a significant increase in the number of proliferative basal keratinocytes, indicating substantial regenerative potential (Figures 1 and 2).



**Figure 1:** Epifluorescence microscopic observation of KI67 immunostaining ( $\lambda$  Em.= 527nm-white spots) in reconstructed epidermis: (A,B) observation of the whole basal surface of the RHE (A: non treated; B: treated by anti-aging cream); (C,D) magnified view of the area highlighted in red (C: non treated; D: treated by anti-aging cream)

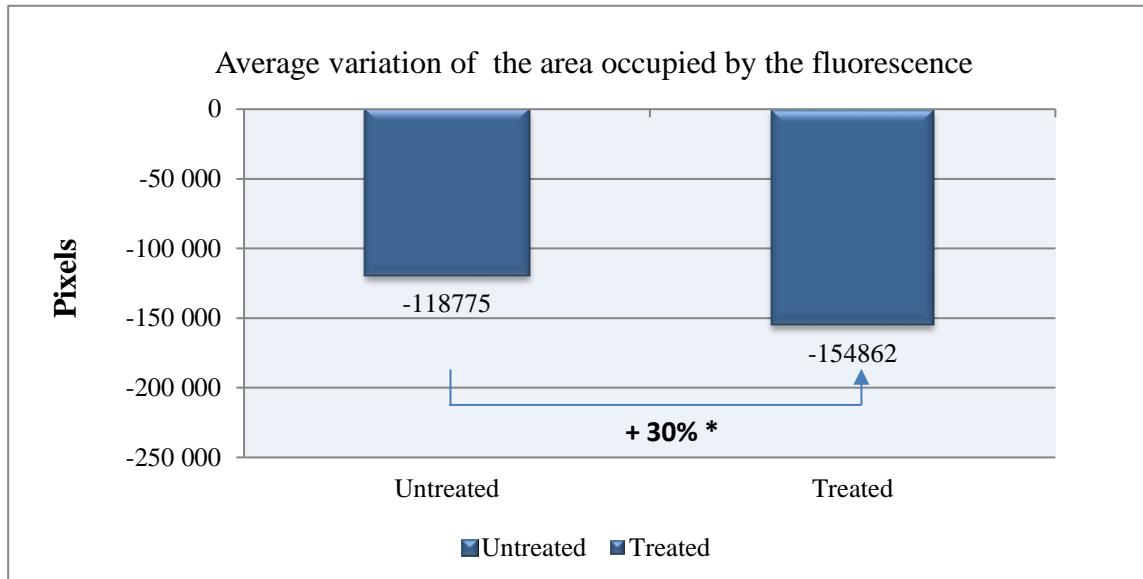


**Figure 2:** Quantification of proliferative basal keratinocytes in RHE.

\* $p<0.05$  vs non-treated Control - Wilcoxon Mann-Whitney test

### 3.2. In vivo assessment of epidermal cell renewal

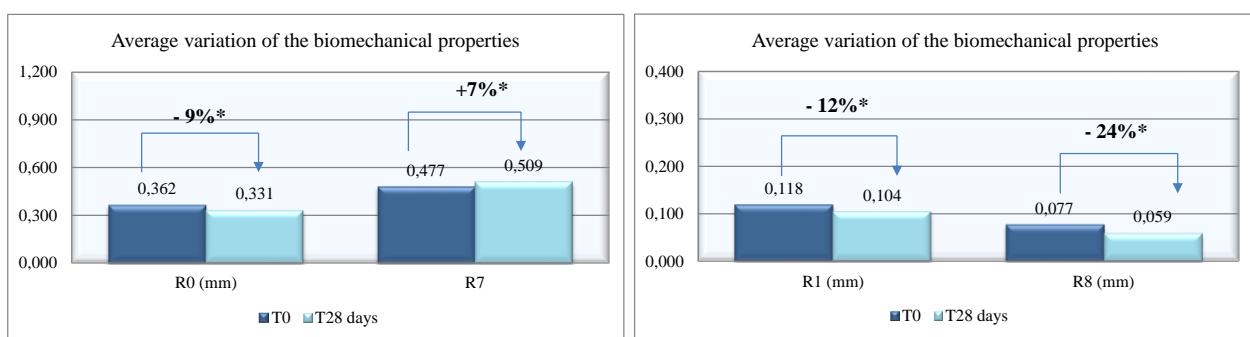
The cell regeneration capacity observed on reconstructed epidermis was confirmed on human volunteers who exhibited a very high increase of +30% in epidermal cell renewal after 10 days of use (Figure 3).



**Figure 3:** Quantification of cell renewal after 10 days

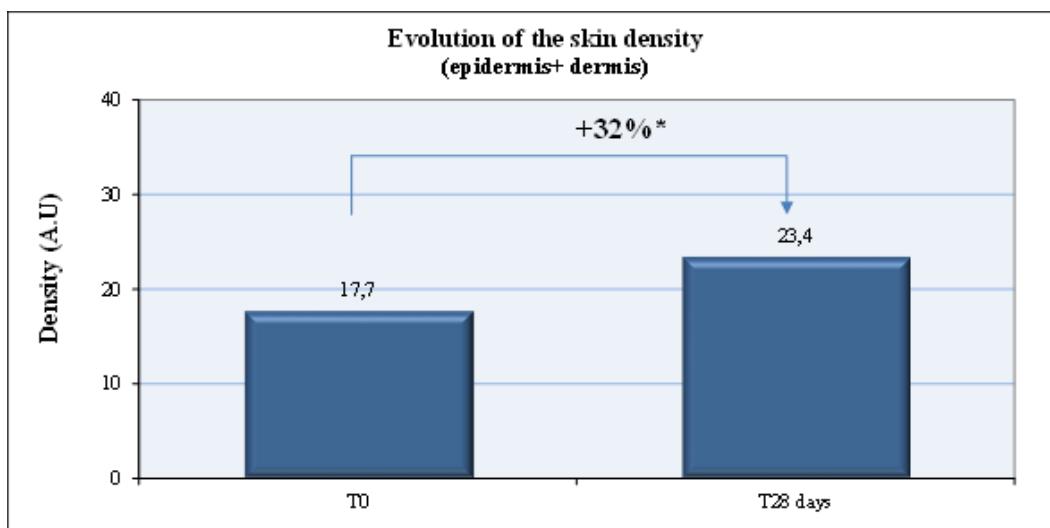
### 3.3. In vivo anti-ageing efficacy

After 28 days, in vivo evaluations confirmed a global improvement in several skin ageing parameters, including significant enhancement of mechanical properties (cutometry, Figure 4): +7% elasticity (R7), -9% extensibility (R0 - firmness), -12% delayed recovery (R1 -tonicity), and -24% fatigability (R8).

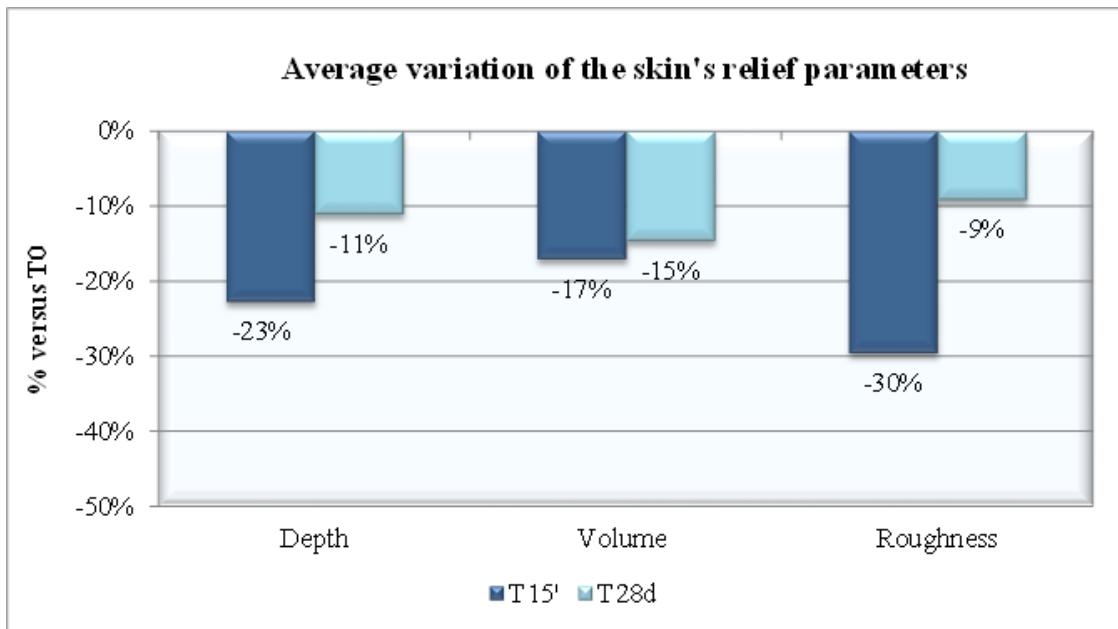


**Figure 4:** Evaluation of skin biomechanical properties by cutometry after 28 days

Cutaneous density (echography) increased by +32% (Figure 5), while roughness of the cutaneous relief (fringes projection method) decreased by -30% after 15 minutes and -9% after 28 days (Figure 6).



**Figure 5:** Evaluation of skin density by echography after 28 days



**Figure 6:** Evaluation of skin's relief parameters by RMD after 15 minutes and 28 days

After 28 days, clinical scoring by a dermatologist showed reduced dryness (-21%), and self-scoring by 102 panelists highlighted enhanced radiance (+97%) and improved tone homogeneity (+94%). Additionally, 72% of 106 participants perceived a resculpted facial contour, 92% a regenerated skin, 89% a firmer skin, 88% a redensified skin, and 67% noted a remodeled neck and décolleté (data not shown).

#### 4. Discussion

The results of this study highlight the potential of a *Syringa vulgaris* extract-enriched anti-aging cream to promote skin regeneration and improve various signs of aging. In an innovative approach, the efficacy of the cream was assessed using a novel ex vivo methodology that quantifies proliferative cells across the entire basal layer of reconstructed human epidermis (RHE). Unlike conventional methods that rely on extrapolations from localized tissue areas, this

comprehensive assessment, utilizing epifluorescence microscopy and image analysis of Ki67-positive cells, provides a more accurate and robust measure of regenerative potential. Using this method, the cream demonstrated a significant increase in Ki67-positive basal keratinocytes after a 5-day treatment. This finding is further corroborated by the significant increase in epidermal cell renewal observed *in vivo* after just 10 days of application. This observed stimulation of cell proliferation is consistent with previous research demonstrating the extract's ability to stimulate markers involved in cell cycle progression and fibroblast proliferation.

The *in vivo* results demonstrate a multifaceted improvement in skin properties after 28 days of cream use. The observed enhancements in elasticity, firmness, tonicity, and fatigability, as measured by cutometry, indicate a positive impact on the skin's mechanical properties. The increase in skin density further supports the notion of improved skin structure and resilience. The reduction in roughness, both short-term and long-term, suggests a smoothing effect on the skin surface. These structural improvements are likely further enhanced by the *Syringa vulgaris* extract's ability to inhibit MMP production, preserving the integrity of the extracellular matrix. These objective measurements are complemented by the panelists' subjective assessments, which revealed significant improvements in radiance and tone homogeneity. The reported perceptions of facial contour resculpting and neck/décolleté remodeling suggest a potential impact on dermal architecture, although further investigation is needed to confirm this effect.

Comparing the cream's efficacy to a known growth factor like KGF in the *ex vivo* model provides a valuable benchmark and suggests that the *Syringa vulgaris* extract may act through similar or complementary pathways. The development and patenting of this novel *ex vivo* methodology for characterizing epidermal cell regeneration strengthens the study's findings by providing a robust and reproducible assessment tool. This methodology could be applied in future research to evaluate the efficacy of other anti-aging interventions.

## 5. Conclusion

This study provides compelling *ex vivo* and *in vivo* evidence supporting the anti-aging efficacy of a *Syringa vulgaris* extract-enriched cream. Utilizing an innovative methodology that assesses the entire basal layer of RHE, the cream demonstrated a significant increase in proliferative keratinocytes, translating into significant improvements in skin structure, resilience, and visible signs of aging *in vivo*. These observed effects are supported by the extract's demonstrated ability to stimulate cell cycle progression, fibroblast proliferation, and inhibit MMP production. These findings highlight the potential of *Syringa vulgaris* extract as a valuable ingredient in anti-aging skincare formulations. Further research is warranted to explore the full extent of the underlying mechanisms of action and to confirm the long-term clinical benefits of this promising botanical extract.