IFSCC 2025 full paper (abstract n° IFSCC2025-1516)

"Dual anti-aging action of new ingredient designed by artificial intelligence"

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1. Introduction

Exposed to intrinsic and extrinsic factors, the skin reflects aging characterized by appearance of wrinkles, loss of elasticity, dryness due to structural and functional perturbation in skin's cells such as the decline of collagen's synthesis. The collagen family comprises 28 members, divided into 8 categories based on their structural and functional properties [1-2]. In skin, 20 collagens are expressed: the majoritarily fibrillar collagens I and III, representing 90% of skin's collagen content, and others collagens so-called minor but also important in the cell anchorage, matrix assembly and signalling [3].

In this context, a new kind of hybrid biofunctional was developed and evaluated to limit skin aging via collagen's study. The composition of this new ingredient was determined by artificial intelligence (AI). The bio-polymer, hyaluronic acid (HA), was identified for its unique physico-chemical properties and its key role in skin hydration and rejuvenation [4-5]. Next, two peptides, known for their anti-aging efficiency, were selected by AI to their strong interactions with HA molecule: tripeptide-1 and hexapeptide-9. Tripeptide-1 is known to upregulate collagen, elastin and glycosaminoglycan production in fibroblasts [6]. Hexapeptide-9 is well-known anti-aging peptide with the ability to facilitate skin regeneration and to reinforce the extracellular matrix [7-8]. The interaction between HA and peptides was strengthened and optimized thank to a unique process used in 2HP™ technology. This unique molecular complex was integrated in cellulose gum allowing dual action: anti-aging by association of HA and peptides, and texturizing by the cellulose gum.

2. Materials and Methods

The computed peptides/HA interaction energy was estimated using the molecular modeling. By Fourier transform infrared spectroscopy (FTIR), the infrared spectrum of the molecular complex from 2HP™ technology was compared with a simple blend composed of the same quantity of peptides and HA. The efficacy of molecular complex *versus* the blend was evaluated on the expression of two key collagens collagen I and IV on *ex vivo* skin.

Secondly, the ingredient, composed of molecular complex integrated in cellulose matrix, was investigated on the expression of 20 collagen-chains distributed in the 8 collagen's categories on *ex vivo* skin or *in vitro* cells either by immunostaining or by qPCR.
The sensorial effect of molecular complex, provided by the cellulose gum part, was assessed by comparing a formula containing 5% ingredient with a formula containing 5% silicone, on two different blind panels (8 experts and 10 non-experts). Both panels, immediately or 5 min minutes after applications, evaluated different parameters such as the ease of spreading, play time, tackiness and greasiness.
Then, two clinical double-blind studies were designed to highlight the interest to combine cellulosic gum element with molecular complex peptides/HA. The first one, was a short-term study conducted on 30 Asian volunteers. They applied during 30 min a mask embedded with 5% ingredient on one side or with placebo on the other side. 30 min and 4h after mask removal, skin elasticity, hydration, glow and wrinkles' appearance were evaluated. The second study was a long-term study carried out on 34 Caucasian volunteers divided in two homogeneous groups. Both groups applied a cream containing 5% ingredient or its placebo for 1 month. At D0, D14 and D28, skin hydration, elasticity were evaluated as well as skin topography on the crow's feet area to measure the differente wrinkles' parameters.

3. Results

- **Characterization and *ex vivo* evaluation of the new technology: 2HP™ technology**

The first part focused on the characterization and the evaluation of molecular complex from 2 2HP™ technology.
By molecular modeling, the computed peptide/polymer interaction energy is determined: strong interactions between the selected peptides and HA were calculated from the self-assembled hydrogen bonding.

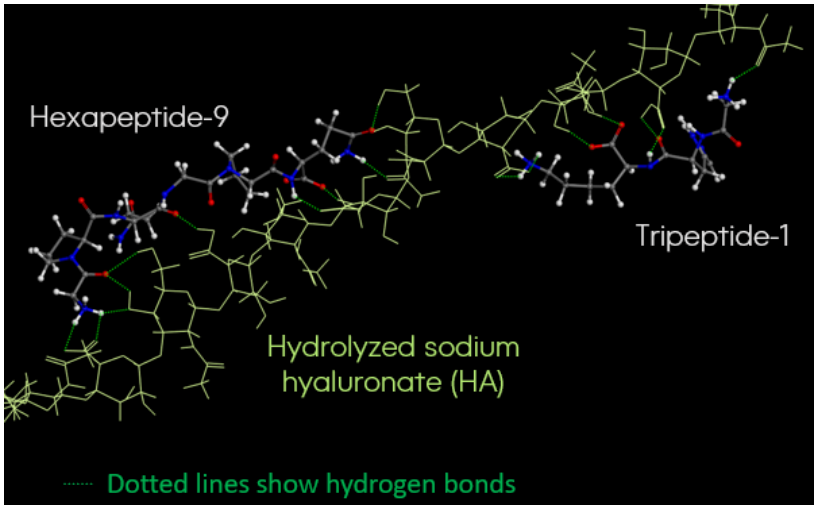

Figure 1: Self-assembled interaction shown by molecular modeling science

These self-assembled interactions were strengthened by 2HP™ technology based on a specific size of HA, optimal concentration of each component and unique process. To prove

the interest of this new technology, a study of the infrared spectrum of molecular complex was performed by FTIR and compared to a blend containing the same components with the same concentration.

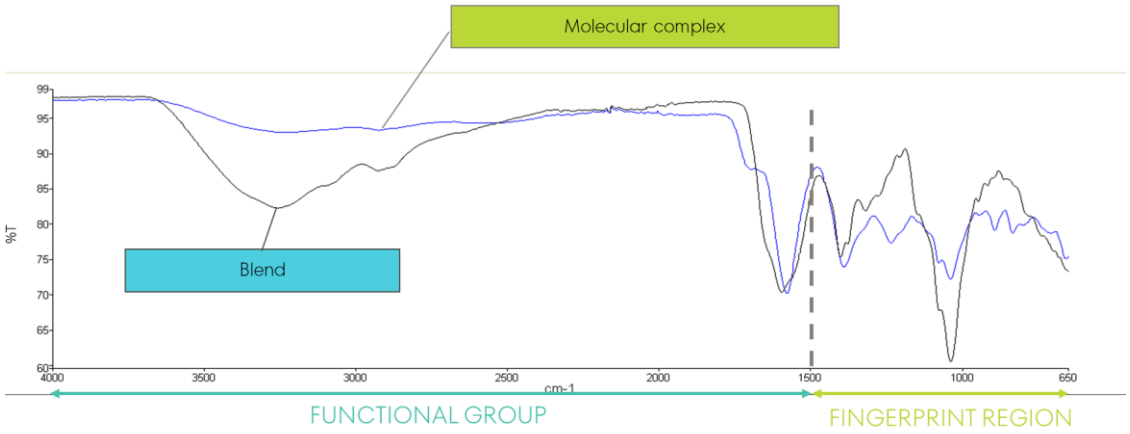

Figure 2: Characterization of molecular complex by FTIR

The molecular complex obtained with 2HP™ technology, and the simple blend of HA/peptides differed in their molecular structure, they have their own «fingerprint region» with a low correlation in spectrum similarity. Thus, this result demonstrated the unique profil of the molecular complex obtained by 2HP™ technology. To finalyze this part, the superior efficacy of molecular complex was investigated on the expression of collagen I and collagen IV on *ex vivo* skin, treated with 5% of molecular complex *versus* 5% of blend for 48 hours.

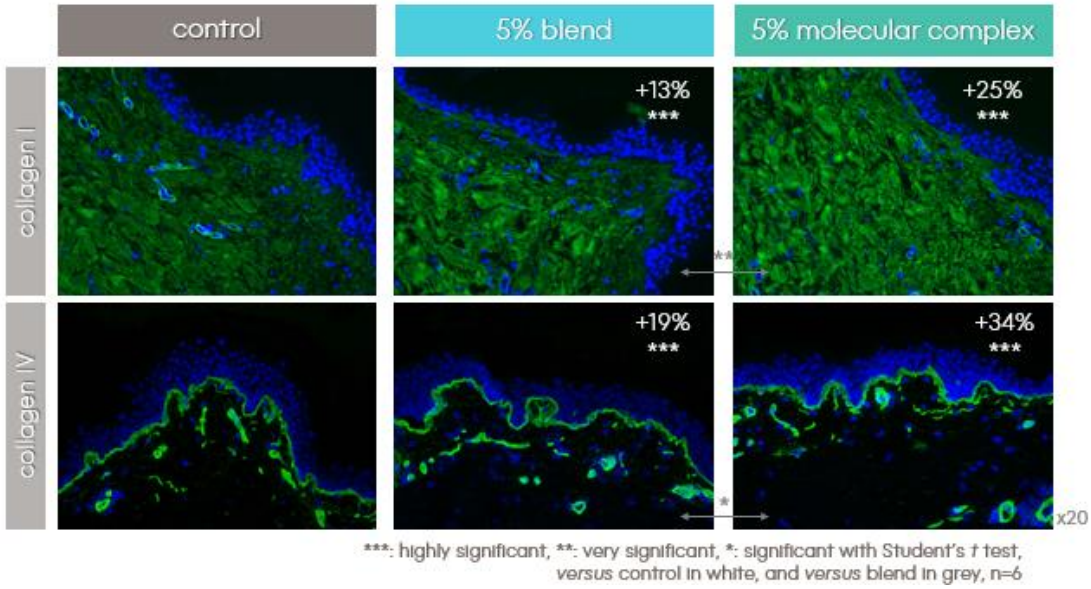

Figure 3: Collagens I and IV immunostaining on *ex vivo* skin treated with blend or molecular complex for 48 h

The application of 5% molecular complex was associated with an increase of collagen I and IV contents, in a greater proportion than biopsies treated with 5% blend.

- ***In vitro and ex vivo* efficacy evaluation:**

After integration of the molecular complex in cellulose matrix, the efficiency of this new ingredient was evaluated on the expression of 20 collagen's chains, distributed in the 8 categories of collagens. The ingredient was applied on the skin biopsies at 5% or applied at 0.5% on *in vitro* cells.

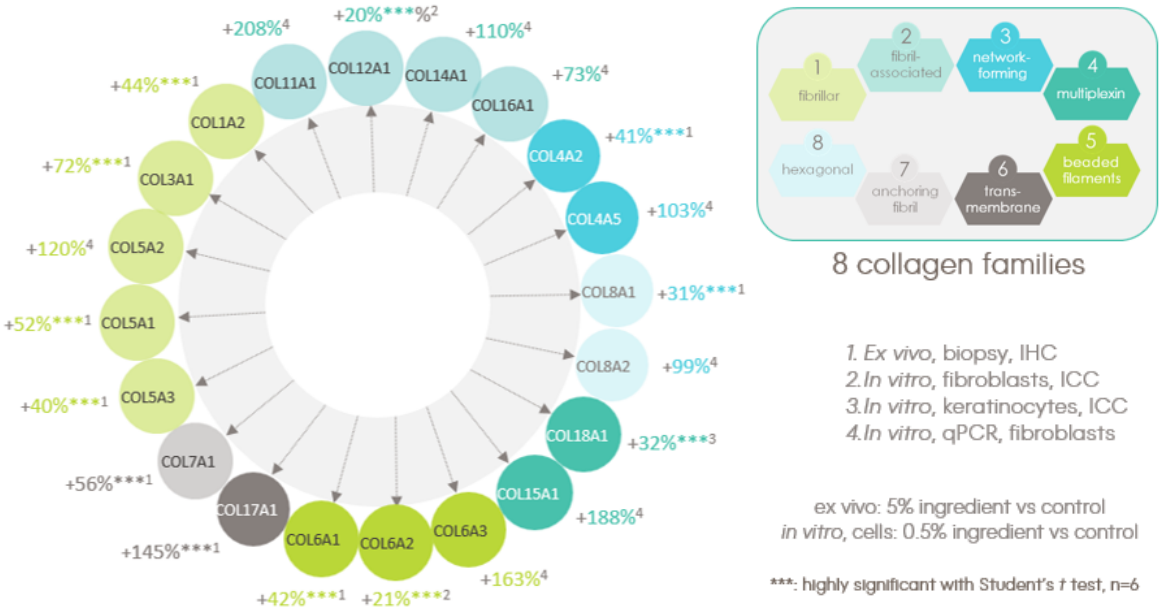

Figure 4: Evaluation of ingredient on 20 skin collagen-chain expressions

The results obtained following the application of the molecular complex in cellulose matrix on *ex vivo* skin or on *in vitro* cells, showed an enhancement of the expression of all collagen-chains studied.

- **Sensorial effect evaluation**:

The sensorial effect of the ingredient was evaluated on the back on the hand by 10 non-experts panel and 8 experts panel versus silicone.

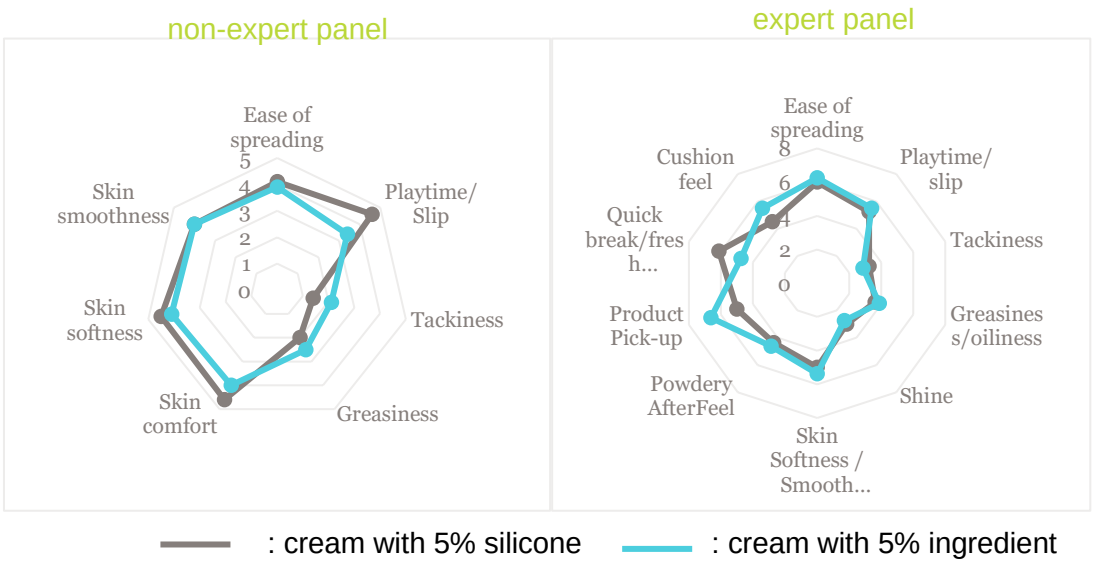

Figure 5: Evaluation of the sensorial effect of ingredient formulated at 5%

Both panelists found that the cream containing the ingredient had the same sensorial effect than the silicone formulation with a better quick break and playtime highlighting the sensorial effect of the biofunctional (figure 5).

- ***In vivo* efficacy evaluation**:

The first clinical study, done in a short-term period on Asian volunteers showed, 30 min after mask removal, an increase in skin hydration with a better elasticity. These effects persisted after 4h of mask removal (figure 6).

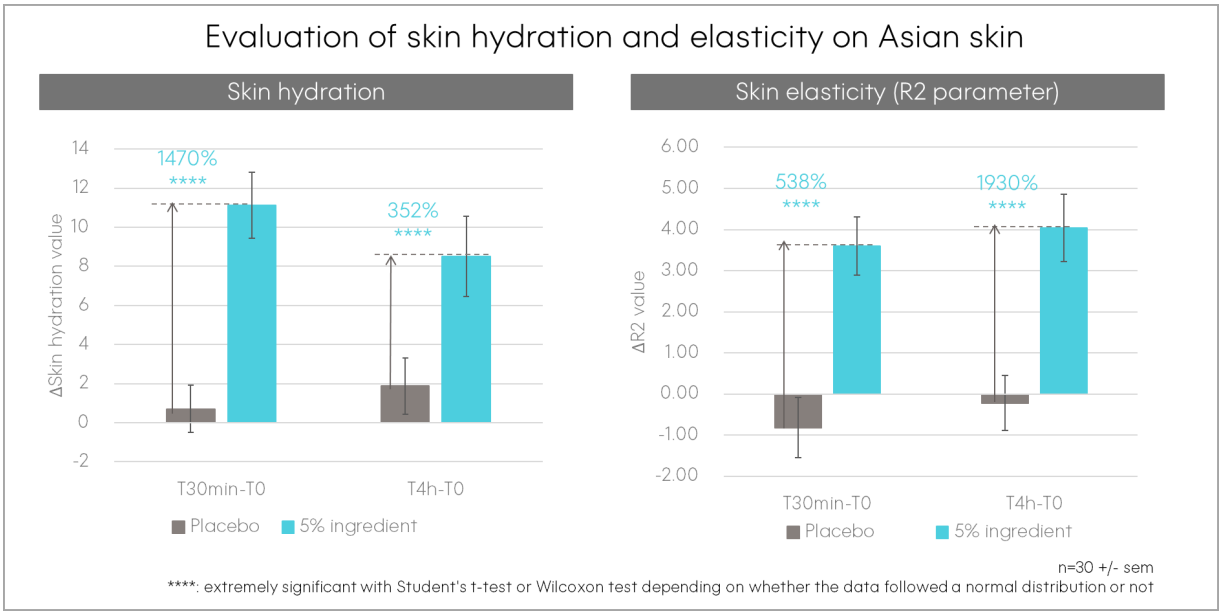

Figure 6: Evaluation of skin hydration and elasticity on Asian volunteers

These results were supported by color pictures showing fewer fine lines on the crow's feet area 4h, after mask removal, on the side of the 5% ingredient compared to placebo (Figure 7).

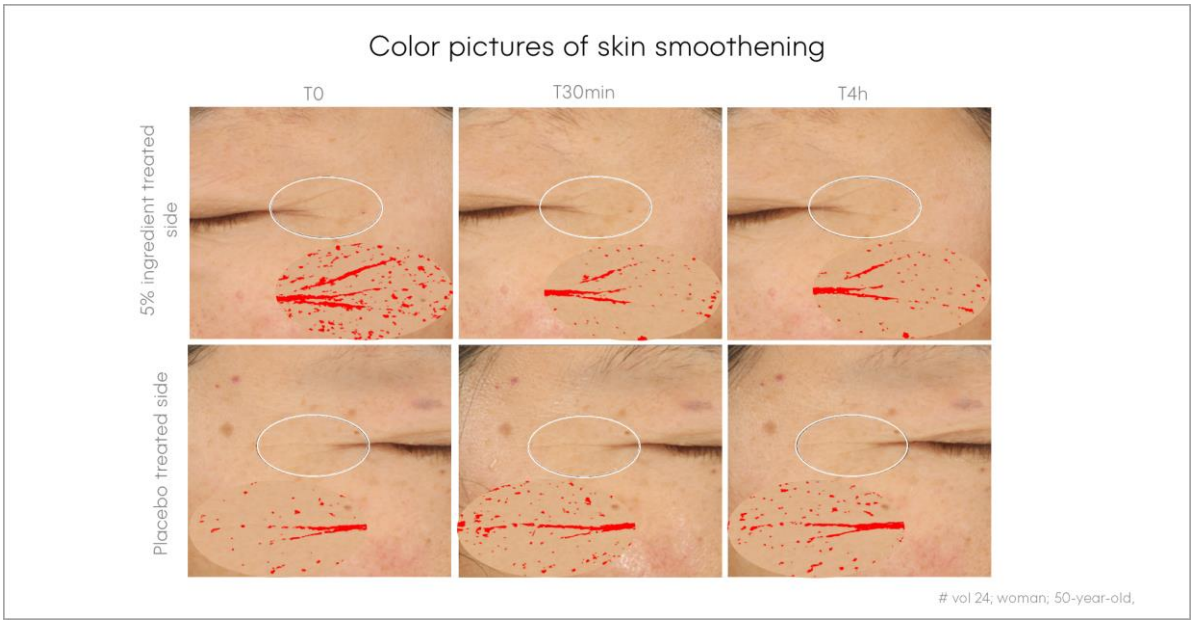

Figure 7: VisiaCR pictures of the crow's feet area on Asian volunteers

The long term clinical study, done on Caucasian volunteers, showed an improvement in skin hydration since D14 with a maintenance of this enhancement after one month of application accompanied by a better skin elasticity (figure 8).

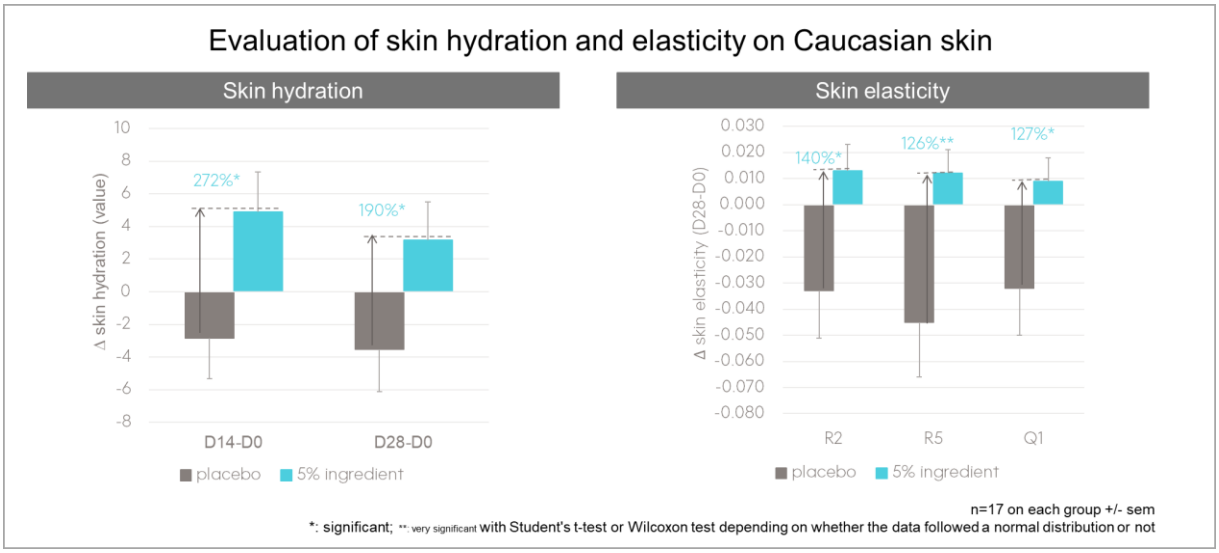

Figure 8: Evaluation of skin hydration and elasticity on Asian volunteers

Moreover, after one month application of the ingredient containing cream, the wrinkles appearance of the crow's feet area but also under the eye, significantly decreased in circumference by -196% and in depth by -105% with an impact on wrinkles's area of all kinds of fine lines (Figure 9).

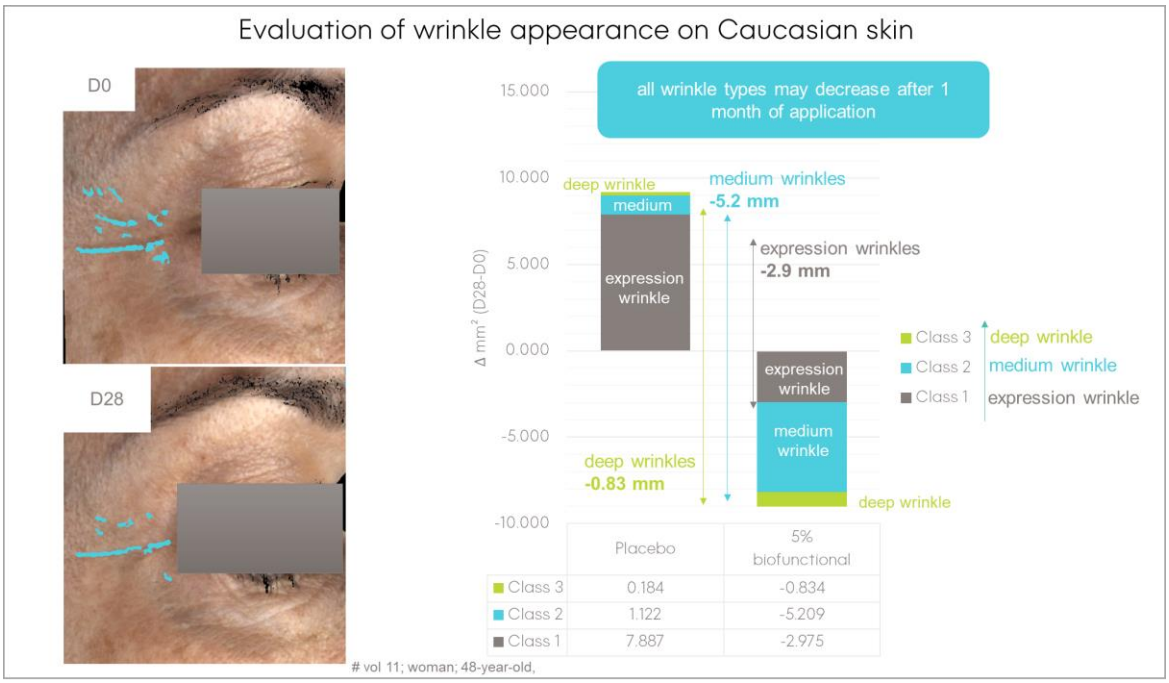

Figure 9: Evaluation of wrinkle appearance on Caucasian skin

4. Discussion

The objective of this study was to evaluate a new ingredient designed by AI and from patented 2HP™ technology. The characterization of the molecular complex by FTIR showed a different infrared profil from a simple blend constitued of the same components. The interest of this unique profil was determined by the evaluation of two key collagens, collagen I and collagen IV. This study was pursued on the evaluation of the expression 20 collagen-chains.

The expression of COL1A2, COL3A1, COL5A1, COL5A3 (collagens belonging to fibrillar collagens), COL4A2 (network forming collagen), COL6A1 (beaded filament-forming collagen), COL7A1 (anchoring collagen), COL8A1 (hexagonal network-forming collagen) and COL17A1 (transmembrane collagen) was increased on *ex vivo* skin following the application of ingredient at 5% quantified by immunodetection.

On *in vitro* fibroblasts, the application of 0.5% ingredient was associated with an increase of COL5A2 and COL11A1 (collagens belonging to fibrillar collagens), COL4A5 (network forming collagen), COL6A3 and COL6A2 (beaded filament-forming collagen), COL14A1, COL16A1 COL12A1 (fibrils associated collagens with interrupted triple helices (FACITs)), COL15A1 (multiplexin collagen) and COL8A2 (hexagonal network-forming collagen). The expression of COL18A1, collagen expressed in basement membrane by keratinocytes and belongs to the multiplexin collagens, was increased in these cells treated with 0.5% ingredient.

Moreover, *in vivo* efficacy of the ingredient was supported by 2 clinical studies. The first one highlited the instant effect on skin hydration and elasticity and also on fine lines appearance. This rapid effect was certainely brought by the cellulosic part of the ingredient known as a good moisturizer. Then, the effectiveness of the molecular complex was put in evidence by the long term study. The ingredient formulated at 5% allowed an improvement in skin elasticity and

wrinkles appearance with a decrease in fine lines circumference and area compared to placebo.
Concerning, the texturizing effect, 5% of ingredient formulated in a cream showed sensorial effect with a better quick break and playtime highlighting the sensorial effect of the molecular complexe.

5. Conclusion

Using a new patented technology including AI and an unique process design, a new hybrid ingredient was developed and revealed dual properties: texturizing and bioactivity. Indeed, this ingredient brought a texturizing effect to creams, with attractive after feel. Moreover, among the 28 members of collagen superfamily, the expression of 20 collagen-chains was increased on *ex vivo* skin, suggesting an overwhelming anti-aging potential. This was confirmed by two clinical studies, where an improvement in skin smoothness was observed after 4 hours application, potentialized by 1 month application with visibly wrinkles faded.

6. References

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