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

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Abstract

Peptides are known for their high efficacy and safety and are commonly used as active ingredients in cosmetic products. With the increasing trend of cosmeceuticals, more and more cosmetic products use active ingredients heavily in their formulations, trying to deliver superior efficacy. However, the action of single active ingredient maybe clear, but the mechanism and performance of active combinations remains unclear.

In this study, the efficacy of Acetyl Hexapeptide-8 and Acetyl Tetrapeptide-2 were tested by *in vitro* 3D reconstructed aged skin model and further validated by a consumer study. It has been found that the peptide combination has a synergistic effect to boost Type I and Type IV collagen, fibrillin-1 and elastin expression *in vitro* and contributes to skin firmness, lifting and skin elasticity *in vivo*. It would be hypothesized that a maximized anti-aging effect would be achieved with the peptide combination in final skincare products.

Keywords: Acetyl Hexapeptide-8; Acetyl Tetrapeptide-2; 3D reconstructed aged skin model; peptide synergy

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 reduce 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 and 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.

Extracellular matrix 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 by asterisks 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 products 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.

1. 3D reconstructed aged skin model

a) Extracellular matrix analysis

Extracellular matrix was analyzed by Masson's trichrome staining and further quantified by image analysis. As shown in Figure 1, 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.

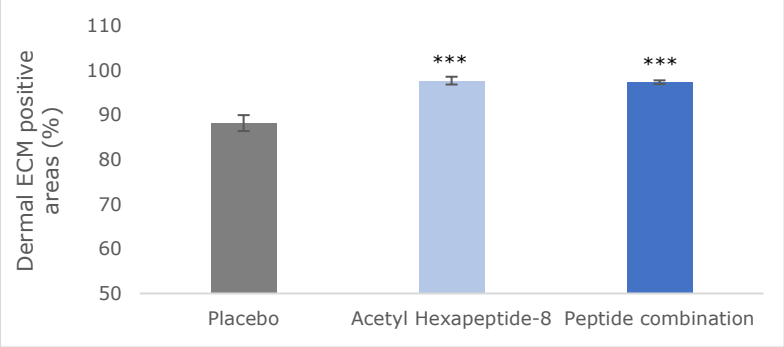
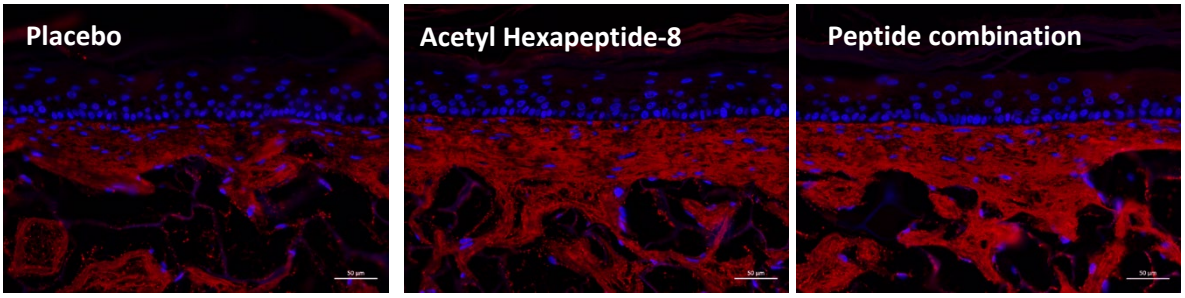


Figure 1. Quantification of extracellular matrix in the upper dermis of the reconstructed skin tissues topically treated with placebo, Acetyl Hexapeptide-8, or the peptide combination

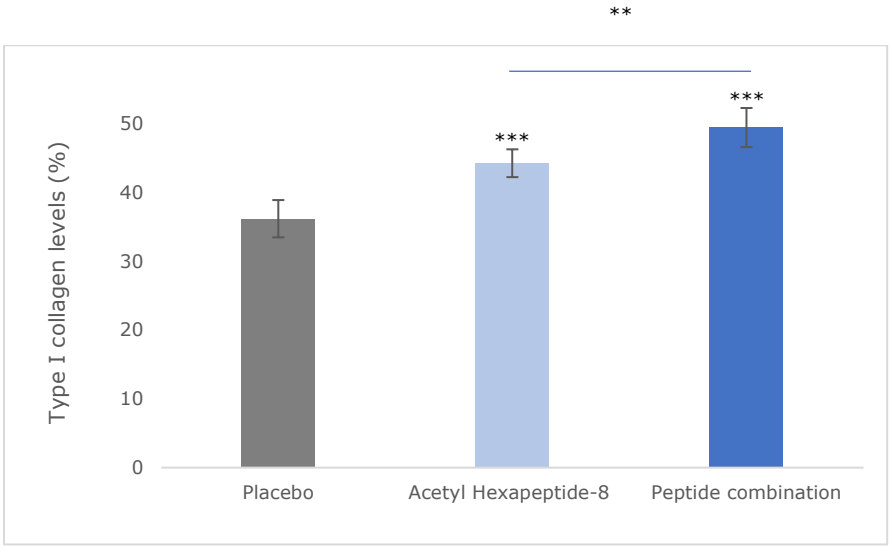
b) Type I collagen analysis

Type I collagen represents the major component of the dermal ECM neo-synthesized by fibroblasts. As shown in Figure 2(a), for both peptide-treated conditions, collagen Type I expression appeared very dense in the dermal ECM, while for placebo, collagen Type I expression was less intense.

The observation was further confirmed by the image analysis quantification. As shown in Figure 2(b), compared with placebo, collagen Type I synthesis was significantly increased by 22.3% for Acetyl Hexapeptide-8 alone ($p<0.001$) and by 36.6% for the peptide combination ($p<0.001$). Interestingly, the peptide combination showed a significant boosting effect compared to Acetyl Hexapeptide-8 alone.



(a) Collagen Type I immunostaining (protein in red fluorescence, nuclei staining in blue)



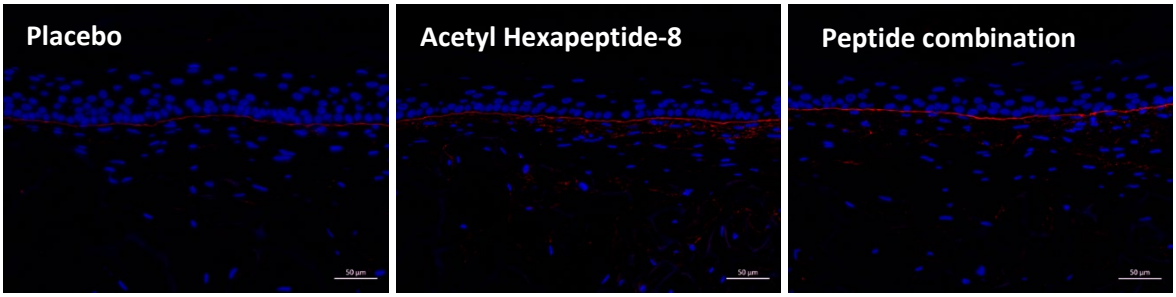
(b) Quantification of Collagen Type I expression in the dermis

Figure 2. Collagen Type I analysis on the reconstructed skin tissues topically treated with placebo, Acetyl Hexapeptide-8, or the peptide combination

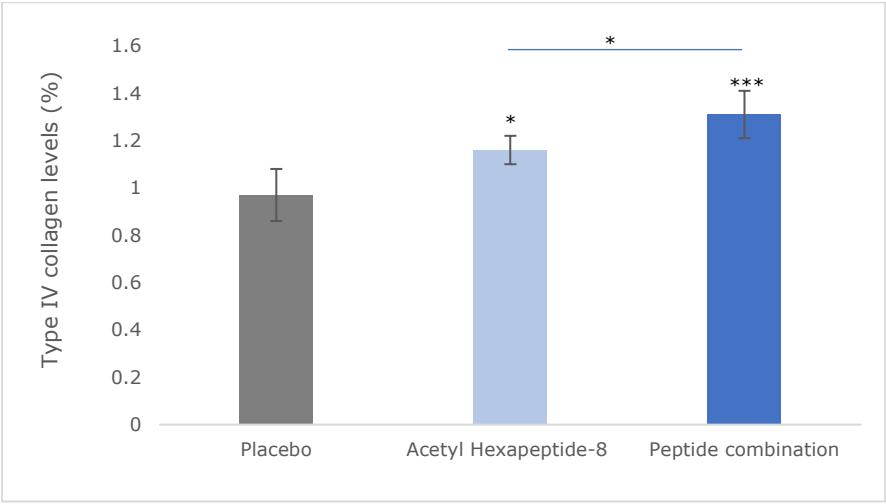
c) Type IV collagen analysis

Type IV collagen represents a main component of the basement membrane in the dermal-epidermal junction and was revealed by immunofluorescence in red (Figure 3).

Image analysis quantification shows that the expression of collagen IV was 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 also showed a significant boosting effect compared to Acetyl Hexapeptide-8 alone.



(a) Collagen Type IV immunostaining (protein in red fluorescence, nuclei staining in blue)



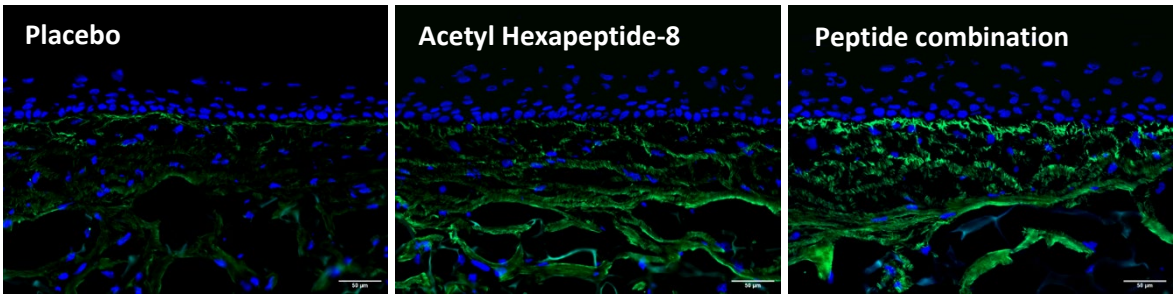
(b) Quantification of Collagen Type IV expression in the dermis

Figure 3. Collagen Type IV analysis on the reconstructed skin tissues topically treated with placebo, Acetyl Hexapeptide-8, or the peptide combination

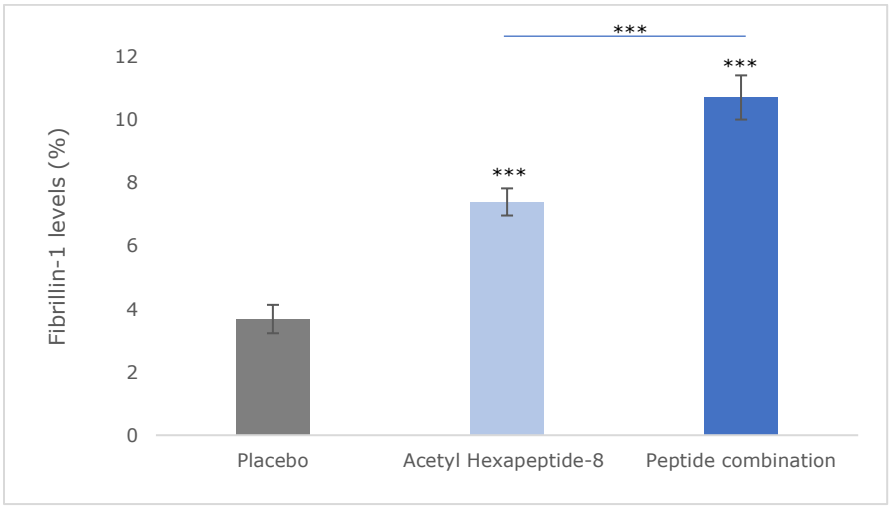
d) Fibrillin-1 analysis

Fibrillin-1 immunostaining allowed studying more specifically this extracellular matrix glycoprotein that serves as a structural component of microfibrils in the dermis. Fibrillin takes part to elastogenesis by providing microfibrillar scaffolds for elastin protein deposit and is thus a major component of elastic fibers.

As shown in Figure 4(a), the peptide-treated conditions show a more intense green color when 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 peptide combination compared to Acetyl Hexapeptide-8 alone.



(a) Fibrillin-1 immunostaining (protein in green fluorescence, nuclei staining in blue)



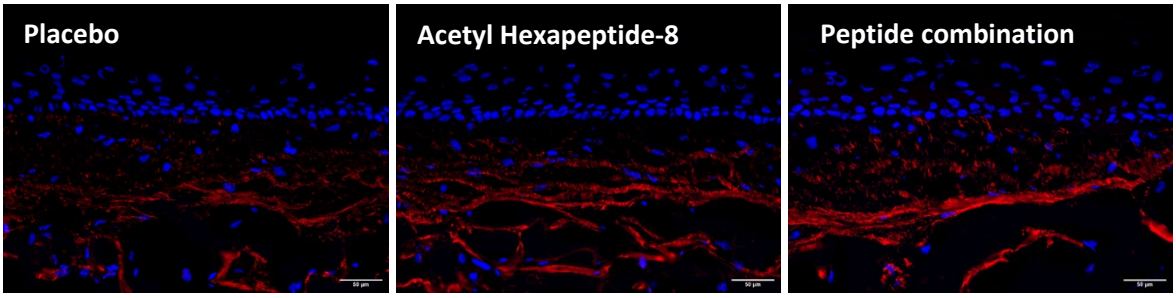
(b) Quantification of fibrillin-1 expression in the dermis

Figure 4. Fibrillin 1 analysis on the reconstructed skin tissues topically treated with placebo, Acetyl Hexapeptide-8, or the peptide combination

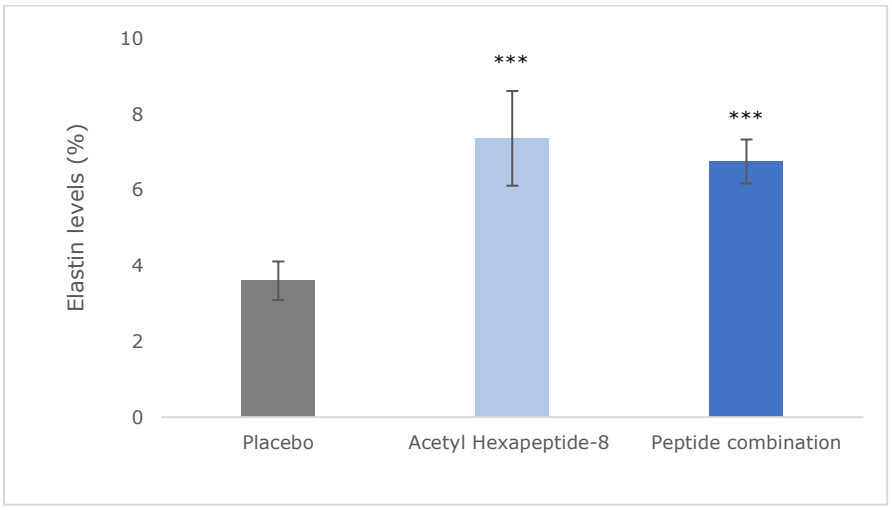
e) Elastin analysis

Elastin immunostaining allowed studying more specifically this protein representing the second main component of dermal ECM and the major component of elastic fibers. Elastin was revealed by immunofluorescence in red as seen in Figure 5(a).

For all conditions, elastin was well synthesized and organized by fibroblasts in the extracellular matrix. For both peptide-treated conditions, elastin expression appeared more intense in the dermal ECM. These observations were confirmed by the image analysis quantification. In the dermis, the percentage of elastin-positive area was significantly higher in Acetyl Hexapeptide-8 treated condition and the peptide combination treated condition compared to placebo by 104% ($p<0.001$) and 87.2% ($p<0.001$) respectively, indicating an increased synthesis of elastin by fibroblasts for both peptide-treated conditions. The two peptide treatments demonstrated no statistical differences between them.



(a) Elastin immunostaining (protein in red fluorescence, nuclei staining in blue)



(b) Quantification of Elastin expression in the dermis

Figure 5. Elastin analysis on the reconstructed skins topically treated with placebo, Acetyl Hexapeptide-8, or the peptide combination

2. Consumer study

A serum containing the peptide combination was used for the 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 6, 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.

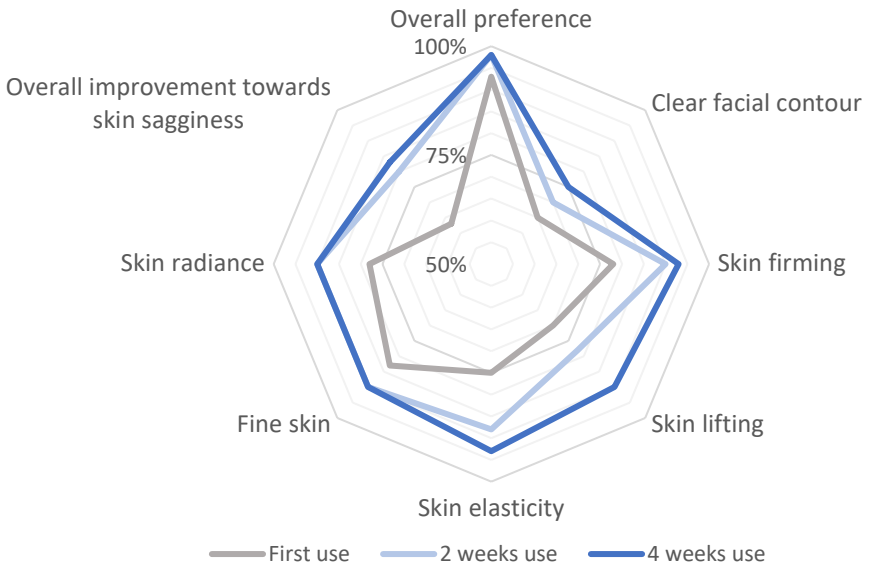


Figure 6. Questionnaire feedback collected after first use, after 2 weeks and 4 weeks use

Discussion and conclusion.

Previous research [4] demonstrates that the new Acetyl Hexapeptide-8 provides superior activity attenuating muscle contraction and speeding-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 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.

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Conflict of Interest Statement.

NONE

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