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# Synergy efficacy on skin firming and elasticity using a 3D reconstructed aged skin model from Acetyl Hexapeptide-8 and Acetyl Tetrapeptide-2 combination

LUBRIZOL LIFE SCIENCE

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## Introduction:

Peptides are one of the most commonly used active ingredients in cosmetic products thanks to the high stability and biocompatibility, low molecular weight, clear mode of action and high efficiency [1]. While the action mechanism for single peptide may be clear, the efficacy of peptide combinations is complicated. The efficacy could be maximized or minimized and deserve to further explore.

Acetyl Hexapeptide-8 is a well-known INCI from the 1st ingredient (Argireline® peptide) to competitively interfere with the assembly of SNARE complex and deliver visible anti-wrinkle efficacy. A new peptide with same INCI but with different amino acid sequence was designed not only with a strong affinity to interfere with the SNARE complex formation and reduce the neurotransmitters release at the pre-synaptic level, but also to attenuate the force of muscle contractions while helping the muscle relax faster and more completely afterwards. Moreover, the peptide is found to inhibit SASPs (senescence associated secretory phenotype) release which enables inhibiting cellular senescence in all skin layers to deliver muti-level anti-aging efficacy [2]. To explore the possibility to strengthen skin firmness, another peptide, Acetyl Tetrapeptide-2 is combined. Acetyl Tetrapeptide-2 is designed to modulate the skin architecture via augmenting the expression of FBLN5 and LOX1, upregulating the collagen gene expression and promoting elastin and collagen I synthesis [3].

In this work, the extracellular matrix (ECM) protein expression relevant to skin firming and elasticity of the Acetyl Hexapeptide-8 and Acetyl Tetrapeptide-2 combination was evaluated using a 3D reconstructed aged skin model to explore the synergy of the peptides. In addition, a 4-weeks consumer study was carried out to validate the effect of perceived efficacy of the peptide combination in finished cosmetic products.

## Materials & Methods:

**1. 3D reconstructed aged skin model**

A 3D full-thickness aged skin model was reconstructed with normal human cutaneous cells from an aged donor (>40 years old). After 40 days, the skin model was treated with 1% Acetyl Hexapeptide-8 solution or the peptide combination (1% Acetyl Hexapeptide-8 solution and 1% Acetyl Tetrapeptide-2 solution) containing serums or placebo for 8 days, after which time samples of the tissues were collected.

ECM production was analyzed by Masson's trichrome staining. The expression of collagen Type I, collagen Type IV, fibrillin-1 and elastin were determined by immunohistochemical staining and image analysis. For all data, the statistical significance was assessed running one-way Student's test, and statistically significant differences are indicated as follows: *p<0.05, **p<0.01 and ***p<0.001.

**2. Consumer study**

40 volunteers aged between 31-51 years old were recruited. Among them, 80% had dry or mixed dry skin and 20% had oily or mixed oily skin. The study duration was 28 days. Volunteers applied the cosmetic product containing the peptide combination to the whole face twice a day. A questionnaire was designed to collect the feedback about the product performance and the questionnaire collection was completed after first use, after 14 days and 28 days of treatment.

## Results & Discussion (I):

**1. 3D reconstructed aged skin model**

***a) Extracellular matrix analysis***

Table 1 shows that in the upper dermis, the percentage of ECM synthesis was significantly increased by 10.8% for Acetyl Hexapeptide-8 alone (p<0.001) and by 10.4% for the peptide combination (p<0.001), compared to placebo. There was no statistical difference between the two treated conditions.

***b) Type I and Type IV collagen analysis***

Type I collagen represents the major component of the dermal ECM neo-synthesized by fibroblasts. For both peptide-treated conditions, collagen I expression appeared very dense in dermal ECM as seen in Figure 1. The observation was further confirmed by image analysis quantification. As shown in Table 1, collagen I synthesis was significantly increased by 22.3% for Acetyl Hexapeptide-8 alone (p<0.001) and by 36.6% for the peptide combination (p<0.001). Type IV collagen represents a main component of the basement membrane in the dermal-epidermal junction and was revealed by immunofluorescence in red as observed in Figure 2. Results show that the expression of collagen IV was also significantly increased in peptide-treated conditions. Compared with placebo, Acetyl Hexapeptide-8 treatment increased collagen IV expression by 19.4% while the peptide combination treatment increased it by 34.5%. Interestingly, the peptide combination showed a significant boosting effect compared to Acetyl Hexapeptide-8 for both Type I and Type IV collagen expression.

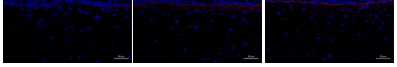



*Figure 1. Collagen I immunostaining (protein in red fluorescence, nuclei staining in blue)*




*Figure 2. Collagen IV immunostaining (protein in red fluorescence, nuclei staining in blue)*

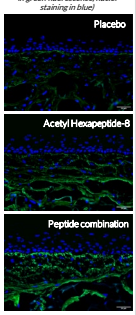
## Results & Discussion (II):

***c) Fibrillin-1 and elastin analysis***

Fibrillin-1 immunostaining allowed studying more specifically this ECM glycoprotein that serves as a structural component of microfibrils in the dermis. As shown in Figure 3, the peptide-treated conditions show a more intense green color compared to placebo, especially for the peptide combination. Image analysis quantification shows that Acetyl Hexapeptide-8 increased fibrillin-1 expression by 100.6% (p<0.001) and the peptide combination by 190.5% (p<0.001) when compared to placebo. Furthermore, fibrillin-1 synthesis was significantly increased for the peptide combination compared to Acetyl Hexapeptide-8.

Elastin immunostaining allowed studying more specifically this protein representing the second main component of dermal ECM and the major component of elastic fibers. Results show that in the dermis, the percentage of elastin-positive area was significantly higher in Acetyl Hexapeptide-8 treated condition and the peptide combination treated condition by 104% (p<0.001) and 87.2% (p<0.001) respectively, compared to placebo, indicating an increased synthesis of elastin by fibroblasts for both peptide-treated conditions. The two peptide treatments showed no statistical difference between them.

*Figure 3. Fibrillin-1 immunostaining (protein in green fluorescence, nuclei staining in blue)*




| | ECM positive area (%) | Collagen Type I levels (%) | Collagen Type IV levels (%) | Fibrillin-1 levels (%) | Elastin levels (%) |
|---|---|---|---|---|---|
| Placebo | 88.12 | 36.14 | 0.97 | 3.68 | 3.60 |
| Acetyl Hexapeptide-8 | 97.62 | 44.20 | 1.16 | 7.39 | 7.36 |
| Peptide combination | 97.27 | 49.38 | 1.31 | 10.70 | 6.75 |

*Table 1. Summary of ECM, Collagen Type I, Collagen Type IV, Fibrillin-1 and elastin analysis on the reconstructed skin topically treated with placebo, Acetyl Hexapeptide-8 or the peptide combination*

**2. Consumer study**

A serum containing the peptide combination was used for a 4 weeks' consumer study. Questionnaire about the serum performance was collected after first use, after 14 days and 28 days of treatment.

As shown in Figure 4, just after first use, 93% of volunteers were satisfied with the product and perceived a more firming, radiant and fine skin. In just 2 weeks, the satisfactory ratio increased to 98% especially showing a great perceivable improvement on skin sagginess and sharper facial contour along with quick improvement in other attributes towards firming, lifting, radiance and fine skin. The product efficacy was further perceived after 4 weeks use. Among all attributes, skin firming, skin lifting, skin elasticity and skin sagginess were largely improved after 4 weeks use when compared to first use.

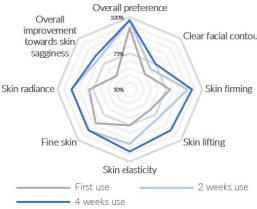



*Figure 4. Questionnaire feedback collected after first use, after 2 weeks and 4 weeks use*

## Conclusions:

Previous research [4] demonstrates that the new Acetyl Hexapeptide-8 provides superior activity attenuating muscle contraction and also speeds-up the muscle relaxation to help recover a relaxed skin appearance after making facial expressions. Its efficacy further regulates the senescence process that drives loss of functionality and age-related changes in all skin layers. The current research verified its efficacy to promote collagen I expression, and interestingly, we found it also promotes collagen IV, fibrillin-1 and elastin expression, indicating that significant stimulation of ECM structures may help improve skin firmness.

Acetyl Tetrapeptide-2 is a peptide that counteracts the sagging and aging effects on the skin by increasing both collagen and functional elastin synthesis. It further contributes to skin firmness by overexpressing genes participating in focal adhesions (FAs), which are the mechanical links between the actin cytoskeleton and extracellular matrix and involved in cellular cohesion [3].

The combination of the new Acetyl Hexapeptide-8 and Acetyl Tetrapeptide-2 showed a significant enhancement in type I and type IV collagen expression, as well as fibrillin-1 levels when compared to Acetyl Hexapeptide-8 alone. Fibrillin molecules assemble to form beaded microfibrils and are functionalized as a scaffold for the correct deposition of elastin [5]. All these three elements are major elements of extracellular matrix and help provide skin firmness and skin elasticity.

To further validate the efficacy of the peptide combination, a 4 weeks' consumer study was carried out. Skin attributes including facial contour, skin firmness, skin elasticity and overall improvement toward skin sagginess were well perceived just after 2- and 4-weeks product use. Particularly, skin firming, lifting, elasticity and skin sagginess were largely improved which is consistent with our findings in the *in vitro* studies. With the *in vivo* and *in vitro* results, it would be hypothesized that a maximized anti-ageing effect would be achieved with the peptide combination in skincare products.

## References:

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