

# Hyaluronic Acid: Elucidating its Penetration and Effect through the Hair Fibre Using Confocal Raman Spectroscopy and Biometric Techniques for a Set of Cosmetic Benefits

Boira Cloe<sup>1\*</sup>, Essendoubi Mohammed<sup>2,3</sup>, Meunier Marie<sup>1</sup>, Lambert Carole<sup>1</sup>, Auriol Daniel<sup>1</sup>, Manfait Michel<sup>2</sup>, Piot Olivier<sup>2</sup>, Scandolera Amandine<sup>1</sup>, Reynaud Romain<sup>1</sup>

<sup>1</sup>Research and Development, Givaudan Active Beauty, Pomacle, France; <sup>2</sup>EA 7506 Biospectroscopie Translationnelle (BioSpectT), University of Reims Champagne-Ardenne, Reims, France; <sup>3</sup>Department of Medicine and Pharmacy of Tangier, Biophysic Laboratory, Abdelmalek Essaâdi University, Tangier, Morocco

## ABSTRACT

**Background:** Hyaluronic Acid (HA) is well known in the cosmetic industry for its hydrating properties linked to its interaction with keratin in the *stratum corneum*. Hair is also mainly composed of keratin, but no evidence of penetration and interaction of HA in hair has been established yet due to a lack of appropriate methods.

**Methods:** Confocal Raman Spectroscopy was used to track HA penetration in hair fibres. Human hair was either UV-irradiated or left untreated before being washed with various shampoo formulations. Low-Molecular Weight hyaluronic acid (LMW at 1% w/v), High-Molecular Weight HA (HMW at 1% w/v), and an optimized blend of low- and high-molecular weight HA (HA-Blend at 1% w/v) were used. The benefits of HA for cosmetic application has been then evaluated at the *ex vivo* level regarding smoothing, reparative and hydrating properties.

**Results:** Raman analysis of non-irradiated samples revealed that HA-blend penetrated the hair cortex 5.9 times more than the placebo, with irradiated samples a 1.8-fold increase in penetration was observed. Whatever the irradiation status, penetration of this optimal HA-blend was significantly higher than that of the other two actives. Compared to the placebo, the HA-blend significantly decreased the spontaneous frizzing by -11%, repaired the hair by increasing the elastic modulus and the break force and increased the water content into the hair shafts.

**Conclusion:** Based on the results obtained, Confocal Raman Spectroscopy can be considered as a powerful, non-invasive technique to investigate the penetration of active ingredients into the hair. The optimized formulation containing both LMW and HMW HA in different proportions penetrates deep into the hair cortex and provides a durable smoothing, reparative and moisturizing effects.

**Keywords:** Raman spectroscopy; Hair penetration; Hyaluronic acid; Keratin conformation

## INTRODUCTION

Hair is a specialized derivative of the skin that is ideally organized to protect the human scalp. The well-known structure of hair consists of an external cuticle composed of overlapping scales that acts as a barrier protecting the inner structure. The central medulla is surrounded by the cortex, which is mainly composed of keratin [1]. This protein plays an important role in the physical and mechanical properties of hair. At the molecular level, keratin

is a helical protein composed of two types of fibres: Type I, with acidic amino acid residues; and Type II, with basic amino acid residues [2]. One strand of each type combine to form a coiled-coil dimer [2]. These dimers coil together in antiparallel tetramers known as protofilaments. Finally, the protofilaments interact to form a single intermediate filament organized into micro-fibrils and larger macro-fibrils [2]. The conformation of the keratin chains is controlled by hydrogen bonds, ionic forces, Van Der Waals interactions, and disulfide bonds. The disulfide bonds,

**Correspondence to:** Boira Cloe, Research and Development, Givaudan Active Beauty, Pomacle, France, Tel: +3322240641; E-mail: Cloe.boira@givaudan.com

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originating from sulfur-containing cysteine residues, create strong crosslinks between adjacent chains, giving each individual's hair its unique shape. Indeed, the more  $\alpha$ -helical conformation the hair keratin is, the greater the curve in the protein chain is, and the curlier the hair fibre is. Conversely, a  $\beta$ -sheet conformation in the keratin decreases the formation of disulfide bonds and produces smoother hair fibres [3]. In the haircare industry, many products have been developed to modify the disulfide bonds, with the aim of shaping or straightening the hair. These include chemical agents such as alkaline reducing agents, thioglycolates, or guanidine which are used to break and rearrange bonds. Unfortunately, all these products damage the hair and reduce its strength [4]. As a consequence, alternative, safe active ingredients that smooth without inducing damage to the hair, the scalp, or the environment are actively sought.

Hyaluronic Acid (HA) is a key active ingredient in skincare products, but its benefits for hair are little described. To date, interactions between HA and keratin have been described in the *stratum corneum* of the skin, where it is known to enhance skin hydration [5]. During previous work, we firstly emitted the hypothesis that HA can also interact with keratin from hair fibre, promoting keratin interconversion from  $\alpha$ -helix to  $\beta$ -sheet conformation. Our data showed an increase of  $\beta$ -sheet conformation after applying hyaluronic acid and a significant reduction of disulfide bounds delivering anti-frizz property [3]. This interconversion has been already observed during mechanical or chemical hair straightening which leads to hair smoothing and ultimately gives an anti-frizz effect [4]. Due to the cuticle on hair fibres, only low molecular weight molecules under 10 kDa have been found to penetrate the cortex [6]. For molecules over 500 kDa, the penetration may occurs when the cuticle is damaged following bleaching procedure for example [6]. In addition, no evidence of hyaluronic acid's interaction deep inside the hair has yet been established, as exploring the penetration of such molecules into the hair fibres presents several challenges. A range of techniques, including confocal fluorescence microscopy, have been used in attempts to examine the penetration of tagged molecules, but auto fluorescence consistently compromises results [7]. Raman spectroscopy is a potential method for this type of evaluation. This non-invasive technique detects the characteristic vibrational energy levels of molecules, and provides structural information. In confocal mode, it can gather information at various depths inside a tissue, and quantification is possible because inelastic Raman scattering correlates linearly with molecule concentration [8]. In a previous study, we were able to track HA penetration *ex vivo* through the skin using this technique [9]. Other studies performed on natural and intact human hair fibres demonstrate that confocal Raman spectroscopy can be used to investigate the influence of cosmetic ingredients on keratin structure. Evaluating hair smoothing or heat-protecting effect by analysing  $\alpha$ -helix and  $\beta$ -sheet keratin conformation and the S-S disulfide bridges is an example [10].

Here, we developed an efficient non-invasive methodology to monitor the penetration of HA in normal and UV-damaged hair. We then developed an optimized HA-Blend combining low- and high-molecular weight HA for haircare applications, based on optimal hair penetration. Once in the cortex, this optimal HA-Blend interacted with keratin to change its conformation,

promoting a  $\beta$ -sheet conformation and producing a durable, visible smoothing effect without any damage of the fibre as it enhanced its strength and hydration.

## MATERIALS AND METHODS

### Shampoo composition for all the tests involving a washing

**INCI composition of the shampoo:** Water, sodium laurylether sulfate, sodium cocoamidopropylbthaine, sodium chlorhyde, phenoxyethanol, guar hydroxypropyl trimonium, citric acid.

This shampoo has been blend with HMW-HA (1-1.4 MDa), LMW-HA (20-50 kDa) or HA-Blend (Confidential ratio between LMW-HA and HMW-HA, commercially available under Resisthial™ tradename supplied by Givaudan Active Beauty).

### Measuring HA penetration by Raman spectroscopy

**Hair fibre preparation and treatment:** One gram of natural, undyed, light blond hair locks measuring 10 cm (n=10) from a white European donor were used to limit fluorescence interference during Raman spectroscopy measurements (INHIP, New York, USA). Half of the hair locks (n=5) were irradiated at 20 J/cm<sup>2</sup> UVA and 0.6 J/cm<sup>2</sup> UVB to open the cuticle (Biosun, Vilber, Eberhardzell, Germany). Hair locks were then washed for 2 min with 0.5 g of shampoo containing 1% (w/v) of LMW, HMW or HA-Blend, rinsed three times with 200 mL distilled water, and dried for 3 min with a hair dryer at 20 cm from the shaft. The washing, rinsing, drying cycle was repeated three times before analysis. The same shampoo without additive was used as placebo. Untreated hair locks were washed with distilled water.

**Raman spectroscopy and confocal measurements on hair fibres:** Raman spectra were recorded using a near infrared confocal Raman micro-spectrometer (Labram, Horiba Jobin Yvon, Villeneuve d'Ascq, France). The set-up comprised an optical microscope (Olympus, BX41, France) coupled to a dispersive Raman spectrometer (Horiba Jobin Yvon, Villeneuve d'Ascq, France) and a Charge-Coupled Device (CCD) detector. Excitation was provided by a 50 mW titanium-sapphire laser (Model 3900S, Spectra-Physics, France) generating a 785 nm beam under the microscope objective. This irradiation is non-destructive for light blond hair samples and causes no thermal or photochemical degradation. Confocal Raman measurements were recorded using an optimized 100X infrared objective (Olympus, France) operating in air with a numerical aperture of 0.8. To obtain axial profiles, the objective was mounted on a high-precision piezoelectric device (Physik Instrumente, Germany) which allowed in-depth vertical scanning by focusing the laser at various depths within the hair fibres. Spectra were recorded over the length of the hair fibre with a 3- $\mu$ m scanning-step. For each spectrum, one 30 seconds accumulation of laser exposure was retained. Data were acquired using LabSpec 5 software (Horiba Jobin Yvon, Villeneuve d'Ascq, France).

**Spectral data processing:** Spectral data were pre-processed using LabSpec 5 software (Horiba Jobin Yvon, Villeneuve d'Ascq, France). First, cosmic radiation was removed and all aberrant profiles were excluded from the database. Remaining raw spectral profiles were corrected to clean up the Raman signal. The data were corrected for spectral shifts, noise was reduced using a 5-point average Savitzky-Golay smoothing filter, and baseline

was adjusted using a fifth-degree polynomial function to remove background fluorescence. Three independent axial profiles were recorded for each hair sample. The processing of corrected data maps was performed by using homemade software based on least squares fitting method that operates in the Matlab environment (The Math Works Inc., Natick, MA, USA). A thorough description of this statistical analysis has been described by Essendoubi et al. [9]. Briefly, the method involves mathematical modeling of HA reference spectra in the overall spectral axial (Z) profiles to determine the contribution and distribution of these spectra within the measured profile.

### **Ex vivo analysis of anti-frizz properties**

Hair locks with various curl levels from wavy to curly were used, n=10 per conditions. At T0, hair locks were divided into equal parts (2 treated parts and one non-treated part) to have an equal distribution of the different curl levels. Photographs were taken before washing. Then hair locks were washed for 10 seconds with shampoos (1 g shampoo/2 g hair) containing 1.5% (w/v) or 3% (w/v) HA-blend, placebo shampoo, or water for non-treated hair locks. All hair locks were rinsed for 30 seconds with distilled water and air-dried. Photographs were taken after treatment and drying.

For the frizz analysis, hair locks were smoothed with a hair straightener and photographed immediately. Then, they were placed in a room under extreme humidity conditions (Relative humidity  $80\% \pm 10\%$  RH) for 8 h and photographed once again. Images were analyzed using Photoshop® to study the anti-frizz effect, by measuring hair strand length before and after exposure to extreme humidity.

### **Ex vivo analysis of restorative properties by tensile test after bleaching**

Natural Caucasian brown hair locks of 1 g each were bleached two-times using a Platifiz bleaching powder made of sodium persulfate, potassium persulfate, ammonium chloride and sodium metasilicate (SP Equation). Then, hair locks were washed for 60 seconds with shampoos containing 3% (w/v) HA-blend, placebo shampoo, or water for non-treated hair locks and rinsed during 20 seconds. Treated hair locks were dried for 30 minutes at 40°C in a helmet hairdresser.

To evaluate the hair damage, a Mobile Tensile Tester (MTT) has been used. The system is based on a circular sample cassette, which allows the automatic measurement up to 100 fibres samples. For this study, 75 hair fibre samples have been mounted using brass crimps and placed onto a rotary cassette. A Laser (Mitutoyo) measures the cross section of the hair fibres to normalize tensile data. After this measurement, a pneumatically operated sample gripper, picks up the sample. The gripper is mounted onto a load cell which measures the force being applied to the sample during the elongation. The test is realized with 100% relative humidity and ambient room temperature.

### **Ex vivo analysis of keratin integrity following UV irradiation**

Hair fibre preparation and treatment: 1 gram of natural, undyed, light blond hair locks measuring 10 cm from a white European donor were used to limit fluorescence interference during measurements (INHIP, USA). Hair locks were washed three times

for 2 min with 500 µL of shampoo containing 3% (w/v) of HA-blend, rinsed three times with 200 mL distilled water, and dried for 3 min with a hair dryer at 20 cm from the shaft. The hair locks were irradiated seven times at  $9 \text{ J/cm}^2$  UVA and  $0.33 \text{ J/cm}^2$  UVB to open the cuticle (Biosun, Vilber, Germany). The same shampoo without active was used as placebo. Non irradiated hair locks was used as a negative control.

### **xPOLAR® analysis for keratin integrity**

For each condition, 30 sections of 1 cm from different fibres were mounted between a slide and a coverslip. We perform 1 xPolar® measurement per segment of hair, so there are 30 measurement points per condition. In each pixel, a dimensionless value called  $K_{\text{max}}$  is dependent on the keratin birefringence (intrinsic physical parameter to the material and whose numerical value depends on the structural state keratin, at the molecular level), and keratin thickness traversed by light. The hair being approximately cylindrical, the thickness value crossed is not constant in the field: This explains the oscillations of the  $K_{\text{max}}$  parameter.

### **Ex vivo analysis of hydration**

Fifteen natural brown hair locks of 10 g each and measuring 10 cm were used for this study. Hair locks were divided in 15 locks of 10 grams each and washed using a neutral shampoo. After the washing procedure the locks were slightly bumped with a towel and then dried with a hairdryer. After drying hair locks underwent to product application. Fifteen hair locks were treated with the products to be tested. 2 ml of product were applied on wet hair locks, rubbed during 20 seconds, rinsed during 30 seconds. After product application, hair locks were dried with a hairdryer, and then measurement was performed. Hair moisture content was measured indirectly using a Tewameter® TM 300 (Courage+Khazaka, electronic, GmbH). Tewameter® is a probe which measures the density gradient of the water evaporation. The measuring head of the probe is a narrow hollow cylinder (10 mm diameter and 20 mm height), in order to minimize influences of air turbulence inside the probe. The water loss from the hair was continuously measured during one hour. A calibration curve was obtained with known amount of water (0, 10, 50, 100, 200, 400, 600 µl).

### **Statistical analysis**

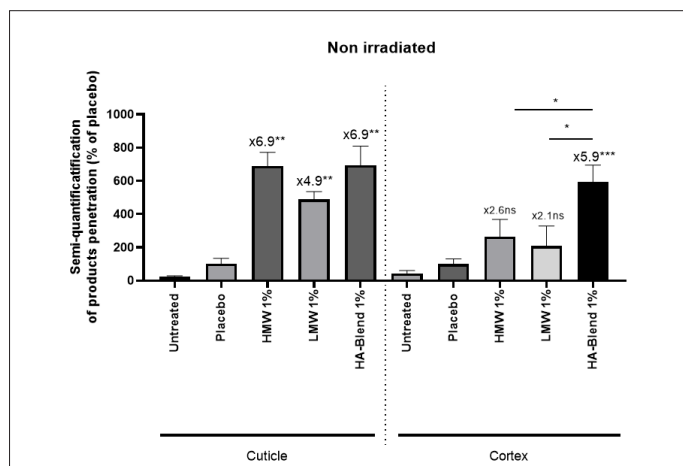
Results that followed a parametric distribution were statistically analyzed by applying a Student's t-test. Non-parametrically distributed results were analyzed using the Mann-Whitney test. Significant p values are presented in figures as follows: \*p value<0.1; \*p value<0.05; \*\*p value<0.01; \*\*\*p value<0.001.

## **RESULTS**

### **HA penetration in hair fibres**

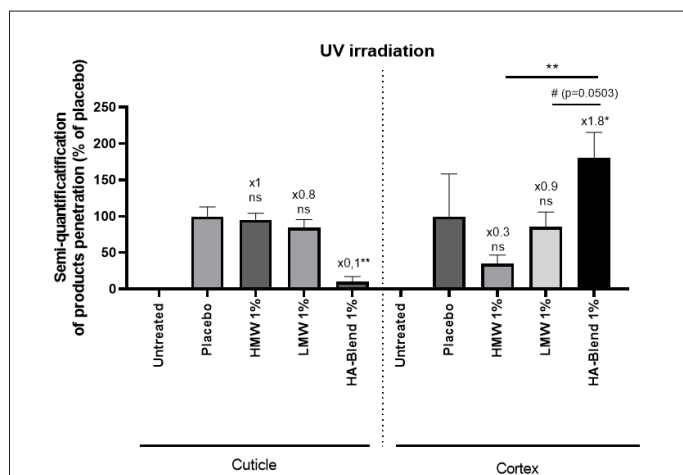
We used the Raman spectroscopy to evaluate the penetration of hyaluronic acid through the hair fibre. Compared to the placebo shampoo (without any HA), we tracked the penetration of LMW, HMW and HA-blend formula in irradiated or non-irradiated hair fibres using Raman spectroscopy. The cuticle was defined as a depth between 0 µm and 12 µm, and the cortex was between 12 µm and 42 µm. With non-irradiated hair locks (Figure 1), penetration of the actives inside the cuticle was significantly higher than the placebo, increasing by x6.9, x4.9, and x6.9 for

HMW, LMW, and HA-blend shampoos, respectively. Only the HA-blend significantly penetrated the cortex, 5.9-fold more than the placebo. HA-blend penetration of the cortex was also significantly higher than the other active shampoos.



**Figure 1:** Semi-quantification of the penetration of LMW 1% (w/v), HMW 1% (w/v) and HA-Blend 1% (w/v) shampoos in non-irradiated hair fibres. Penetration was monitored in the cuticle (left) and the cortex (right). **Note:** Mann-Whitney test with \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ , ns: Not significant.

When hair fibres were irradiated with UVA and UVB, the difference in penetration inside the cuticle relative to the placebo was significantly reduced. Indeed, the HA-blend showed only 0.1-fold penetration compared to the placebo. In contrast, the quantity penetrating the cortex was significantly increased, at 1.8-fold the placebo level. Once again, the amount of 1% (w/v) HA-blend detected inside the cortex of the irradiated hair fibres was significantly higher than the amount of 1% HMW (w/v) or 1% (w/v) LWM (Figure 2).



**Figure 2:** Semi-quantification of penetration of LMW 1%, HMW 1% and HA-Blend 1% shampoos in hair fibres irradiated with UVA and UVB. Penetration was monitored in the cuticle (left) and the cortex (right). **Note:** Mann-Whitney test with # $p<0.1$ , \* $p<0.05$ , \*\* $p<0.01$ .

**Anti-frizz study**

To highlight a straightening effect, we have worked at the *ex vivo* level on hair shafts. Hair locks were washed with shampoos containing HA-blend at 1.5% (w/v) or 3% (w/v). After

straightening with a hair straightener, hair was placed in extreme humidity conditions for 8 h. The smoothing effect was calculated based on variations in hair length on photos taken before and after exposure to humidity (Table 1). The effect of HA-blend at 3% (w/v) was significant, smoothing the hair 11% more than the placebo shampoo, and producing a visible effect (Figure 3).

**Table 1:** Comparison of anti-frizz effect of shampoos after exposure to extreme humidity conditions for 8 h, ns: Not significant.

Condition and parameters	Smoothing percentage (mean ± SD) and <i>p</i> value (Student <i>t</i> test)	Δ(%) versus placebo
Untreated	69 ± 3 ( $p<0.001$ )	
Placebo	83 ± 4 ( $p<0.01$ )	
HA-blend 1.5%	87 ± 2 ( $p<0.01$ )	4% (ns)
HA-blend 3%	94 ± 2 ( $p<0.01$ )	11% ( $p<0.05$ )



**Figure 3:** Macro photographs illustrating the anti-frizz effect of HA-Blend compared to the placebo.

**Reparation study**

To go further on the benefit of hyaluronic acid for haircare application we have analyzed several mechanical properties. After

bleaching with a chemical bleaching powder, the Elastic Modulus and the maximum force needed to break a fibre significantly decreased by 20% and 11% respectively. The placebo has not shown any statistical effect in comparison to the damaged hair lock. Following the treatment with HA-blend at 3% (w/v), the elastic modulus and the break force significantly increased by 4% for both parameters in comparison to the damaged condition. The effect was also significant in comparison to the placebo (Figures 4 and 5).

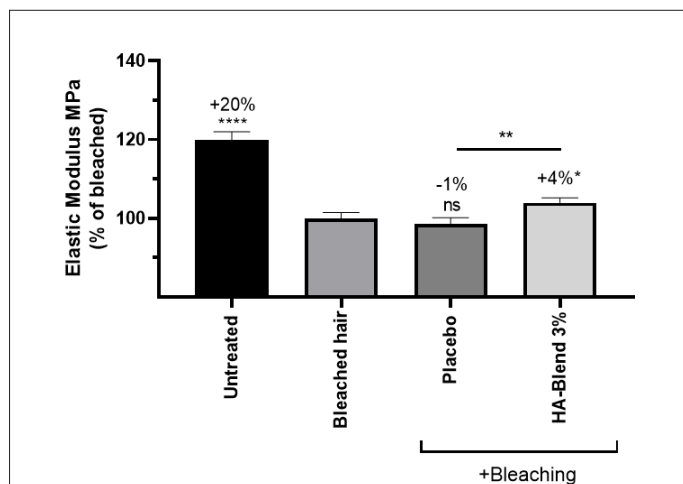


Figure 4: Elastic Modulus evaluated after tensile-test analysis on hair locks damaged or not with bleaching powder and washed with HA-blend 3% (w/v) or placebo shampoos. Note: Mann Whitney test with \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\*\* $p < 0.0001$ , ns: Not significant.

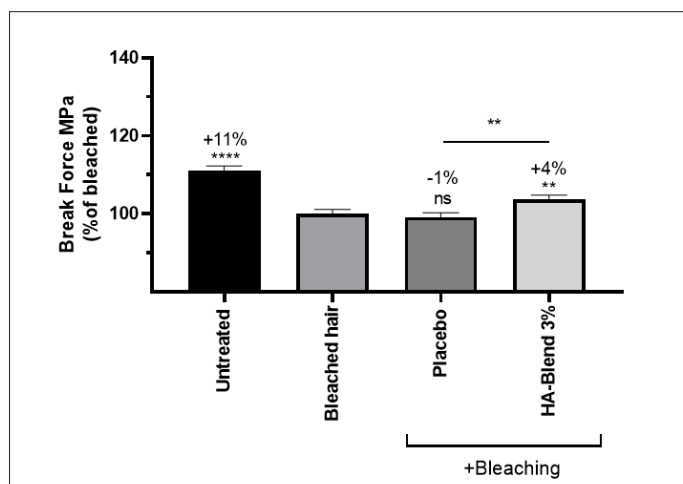


Figure 5: Maximum force needed to break fibres of hair locks damaged or not with bleaching powder and washed with HA-blend 3% (w/v) or placebo shampoos. Note: Mann Whitney test with \*\* $p < 0.01$  and \*\*\*\* $p < 0.0001$ , ns: Not significant.

### Keratin integrity

The HA-blend at 3% was able to repair damaged hair following bleaching. UV irradiation also damaged the hair and impacted keratin structure. We analyzed keratin integrity using specific Xpolar® technology which is an imaging mode for measuring the birefringence of photons on the keratin. The birefringence value

is measured in each pixel of the image. After irradiation, the keratin integrity was significantly reduced by 16%. The placebo has not shown any significant effect in comparison to the damaged hair lock. Following the treatment with HA-blend at 3% (w/v), the keratin integrity was improved by 31% in comparison to the damaged condition. The effect was also significant in comparison to the placebo (Figures 6 and 7).

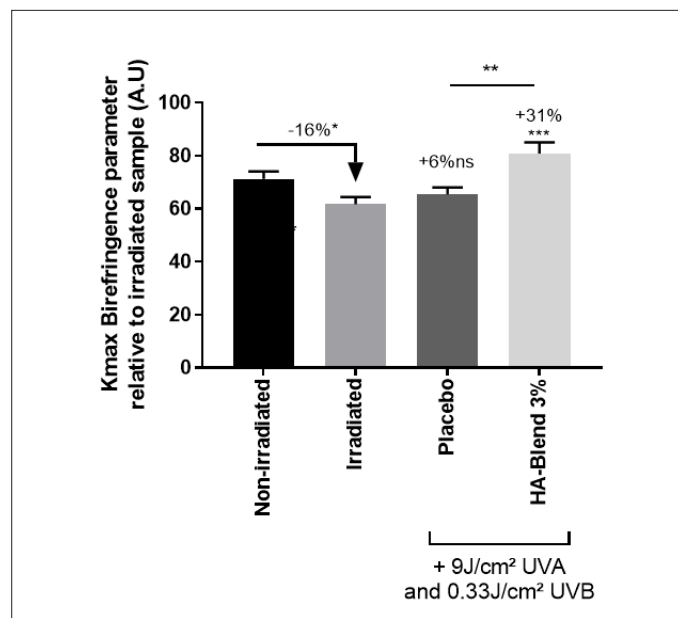


Figure 6: Keratin integrity measured using the  $K_{max}$  parameter based on keratin's photonic birefringence. The hair locks were washed with HA-blend 3% (w/v) or placebo shampoos before irradiation. Note: Mann Whitney test with \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ , ns: Not significant.

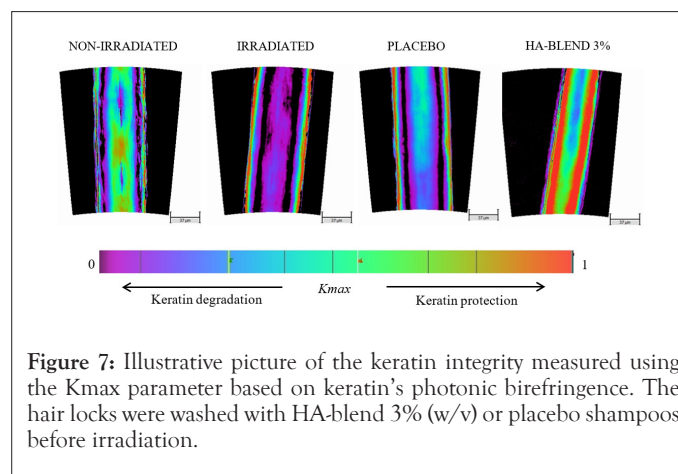
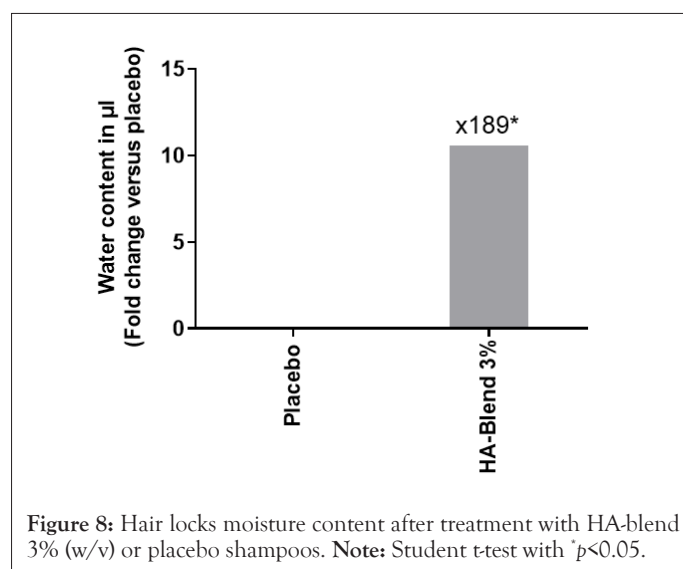


Figure 7: Illustrative picture of the keratin integrity measured using the  $K_{max}$  parameter based on keratin's photonic birefringence. The hair locks were washed with HA-blend 3% (w/v) or placebo shampoos before irradiation.

### Hydration study

Hyaluronic acid is a hero molecule to preserve the hydration of the skin. To evaluate if HA is also able to moisturize the hair, we have measured the water evaporation using a Tewameter®. Under the tested experimental conditions, HA-blend 3% (w/v) increased the hair water content when compared to the same formulation without the active ingredient (placebo) by 189-times (Figure 8). The increase of hair moisture content was as follows, see Table 2.



**Figure 8:** Hair locks moisture content after treatment with HA-blend 3% (w/v) or placebo shampoos. **Note:** Student t-test with \* $p < 0.05$ .

**Table 2:** Water content of hair locks after treatment with HA-Blend 3% (w/v) in comparison to placebo.

Condition	Water content ( $\mu\text{L}$ ) (mean $\pm$ SD)	Fold change and $p$ -value versus placebo (Student t test)
Placebo	0.056 $\pm$ 0.01	
HA-Blend 3%	10.567 $\pm$ 1.18	$\times 189$ $p < 0.05$

## DISCUSSION

The aim of this study was to develop a method to measure HA penetration in hair, and to assess the benefits of a blended and optimized HA formulation for use in haircare products.

We established an innovative method to demonstrate the penetration of hyaluronic acid inside the hair fibre in rinse-off conditions. It has been mainly described that UV irradiation damage the hair leading to protein loss and colour change [11]. Using UVA and UVB irradiation, we damaged the hair to open the cuticle and enhance product penetration. The semi-quantification of the total Raman signal has shown an increase in the quantity of LMW, HMW and placebo (shampoo without the active) detected into the hair after the irradiation in comparison to undamaged hair (Data not shown). Interestingly, the total quantity of HA-Blend detected is the same but the penetration profile is different with a lower quantity of HA-blend in the cuticle and a higher amount in the cortex for irradiated hair fibre. We have thus compared the penetration of HA in normal or damaged hair locks to gain a greater understanding of how the product works. When using non-irradiated hair locks, the three active shampoos (LMW, HMW and HA-Blend) had a similar capacity to penetrate the cuticle, which was consistently better than the placebo. In contrast, when examining the cortex, the HA-Blend outperformed all the other formulations. With UV-damaged hair, penetration of the HA-Blend inside the cuticle was significantly reduced, but penetration into the cortex was still significantly higher than the placebo or the two other active shampoos. This apparent discrepancy is due to the fact that when hair has undergone UV-induced damage, the placebo more readily penetrates the cuticle, whereas hydrophilic compounds such as HA bind more tightly to proteins such as keratin

composing cortex [12]. In other words, once the HA-Blend penetrates the cuticle in damaged hair, this optimal formulation may be attracted toward the keratin inside the cortex, leaving only small amounts on the cuticle. Interestingly, even in the non-irradiated condition, only the HA-Blend significantly penetrated the cortex, due to its optimized low- to high-molecular weight HA formulation, and its capacity to bind to keratin. Besides, the optimal process behind the HA-Blend involved the addition of lactic acid to reduce the pH of the formula. It has been described that pH plays a critical role on molecule absorption and has an opposite effect depending on the initial ionization of the molecule (cationic or anionic compound). Indeed, change in pH could lead to structural changes in keratin including changes in the binding affinity and the number of available binding sites [12]. At pH over 6.0, hair keratin is negatively charged and attracts positively charged molecules. Inversely, at pH 2.0, cationic species showed a low level of binding to keratin due to the electrostatic repulsion [12]. HA is an anionic compound and we hypothesized that an acidification of the pH induced by the lactic acid below 6.0 improved the binding of HA-Blend to keratin.

By binding to the keratin, this optimized HA-Blend modified the keratin conformation, decreasing  $\alpha$ -helices, and promoting the formation of  $\beta$ -sheets, leading to smoothing of the hair. This data corroborated results from our previous study, where analyses showed that this HA-Blend decreases the number of disulfide bonds in keratin and induce a change in the  $\alpha$ -helix/ $\beta$ -sheet ratio [3]. The results presented here establish that the change in keratin conformation is correlated to the effective penetration of the HA-Blend, allowing a direct interaction between HA and keratin. This anti-frizz potential was confirmed in *ex vivo* experiments, with the HA-Blend producing a significant smoothing effect 11% better than the placebo which resulted in a visible benefit.

It has been described that current straightening methods involving thermal method (with an iron) or chemical agents damaged the hair by reducing their physico-mechanical properties [13]. Those treatments break and rearrange disulfide bonds, change the structure of keratin in favor of a  $\beta$ -sheet conformation and disrupted cuticle [14,15]. We have shown that HA is able to straight the hair and to change the keratin conformation without damaging the fibre as proved by the tensile test analysis. Indeed, we have demonstrated that HA treatment smooth the hair while improving the Elastic modulus and the maximum strength needed to break a fibre by filling damaged hair from inside. We have demonstrated that the HA-Blend is also able to repair keratin integrity following UV irradiation.

Moreover, it has been described that thermal straightening leads to a reduction of water content into the hair fibre [16]. The authors explained that the changes in protein conformation and a reduction of the  $\alpha$ -helix conformation may change the water accessibility. Interestingly, we have shown that even if HA interact with keratin and change its conformation, it is able to significantly increase water content into the fibre. This interaction between HA and keratin has already been described in the skin to provide hydration and maintaining skin barrier integrity [5]. We hypothesize that the damage caused at the cuticle level by the thermal straightening is mainly responsible of water loss, but using high molecular weight HA into the HA-Blend may coats the fibre and improved hair hydration while smoothing the fibre.

## CONCLUSION

Our results demonstrate that Confocal Raman Spectroscopy is a powerful, non-invasive technique to investigate the penetration of cosmetic ingredients into human hair fibres. To the best of our knowledge, this study is the very first evidence of hyaluronic acid penetration inside hair fibres, and the first comparison of the penetration of different molecular weights of HA. Our results show that an optimized formulation containing different molecular weight HA and lactic acid penetrates deeply into the hair cortex to interact with keratin and exert a smoothing, hydrating and reparative effect. This insight opens new doors for the haircare industry to understand the mechanisms of action of active molecules for applications in haircare.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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