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ORIGINAL RESEARCH

Evaluation of Human Hair Absorption and Retention

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Objective: Adequate nutrition and moisture are crucial for preventing hair loss and maintaining hair health and appearance. Hair loss (alopecia) affects up to 50% of the population, causing significant social and psychological impacts. Despite the popularity of hair care products and their ingredients known to be beneficial, existing methods for evaluating the absorption of these substances into hair remain limited. This study assesses the absorption and retention of nutrients and moisture within human hair via 3D Raman spectroscopy.

Methods: Three variables were structured for a double-arm study, involving untreated hair, water-treated hair, and hair treated with a supplemental hair care product. Hair samples were analyzed for absorption amount, depth, and dryness at 30 min intervals using Raman spectroscopy with 3D imaging technology.

Results: Hair treated with hair care product indicated significantly higher in absorption amount, deeper penetration, and reduced dryness, confirmed by statistical analysis ($p < 0.05$). After 30 min of treatment, hair care product-treated samples maintained their absorption parameter, amount, depth and dryness ($p < 0.05$). This was further validated by the 3D Raman visualization which provided detailed spatial distribution and retention of absorbed substances within the hair fibers over time.

Conclusion: These findings demonstrate the superior absorption and retention of hair care product in hair compared to untreated and water-treated hair, setting a new standard for evaluating hair absorption and product efficacy. Our method offers a promising tool for future clinical research and hair care product development.

Keywords: hair absorption, hair moisture, hair treatment, hair growth, hair loss, Raman spectroscopy

Introduction

Maintaining healthy hair status plays a vital role in preventing various hair-related issues.[1] Hair loss medically referred to as alopecia, is one of the common issues individuals encounter throughout their lifetime.[2] It is widely perceived that hair loss acts as a social and psychological stress factor and adversely affects the quality of life.[1–4] Prevalence estimates revealed that up to 50% of the population were reported to suffer from hair loss issues.[2] These statistics underscore the importance of hair loss as a major health concern among diverse populations. Among the symptoms associated with hair loss are telogen effluvium and predominant thinning of the hair shaft in the scalp, axillary and pubic areas which contributes to an overall reduction in hair density.[1–3]

Providing adequate nutrition to hair is one of the preventative methods for hair loss also maintaining healthy and aesthetically pleasing hair.[5–7] Hair moisture is an important factor preserving the appearance of hair, as it prevents breakage, enhances elasticity, and reduces split ends thereby maintaining healthy and exquisite hair.[5,6,8] The hair follicles in the scalp are among the most metabolically active tissues in our body, making them particularly sensitive to nutrient supply and physiological changes.[9–11] Therefore, ensuring appropriate hair nutrition and moisture is vital for both the health and aesthetic quality of hair.[5,7,8]

Consequently, there has been a growing demand for hair care products and medications that are directly applied to the hair and designed to deliver essential nutrition and moisture absorbed to hair follicles, thereby mitigating hair shedding

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and reassuring psychologic well-being.[9,12–14] Despite the availability of standardized diagnostic methods, such as the hair pull tests and trichoscopy commonly used for assessing hair loss and scalp condition, there remains a deficiency in methodological approaches for evaluating the absorption of hair care products within hair shafts and retaining its efficacy.[2,3,15]

Raman spectroscopy is a non-invasive analytical device that utilizes various light frequencies to scatter across the spectrum, activating specific molecular vibrations and rotations within a substance.[16–19] 3D Raman spectroscopy transforms these molecular composition and structural changes into detailed 3D visualization, extending beyond mere identification of molecule peaks and values.[20,21] This 3D imaging technique is a versatile technology to confirm the absorption extent and substance retention in hair fibers.

This study aims to evaluate the absorption of nutrition and moisture to the hair fiber and its efficacy retention from hair care products via 3D Raman spectroscopy. We seek to advance the measurement of substance absorption into hair fibers by this novel approach with 3D Raman spectroscopy.

Methods

Hair Samples

Virgin hair samples, chemically untreated, were arranged in multiple strands and affixed to polystyrene plates for this study (Figure 1). Each hair strand was thoroughly washed with a 3% sodium dodecyl sulfate (SDS) solution (w/w), followed by rinsing with tap water. Post-drying, the samples were dried and allowed to acclimate for 20 minutes in a controlled environment with minimal movement and absence of UV radiation (Room Temperature (RT) 22 °C ±2°C; Relative Humidity (RH) 50% ± 10%) prior to measurement.

Hair Treatments

Virgin hair strands were divided into three treatment conditions for experimental analysis. No treatment, which served as untreated regular conditioned hair strands. Water treatment, immersing the hair strands in tap water for three minutes. Product treatment, where "Minoxell Scalp Intensive Ampoule by Labcle (Hyundai Pharm Co., Ltd., Seoul, Korea)" was applied as a hair nutrition and moisture supplement product (SP). SP is recognized as an efficacious hair care product for therapeutic hair loss prevention by providing nutrition and moisture to hair. An appropriate dose of 1mL was uniformly applied to the target site of the hair strands. Three conditions were organized into a double-arm study. The control group comprised untreated hair, samples assessed immediately following water treatment, and samples evaluated 30 minutes post-treatment. The test group comprised untreated hair, samples immediately treated with SP, and those assessed 30 minutes thereafter. To enable comparative analysis, untreated samples were included in both groups as a baseline.

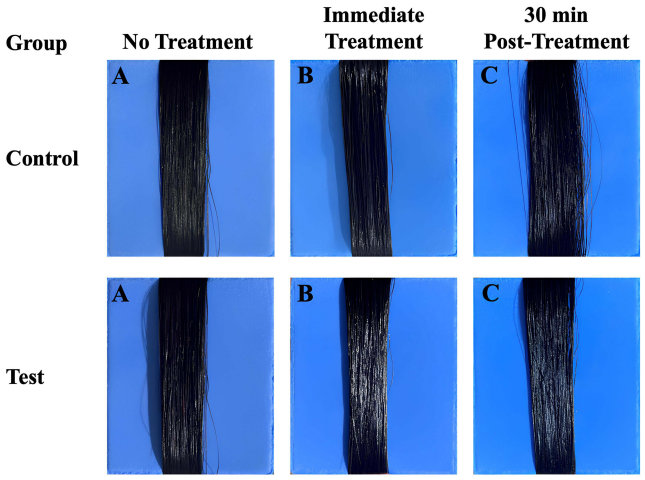

Figure 1 Experimental setup for Raman spectroscopy measurement. The three plates separated for each group. (**A**) No treatment (**B**) Water/SP treatment (**C**) 30 min post-treatment with Water/SP. The control group was administered water; the test group was administered with SP.

This design allowed comparison of absorption effects from no treatment to active treatment, and between water and SP treatments under standardized conditions.

Hair Absorption Measurement

To evaluate the extent of treatment absorption and its retention over time, measurements for each treatment were conducted twice, with 30 min intervals between assessments. This approach aimed to determine whether the absorbed properties remained in hair strands after 30 min for both the control group and the test group. For each treatment condition, 20 measurements were conducted across 3 independently prepared hair strands, yielding a sample size of n = 20 per condition. The absorption properties of hair samples were assessed non-invasively in real-time using the Nanofinder_FlexG Raman Spectroscopy system (Tokyo Instrument, Tokyo, Japan).

Three key 3D Raman spectroscopy parameters were analyzed, Absorption amount (A.U., Arbitrary Units) was quantified to assess the relative concentration of treatment absorbed into the hair. Arbitrary units are dimensionless values derived from the Raman signal intensity, which reflects the magnitude of molecular vibrational energy changes induced by light–matter interactions. Absorption depth (μm) indicates the maximum depth of treatment penetration, with measurements taken at intervals of 5 μm along the X-axis, up to a depth of 70 μm. Dryness (A.U., Arbitrary Units) was evaluated by determining the relative proportion of unabsorbed or evaporated constituents, based on spectral regions characterized by attenuated or absent vibrational activity. To evaluate intragroup differences and the retention of treatment efficacy, the change amount in absorption parameters within the treatment group was measured, comparing how each absorption parameter changed over time relative to the intermediate initial treatment and 30 min after the treatment application.

All measurements are based on the principles of Raman scattering, utilizing a 785 nm laser to detect specific wavelength regions of the Raman spectrum.[16–19] The measured data were processed to generate 2D and 3D visualizations of the absorption properties, with 3D imaging derived from the automated assembly of 2D Raman spectral data via advanced 3D Raman imaging technology.

Statistical Analysis

The data acquired from the study were analyzed using SPSS Statistics Software ver. 29 (IBM Corporation, Armonk, NY, USA), with results expressed as mean ± standard deviation. The normality of the data distribution was assessed using the Shapiro–Wilk test, yielding a p-value of $p < 0.05$, at a 95% confidence level.[22] For statistical comparisons between the intermediate treatment application and the measurements taken 30 minutes post-application, a parametric paired *t*-test was employed. An independent parametric *t*-test was utilized to evaluate the statistical significance of inter-group comparisons concerning absorption amounts and dryness, while the non-parametric Mann–Whitney *U*-test was employed for assessing absorption depth.[23]

Results

The results of our research were derived from three treatment variables, which were organized into a control group and a test group, receiving treatment with water and SP products, respectively. Raman spectroscopy measurements were taken at 30 min intervals post-application. Figures 2–4 illustrate the comparison of absorption efficacy among untreated, water-treated, and SP-treated conditions across three key parameters. Table 1 and Table 2 represent the numerically quantified data for the measured parameters with calculated averages and standard deviations. Statistically analyzed p-values were applied to each variable to confirm significant changes and validate the absorption efficacy and retention of the treatments.

Figure 2 depicts 3D mapping images of target hair strands subjected to different treatments, illustrating their absorption of substances for both the control and test groups across the visible light spectrum (380–740 nm). The brighter green areas signify a higher intensity of absorption, and the transition from the darker green to transparent red region represents a gradual decrease and lower absorption. These images visually confirm the position and distribution of active ingredients absorbed into hair samples. Untreated hair samples predominately display red and minimal green regions, indicating little absorption (Figure 2). In contrast, both water-treated and SP-treated hair samples exhibit a wider

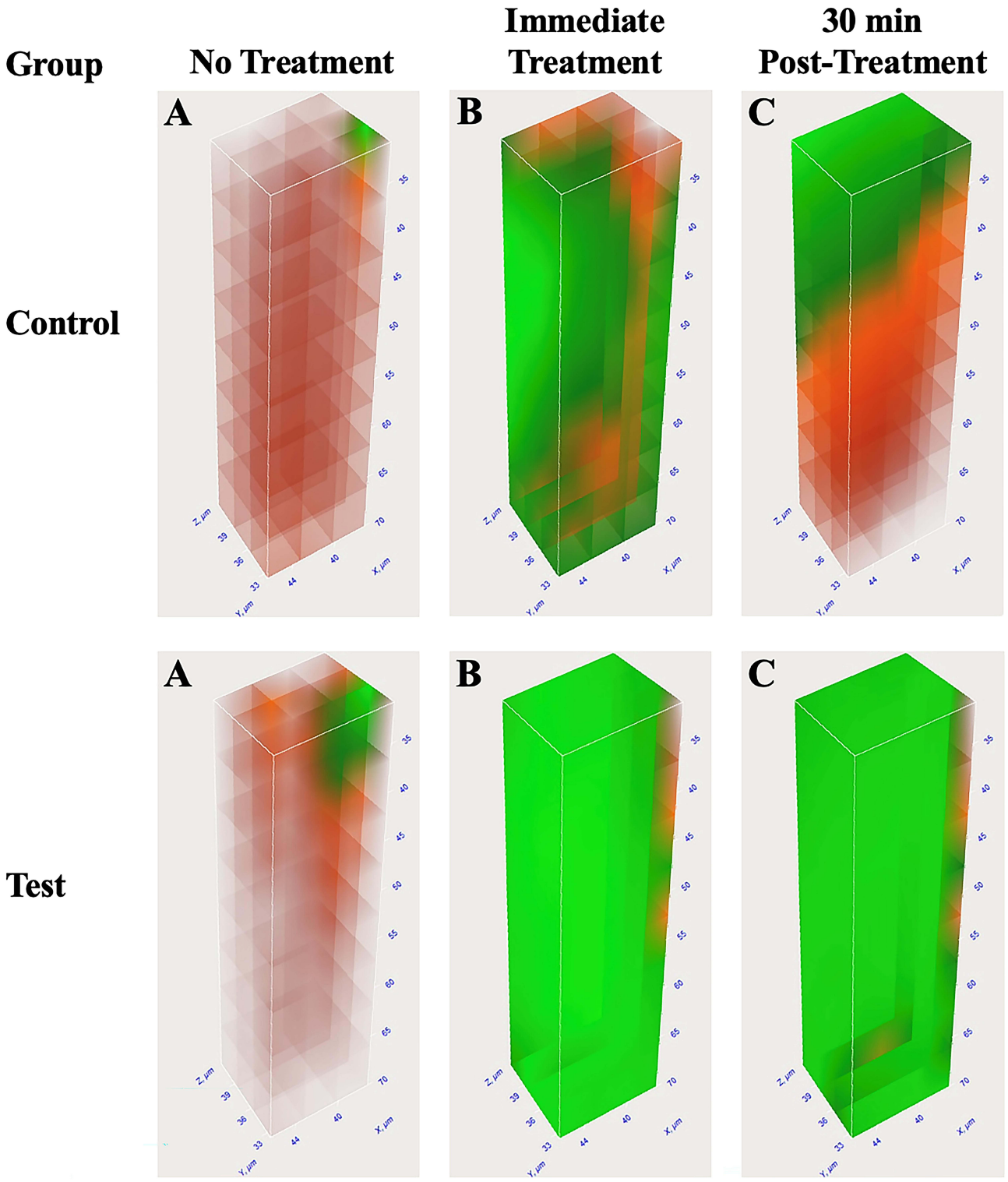

Figure 2 3D visualization of hair absorption at immediate treatment and 30 min post-treatment in control group and test group. The three images separated for each group. (**A**) No treatment (**B**) Water/SP treatment (**C**) 30 min post-treatment with Water/SP. The control group was administered water; the test group was administered with SP.

distribution of green areas compared to untreated samples. Notably, the SP-treated samples indicate relatively a greater extent of darker green coloration, suggesting higher absorption, with only a minimal decrease in green regions 30 min post-treatment (Figure 2). This indicates that SP treatment not only has higher absorption but also improves the retention

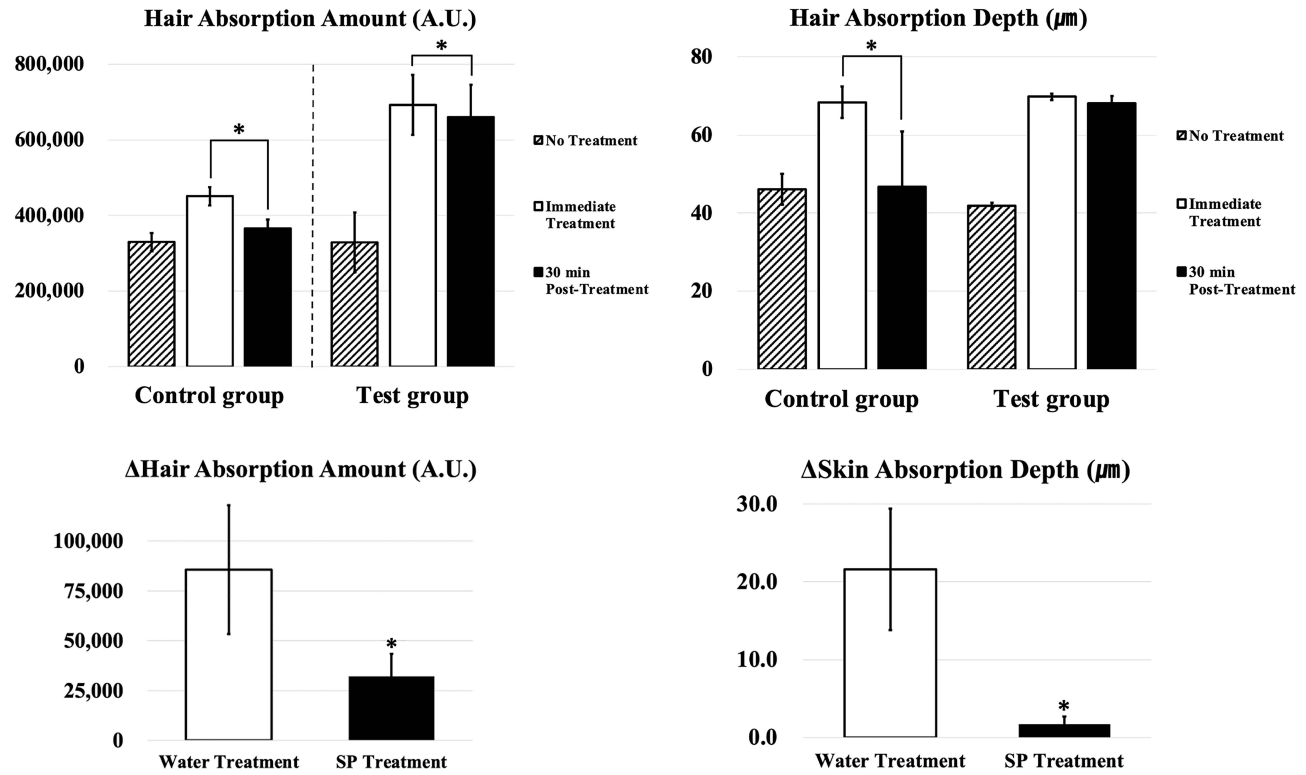

Figure 3 Effect of treatment on hair absorption amount and hair absorption depth at immediate treatment and 30 min post-treatment in control group and test group, and comparative changes over time. *Denotes significant difference at $p<0.05$ compared between time points by paired *t*-test.

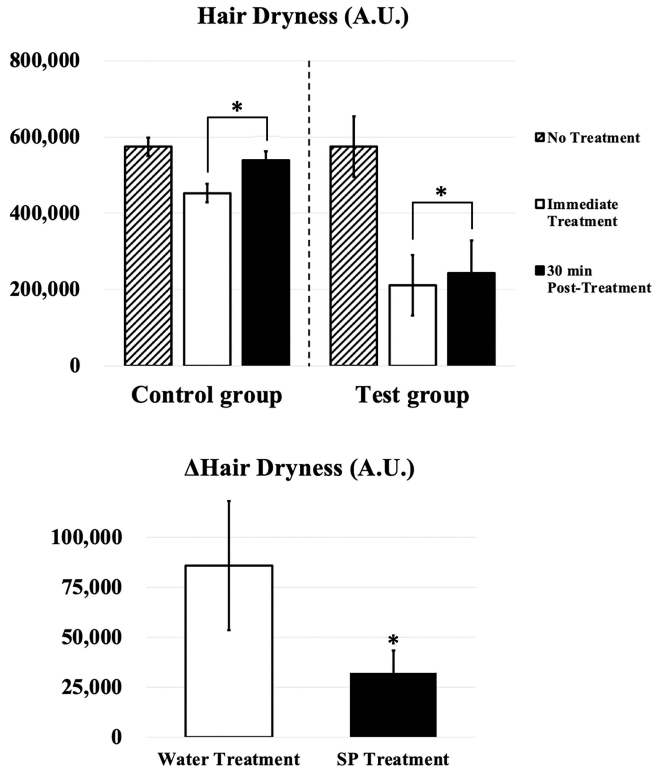

Figure 4 Effect of treatment on hair dryness at immediate and 30 min post-treatment in control and test group, and comparative changes over time. *Denotes significant difference at $p<0.05$ compared between time points by paired *t*-test.

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Table 1 Comparative Values of Hair Absorption Amount (A.U.) and Hair Absorption Speed (μm/h) Between Control and Test Group at 30 min Intervals. Quantified Values with Calculated Mean ± Standard Deviation (X ± Y), 20 Measurements for Each Value

Parameters	Group	Treatments	Amount (A.U)	Change (Δ)	*p*-value
Hair absorption amount (μm/h)	Control	No treatment	329647.0±6829.8		
		Immediate Treatment	451131.4±24,109.4	85,761.9±32,283.2	<0.05
		30 min Post-Treatment	365369.5±24,109.4		
	Test	No treatment	329178.1±7635.9		
		Immediate Treatment	692126.5±78,901.4	32,218.5±11,196.4	<0.05
		30 min Post-Treatment	659908.0±85,311.6		
Hair absorption depth (μm/h)	Control	No treatment	46.1±15.0		
		Immediate Treatment	68.4±4.0	21.6±7.8	<0.05
		30 min Post-Treatment	46.7±14.2		
	Test	No treatment	41.9±12.6		
		Immediate Treatment	69.8±0.8	1.7±1.0	0.256
		30 min Post-Treatment	68.1±1.9		

Abbreviation: A.U, arbitrary unit.

Table 2 Comparative Hair Dryness (A.U.) Between Control and Test Group at 30 min Intervals. Quantified Values with Calculated Mean ± Standard Deviation (X ± Y), 20 Measurements for Each Value

Parameters	Group	Treatments	Amount (A.U)	Change (Δ)	*p*-value
Hair dryness (A.U)	Control	No treatment	574288.0±6829.8		
		Immediate Treatment	452803.7±24,109.4	85,761.9±32,283.17	<0.05
		30 min Post-Treatment	538565.6±24,109.4		
	Test	No treatment	574756.9±7635.9		
		Immediate Treatment	211808.5±78,901.4	32,218.5±11,196.4	<0.05
		30 min Post-Treatment	244027.0±85,311.6		

Abbreviation: A.U, arbitrary unit.

of substances over time. These 3D Raman images representations visually validate that SP treatment is more effective in retaining absorbed substances regardless of time.

Figure 3 presents the absorption amount and depth across the different treatments and the quantified changes within the control group and test group measured over the 30 min intervals. The SP treatment exhibited a substantially greater increase in absorption amount compared to the water treatment, with both treatments showing a significant rise relative to the untreated hair samples ($p < 0.05$) (Figure 3 and Table 1). However, the control group demonstrated a marked decline in absorption 30 min post-water treatment, whereas the test group sustained a stable absorption level, with only a minimal decrease observed over the same intervals ($p < 0.05$) (Table 1). This suggests that the SP treatment effectively maintains absorption, indicating superior retention capabilities compared to the water treatment.

In contrast to the absorption amount, there was a similar increase in absorption depth for both water and SP treatments (Figure 3 and Table 1). A reduction in absorption depth was also observed in the water-treated samples after 30 min, whereas the SP-treated samples maintained a deeper absorption, sustaining a consistent depth even 30 min post-application ($p < 0.05$, $p = 0.256$) (Figure 3 and Table 1). As a result, while water-treated hair initially achieved a depth of 70 μm resembling SP treatment, this level was not sustained, indicating that the water-treated hair dried rapidly, with absorption depth decreasing, shifting outward. This trend is further visualized in the 3D representation in Figure 2.

The test group with SP treatment showed significantly less change in absorption amount and depth compared to the control group, indicating better retention across both parameters ($p < 0.05$, $p = 0.256$) (Figure 3 and Table 1). While the control group experienced greater fluctuations over time, the test group maintained stable absorption levels, demonstrating superior efficacy in retaining the absorbed substances ($p < 0.05$) (Figure 3 and Table 1). This suggests that SP treatment is more effective in preserving the absorbed amount over the measured intervals.

Dryness was highest in the untreated hair group, significantly decreasing after both water and SP treatments, indicating increased moisture (Figure 4 and Table 2). Particularly, the SP-treated samples exhibited a greater reduction in dryness compared to the water treatment ($p < 0.05$). The differences between the two groups emerged after 30 min. The dryness level of the control group returned closer to those of the untreated group, while the test group maintained lower dryness levels. This difference in moisture retention over the 30 min intervals was statistically significant ($p < 0.05$). Figure 4 further supports these findings, showing that the change in dryness for the control group was greater than that of the test group, indicating inferior efficacy in retaining reduced dryness (Table 2).

Discussion

Our study elucidates the absorption of nutritional and moisturizing components from the supplemental hair care product into hair fibers, along with the retention of these components within the fibers over a sustained period.

Recent studies have employed Raman spectroscopy to assess the efficacy of personal hygiene products.[24,25] Our research extends the application potential of this technique to the realm of hair supplement products. Both earlier studies demonstrated the utility of Raman spectroscopy in evaluating product absorption and skin penetration of various supplement application products, and the findings which resemble our observed outcomes. Our incorporation of 3D Raman spectroscopy further enhances the capabilities of this analytical method, providing a more comprehensive spatial visualization of absorption within hair fibers. Thus, this approach not only enriches the understanding of absorption patterns but also facilitates clearer communication of the results to a broader audience.

The greater absorption amount, deeper penetration, and reduced dryness were observed with the SP treatment relative to the water treatment with the higher retention after 30 min. These effects may be attributed to the presence of emollients, humectants, and occlusive agents in hair care products.[13,26] The properties of these components to potentially bind to hair proteins likely have contributed to their superior efficacy in these parameters, which should be subjected to further investigation.

Hair follicle cells require a sufficient amount of nutrients and energy for their active metabolism, given their rapid turnover rate.[27,28] Hair nutritional deficiencies are often associated with their growth disorders.[27,29] Providing adequate nutrition and moisture to hair is regarded as a prominent way to prevent hair loss and maintain healthy, aesthetically pleasing hair.[5–8] Despite the highly valued market demand for evaluating hair absorption and the nutrition and moisture

deficiencies contributing to hair loss, there is a current lack of available clinical hair analysis methods. Therefore, our approach with 3D Raman spectroscopy will bring a meaningful development in clinical studies, enabling the evaluation of product efficacy and content absorption in hair.

Hair cuticles, the outermost layer of hair fiber act as a major path for nutrition and moisture to be absorbed into further hair cells or spread to the scalp.[9,30] Their condition is also closely linked to hair luster and texture.[5,8,12] In this study, the evaluation of nutrition and moisture absorption within the length of human hair cuticles in-vivo enabled by non-invasive Raman spectroscopy, which measures the absorption up to 70 μm, corresponding to the cuticle layer.[31]

Previous studies by Kourbaj et al demonstrated the use of confocal Raman spectroscopy of hair-cleansing products on the scalp.[28] Building upon this foundation, our study aims to evaluate how a hair care product enhances absorption, penetration, and moisture retention in hair fibers, providing a novel perspective on using Raman spectroscopy between hair care products and hair fibers. When comparing our results with this research, they observed a reduction in natural moisturizing factors (NMFs) and stratum corneum (SC) lipids, contributing to skin dryness after repeated hair washing, suggesting that cleansing products and water treatment can strip away essential components.[28] Our study revealed that using SP not only restored nutrition and moisture but also showed enhanced absorption and retention, suggesting its effectiveness in the application of the hair care products after washing of hair for replenishing lost hydration and nutritional contents.

Dryness is a newly developed parameter designed to measure the loss of a substance that does not induce specific rotation and vibration of molecules across the hair shaft at various light spectra frequencies. Moisture from external hair care products also plays an important role in the outer layer of hair fibers, influencing overall aesthetical hair attributes.[5,6,8] Unlike human skin which consists of multiple layers and is subsequently absorbed into the inner layer, the hair shaft has a relatively shallower structure in depth.[30,32] As a result, substances absorbed by the hair tend to evaporate from the outer layer. This is evident in Figure 2, where the visualized absorption area decreases, and the remaining absorbed areas shift upward, highlighting the drying process of the hair. This inclination can be measured by calculating the evaporated amount, which can be justified as the area of inactivated molecular changes, establishing dryness as our novel parameter.[30,33] Raman spectroscopy has a wide range of application potential in diverse fields.[17,18] This parameter provides valuable insights and a methodological approach applicable to hair and other absorbents, and evaporative materials in vivo, allowing the quantification of substance loss over time.

To assess the retention of hair absorption specifically for the selected treatments, 3D Raman spectroscopy obtained data enabled a comparative analysis of treatment retention via measuring the remaining contents in the hair samples at 30 min intervals. This timeframe minimized the interference from subsequent reactions related to the absorbed properties of the treatments due to additional biochemical reactions and physiological responses. Sebum production from sebaceous glands and lipidation in scalp tissues and hair typically occur within a few hours of application.[34,35] In addition, the nutrition and moisture are absorbed more deeply into hair fibers and scalp epidermis after 30 min in physiological condition.[36] Therefore, extending the measurement time beyond 30 min will distort the results by affecting the changes in intermolecular forces observed in the Raman spectrum.

Conclusion

In conclusion, our study evaluates the absorption and retention of absorbed properties within human hair fibers from three selected treatments, highlighting their impact on the absorption parameters of hair. Utilizing 3D Raman spectroscopy, we quantitatively and visually assessed the absorption of substances from three treatments in vivo. Our study demonstrates that the SP, the hair care product is relatively more effective at absorbing and retaining their contents within the hair fibers over time.

Raman spectroscopy, corroborated by statistical analyses, demonstrates that the SP, hair care product achieves greater absorption amount, deeper penetration and reduced dryness compared to untreated hair and water-treated hair. Moreover, the absorbed components remain stable in amount, depth and moisture, indicating prolonged efficacy.

This was further validated by the 3D imaging Raman spectroscopy technology which provided detailed spatial visualization of the distribution of product contents of SP. This technique establishes a more comprehensive assessment beyond the conventional wavelength analysis principle of Raman spectroscopy. Our findings and methodology not solely

provide the potential for future clinical research opportunities but also set a new standard for evaluating the absorption of human hair and the efficacy of hair care products.

Ethical Statement

This clinical research received approval from the Institutional Review Board (IRB) of Sunjin Beauty Science, Inc. on August 26, 2024. The research was conducted in full compliance with the ethical principles outlined in the Declaration of Helsinki and followed Good Clinical Practice (GCP) guidelines as per ICH E6 [R1, R2]. All participants provided written informed consent prior to inclusion in the research. The IRB approval number for this study is SCRC_24_IRB_HD0013_0077.

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Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflict of interest in this research.

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