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The impact of AP collagen peptides (APCPs) on hair shaft elasticity and gloss: A comprehensive analysis

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ABSTRACT

AP collagen peptides (APCPs) are enzymatically decomposed collagen peptides that have been shown to promote hair growth and improve hair cuticle structure and thickness in mice. However, their impact on the elasticity and gloss of human hair has not been thoroughly investigated. Therefore, this study sought to identify the factors affecting hair elasticity and gloss in APCPs-treated human hair follicles (hHFs). Using Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) analysis, changes in amino acid content related to hair elasticity and lipid components associated with hair gloss were measured. As a result, APCP treatment led to increases in proline and cysteine amino acid, as well as in lipid components such as 18-methyleicosanoic acid (18-MEA), lauric acid, oleic acid, 11,13-eicosenoic acid, behenic acid, methyleicosanoic acid and squalene. Fourier-transform infrared spectroscopy (FT-IR) analysis of hair cross-sections indicated an increase in the intensity of bonds such as S=O, C=O, cystine, C–N and C–H in APCPs-treated hHFs. Additionally, APCPs elevated the expression of Keratin 17, integral to the inner root sheaths (IRS) and outer root sheaths (ORS), as well as Keratins 31, 85, and 86, components of the hair cortex and medulla. APCPs treatment also resulted in increased bonded water and moisture content in hHFs, along with a reduction in cuticle surface roughness. These findings suggest that APCPs may have the potential to enhance hair elasticity and gloss.

1. Introduction

Each hair consists of a hair shaft (HS) and a hair root. The shaft is the visible portion that protrudes from the skin, while the root resides within the skin, extending into its deeper layers. The hair root is encased by the hair follicle (HF), which is also linked to a sebaceous gland. The HS, a filamentous structure, is derived from the epidermis and originates from the HF (Powell, Crocker, & Rogers, 1992; Randall & Botchkareva, 2009). Its primary functions are to protect the body from ultraviolet (UV) rays, cold, heat, and physical impacts (Buffoli et al., 2014). Healthy hair is characterized by its glossiness, absence of damaged ends, frizz-free smoothness, volume, and strength without breakage (Marsh et al., 2015). Notably, hair elasticity and glossiness are crucial indicators of overall hair health.

The hair shaft (HS) is primarily composed of keratin protein fibers (85 %), which impart strength and resilience to the hair. Additionally, it contains lipids (1–5 %) and moisture (10 %). The HS has a distinct microstructure consisting of three distinct zones: the cuticle (outer layer), the cortex (main part of the fiber), and the medulla (inner layer) (Marsh et al., 2015; Yang, Zhang, & Rheinstädter, 2014). Keratin—the smallest structural unit of hair—comprises amino acids, including a high concentration of sulfur-containing amino acids such as cysteine and methionine, as well as histidine and proline (Bantignies et al., 2000; Strnad et al., 2011). These amino acids are connected by various bonds such as peptide, amide, disulfide, and hydrogen bonds, forming the keratin fiber structure and ensuring its stability (Harland et al., 2022; Popescu & Höcker, 2007).

The hair follicle (HF) is a complex anatomical structure composed of multiple distinct layers, including the outer root sheath (ORS), the companion layer, and the inner root sheath (IRS). Each of these layers

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expresses specific sets of cytokeratins, which are structural proteins critical for maintaining the integrity and functionality of epithelial cells. Cytokeratins are categorized into type I (acidic) and type II (basic to neutral) proteins, which interact to form heterodimeric pairs, contributing to the structural stability of the HFs (Jacques et al., 2005). Conversely, the hair-forming compartments, including the matrix, cortex, and cuticle, demonstrate complex expression patterns of hair keratins based on hair differentiation (Rogers, Winter, Langbein, Krieg, & Schweizer, 1996). Hair keratins are classified into two main types based on their isoelectric points (pI): acidic keratins and basic to neutral α-keratins. These keratins form heterodimers in a 1:1 ratio, creating microfilaments with a width of 7–11 nm (Rogers et al., 1996). Acidic α-keratins, known as type I keratins, include 11 types (the numbers 31–40; KRT31-40). Basic to neutral keratins, called type II keratins, consist of six types (the numbers 81–86; KRT81-86) (Schweizer et al., 2006). Among these, KRT32 (type I) and KRT85 (type II) are predominantly found in the hair cuticle layer, while KRT31, 35 (type I), KRT81, 83, 85, 86 (type II) are expressed in the hair cortex layer (Langbein, Rogers, Winter, Praetzel, & Schweizer, 2001). The hHFs comprise epidermal and dermal layers. The epidermal layer is further divided into the ORS and IRS, where epithelial cytokeratins, including keratin 17 (type I), are predominantly expressed (Langbein, Yoshida, Praetzel-Wunder, Parry, & Schweizer, 2010).

Hair lipids prevent hair breakage and thinning by protecting against environmental and chemical damage. Furthermore, they serve as a protective barrier against moisture loss while improving the gloss, elasticity, and tensile strength of the HSs. Hair lipids are characterized as exogenous or endogenous. Exogenous and endogenous lipids originate from sebaceous glands and hair matrix cells, respectively (Luisa Coderch, Alonso, García, Pérez, & Martí, 2023). Exogenous lipids comprise free fatty acids (FFA), triglycerides, cholesterol (CH), wax esters, and squalene (SQ). Endogenous capillary lipids include FFA, CH, ceramides (CER), glucosylceramides, cholesterol sulfate (CS), and 18-methyleicosanoic acid (18-MEA) (L Coderch et al., 2017). Among them, 18-MEA is the sole component chemically bound to the cuticle surface through a thioester bond, enhancing hair gloss by contributing to the smoothness and reflectivity of the hair surface (Tokunaga, Tanamachi, & Ishikawa, 2019). The presence of 18-MEA is crucial in preserving the structural integrity of the hair cuticle, which is essential for overall hair health. Depletion of 18-MEA owing to environmental factors or grooming practices decreases hair gloss, increases surface friction, and heightens susceptibility to dryness and damage (Tanamachi, Tokunaga, Tanji, Oguri, & Inoue, 2010). Therefore, maintaining adequate 18-MEA levels is crucial for healthy, gloss, and resilient hair.

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) enables mass-specific quantitative analysis of constituents, such as bound water, amino acids, fatty acids, and SQ, using high chemical selectivity and surface sensitivity for small samples (10–100 mg) or areas (10–100 μm^2) by analyzing the inner cortex or surface cuticle of a single hair strand (Lhoest, Wagner, Tidwell, & Castner, 2001). Fourier transform infrared (FT-IR) spectroscopy is used to quantitatively measure amino acids and chemical bonds in hair cross-sections to assess the density and internal composition of the hair (K. S. Kim & Park, 2013).

Collagen, a key extracellular matrix (ECM) component, is found in the skin, hair, nails, joints, bones, tendons, and cartilage (M. Lee, Kim, Ahn, Son, & Lee, 2023). Recent studies have highlighted the efficacy of collagen peptides in promoting hair growth and improving hair quality. Oral supplementation with Bioactive Collagen Peptides (BCP) has been shown to significantly increase hair thickness and stimulate follicle cell proliferation (Oesser, 2020). Similarly, collagen peptides derived from fish, particularly Mozambique tilapia, have been found to enhance hair regrowth and promote human dermal papilla cells (hDPCs) proliferation by modulating key signaling pathways, including Wnt/β-catenin and BMP (Hwang, Park, & Lee, 2022). Moreover, low molecular weight collagen peptides have demonstrated the ability to promote hair growth by activating the Wnt/GSK-3β/β-catenin signaling pathway (Y. Kim et al., 2024). In clinical research, oral intake of hydrolyzed collagen has shown promise in improving hair conditions, with studies reporting increased hair mechanical resistance and beneficial effects on hair loss in individuals with androgenetic alopecia or alopecia areata (Campos, de Melo, Shirata, & Leite, 2022; Milani, Colombo, & GFM-O-Trial Investigators Group, 2023).

AP collagen peptides (APCPs) are food ingredients made of enzymatically decomposed collagen peptides that are rich in tripeptides, including glycine-proline-hydroxyproline. According to previous research, APCPs stimulate hDPCs proliferation, enhance keratin expression, and activate the GSK-3β/β-catenin signaling pathway, which plays a crucial role in transitioning hair follicles from the anagen to the telogen phase, thus prolonging hair growth (Lee et al., 2024). In addition, APCPs confer protection to hDPCs against oxidative stress and elevate the mRNA expression levels of keratins 32 and 42 in hHF keratinocytes (Shim et al., 2024). These studies collectively underscore the potential of collagen peptides in improving hair growth, thickness, and strength through mechanisms such as cell proliferation, keratin synthesis, and the modulation of critical signaling pathways.

In this study, to thoroughly investigate the effects of APCPs on hair elasticity and gloss, we employed fluorescence imaging and quantitative measurements to analyze the keratin, amino acids, fatty acids, moisture levels, and chemical bonds in APCPs-treated hHFs that influence hair elasticity and gloss.

2. Materials and methods

2.1. APCPs preparation

APCPs were produced using enzymatically degrading collagen from the scales of golden threadfin bream (*Nemipterus virgatus*). APCPs contained at least 15 % glycine -X-Y tripeptides (X and Y are often occupied by a proline, hydroxyproline, or alanine), including 3 % glycine-proline-hydroxyproline.

2.2. Human hair follicle (hHF) organ culture

All hHFs were obtained from Dankook University Hospital (IRB approval numbers: DKUH 2021-12-005-003 and DKUH 2021-12-025), with written informed consent obtained from all patients. The isolated anagen follicles were cultured in 500 μL of Williams E medium (Gibco, Grand Island, NY, USA) in 24-well plates at 37 °C and 5 % CO_2 for 24 h. Next, to measure cuticle surface roughness and the expression of keratin, the hHFs were cultured with APCPs (0, 25, or 50 μg/mL) for 7 days. To analyze the amino acid content, chemical bonds, and the types and quantities of elements and molecules on the hair surface, hHFs were cultured with APCPs (0 or 30 μg/mL) for 10 days. Images of the hHFs were captured using a confocal microscope (LSM 880, Zeiss AG).

2.3. Hair shaft sample preparation

Hair samples (4–8 mm) were isolated from hHFs cultured *ex vivo* for 10 days using fine forceps and prepared as samples for analysis. For ToF-SIMS analysis of hair surface fatty acid composition, the samples were attached to a SIMS assay plate with a double-sided carbon tape. Hair samples for FT-IR analysis of chemical bonding were prepared by immersing approximately 10 strands of hair in an OCT embedding medium (Sakura Finetek, CA, USA), freezing at −20 °C, making 40 μm thick cryosections, and mounting them on glass slides. For ToF-SIMS analysis of hair components, samples were similarly immersed in OCT embedding medium (Sakura Finetek, CA, USA) in 10 hair strands, frozen at −20 °C, cry sectioned at 40 μm, and mounted on SiO_2 wafers.

2.4. ToF-SIMS analysis

ToF-SIMS measurements were conducted using a ToF-SIMS V instrument (ION-TOF GmbH, Münster, Germany) at the Sungkyunkwan University (SKKU) Cooperative Center for Research Facilities with a focused 25 keV Bi3++ primary ion beam. The hair surface and cross-section were scanned with primary ions emitted at 25 keV and an incidence angle of 45° over an area of 50x50 μm with PI doses ranging from 1.1E + 09 to 1.2E + 09. The mass of secondary ions, released as anions after primary ions struck the hair cuticle or cortex surface, was measured to analyze the types, and amounts of elements and molecules comprising the hair surface. Several studies have focused on the identification and quantitative comparison of amino acids, fatty acids, and squalene in human hair using TOF-SIMS analysis (Amaya, Sweedler, & Clayton, 2011; Kezutyte, Desbenoit, Brunelle, & Briedis, 2013; Lhoest et al., 2001; Okamoto & Aoyagi, 2016; Okamoto et al., 2012; Siljeström, Parenteau, Jahnke, & Cady, 2017).

2.5. High-Resolution FT-IR analysis

To investigate the chemical bonds within the cortex inside of a single hair strand, FT-IR spectra were obtained with high-resolution vacuum FT-IR spectroscopy (Vertex-80v/Hyperion2000, Bruker, MA, USA) at the National Instrumentation Center for Environmental Management (NICEM) at Seoul University. The hair sample was positioned in a vacuum for analysis, focusing on the hair cortex segment. The FT-IR spectra ranged from 4000–650 cm^{-1} at a resolution of 0.06 cm^{-1} using a deuterated L-alanine-doped triglycine sulfate detector (DLATGS). Experimental studies conducted on FT-IR spectroscopic analysis on human hair were referenced to identify chemical functional groups associated with specific wavenumbers and vibrations (Aziz, Jaleeli, & Ahmad, 2017; Bantignies et al., 2000; Kim & Park, 2013).

2.6. Hematoxylin and Eosin (H&E) staining

The hHFs were fixed in 10 % formalin, embedded in paraffin, cut into sections stained with hematoxylin and eosin (H&E), photographed with a slide scanner (Pannoramic MIDI; 3DHISTECH Ltd, Budapest, Hungary), and analyzed using Case Viewer software. The hHF count was performed on a cropped image within a standardized area (1 × 1 mm).

2.7. Immunocytochemistry (ICC)

The hHFs were fixed in 10 % Neutral Buffered Formalin (NBF), paraffin-embedded, and sectioned. Subsequently, the paraffin was removed with xylene, followed by ethanol treatment and hydration. To inhibit peroxidase activity, a 10-min reaction with 3 % hydrogen peroxide was followed by four washes with 1X Tris-buffered saline containing 0.1 % Tween-20 (TBS-T). To reduce non-specific reactions, incubation with Ultra V Block was performed at room temperature for 1 h, followed by overnight incubation with keratin 17 (Abcam, Cambridge, UK), keratin 31 (Invitrogen, MA, USA), keratin 85 (Biorbyt, NC, USA), keratin 86 (Biorbyt, NC, USA) or Hair keratin type I and II (Progen, Heidelberg, Germany) primary antibodies at 4 °C. The sections were incubated with anti-rabbit or anti-goat IgG-FITC secondary antibodies (Santa Cruz Biotechnology, CA, USA) in the dark at room temperature (RT) for 1 h after washing with PBS. Cell nuclei were counterstained with 4′,6-Diamidino-2-Phenylindole, dihydrochloride (DAPI) (Immuno Bioscience Corp., WA, USA) and observed using confocal microscopy (LSM 880, Zeiss, Oberkochen, Germany).

2.8. Measurement of cuticle surface roughness

The hHFs were cultured with APCPs (0, 25 or 50 μg/mL) for 7 days. A scanning electron microscope (SEM; JEOL FESEM J-7100) was used to capture magnified images to study hair shape and structure. Hair surface roughness was assessed using the Image J 1.52 program (NIH, Bethesda, MD, USA) to calculate the average roughness (Ra).

2.9. Cell culture

The hDPCs were obtained from Kyoungpook university. hDPC growth medium (Promocell, Heidelberg, Germany) was prepared with fetal calf serum (0.04 mL/mL), bovine pituitary extract (0.004 mL/mL), basic fibroblast growth factor (1 ng/mL), insulin (5 μg/mL), and 1 % Gibco™ penicillin–streptomycin (Thermo Fisher Scientific, Waltham, MA, USA). hDPCs were cultured in a humidified incubator at 37 °C with 5 % CO_2.

2.10. Preparation of libraries for mRNA sequencing

For the transcriptome analysis, hDPCs were each seeded in 100 mm dishes (Corning Inc., NY, USA) at a density of 1 × 10^6 cells/dish and cultured for 24 h. Afterward, hDPCs were treated with 30 μg/mL of APCPs and incubated for an additional 3 h, while the non-treated control was treated with an equivalent volume of solvent (DPBS). The cells were then collected in RNA later RNA stabilization reagent (QIAGEN, Hilden, Germany) and mRNA were extracted. Six different libraries for mRNA sequencing were prepared using the TruSeq Stranded mRNA LT Sample Prep Kit according to the manufacturer's instructions, and paired-end sequencing was performed using Illumina's Nova-Seq 6000 system (Macrogen, Seoul, Korea).

2.11. Transcriptome analysis and Gene Ontology (GO) term enrichment analysis

Trimmed reads were mapped with reference genome(GRCh38) using HISAT2 (Hierarchical indexing for spliced alignment of transcripts 2, Maryland, USA). After mapping, transcript assembly was performed using StringTie (V2.2.0, Maryland, USA). Transcript abundance was then estimated based on the number of reads and normalized values, FPKM (Fragments Per Kilobase of transcript per Million mapped reads), and TPM (Transcripts Per Million kilobases). Differential expression analysis between APCP-treated hDPCs (30 μg/mL) and not treated hDPCs was conducted using the DESeq2 package implemented in GENAVi. DEGs were identified based on P-values <0.05 and log2 fold changes greater than 2. A volcano plot was generated using the DESeq package in R Studio to visualize the distribution of gene expression. To investigate the biological function of APCPs in hDPCs, we performed Reduce and Visualize Gene Ontology (RRVGO) analysis using the Macrogen DEG Viewer program and generated a Similarity Heatmap, Treemap, and Scatter plot for each cell type. Enriched GO term groups were identified according to biological processes, cellular components, and molecular functions using the GO database and the NCBI GEO dataset (https://www.ncbi.nlm.nih.gov/gds/).

2.12. Statistical analysis

All data are presented as mean ± standard deviation (SD) from at least three independent experiments. Data were analyzed using one-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test. All statistical analyses were conducted using GraphPad Prism 7.0 (GraphPad Software Inc., CA, USA). Results with a p-value of < 0.05 were considered statistically significant ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$).

3. Results

3.1. APCPs increase amino acid content in the hHFs

For hair to exhibit elasticity, it requires density and sufficient proteins and amino acid components. Hair cross-sectional samples were

Table 1
Effect of APCPs on amino acid content in hHFs. ToF-SIMS was used to measure the increase in amino acid content in the internal cortex of human hHFs treated with APCPs (0 or 30 μg/mL) for 10 days.

Amino acid	Molecular weight (MW)	Peak assignment	Center mass (u)	APCPs (μg/mL)	
				0	30
Proline	115.1	$C_4H_6N^-$	68.02	11,837	16,553*
Threonine	119.1	C4H5O-	69.01	17,187	74,993*
Cysteine	121.2	CHS^-	45.00	15,210	43,902*
Lysine	146.2	$C_5H_{10}N^-$ $C_5H_{12}N^-$	84.01	23,763	26,771*
Phenylalanine	165.2	C8H10N-	120.05	3,218	6,438*
Tyrosine	181.2	C7H7O-	107.05	4,401	7,664*
		C8H10NO-	136.04	3,961	9,051*
Arginine	174.2	CH_3N_2-	43.03	54,511	100,955*

** $p<0.01$, * $p<0.05$.

prepared and quantitatively analyzed for amino acid content using ToF-SIMS. Proteins consist of 21 different α-amino acids categorized as electrically charged, polar uncharged, hydrophobic nonpolar, and, in special cases, amino acids based on their side chains. ToF-SIMS analysis can be used to identify and measure seven amino acids, including proline, threonine, cysteine, lysine, phenylalanine, tyrosine, and arginine, whose contents were statistically significantly increased in hair treated with 30 μg/mL of APCPs (Table 1).

3.2. APCPs enhance the strength of chemical bonds in the hHFs

The strength of chemical bonds between hair components affects hair tensile strength and elasticity, typically showing a positive correlation. In this study, FT-IR was used to demonstrate that chemical bond strength increased in APCPs-treated hair compared to the untreated group (Fig. 1). Hair keratin has a high cysteine content compared to skin keratin, and the strength of cysteine bonds and disulfide bonds correlates with hair tensile strength, potentially enhancing hair elasticity (Deb-Choudhury, 2018; Harland et al., 2022). APCPs-treated hair exhibited an increase in S=O bonds, cysteine, amide I, III band which comprise C=O stretching, and amide II corresponding to C-N stretching and N–H bending (Table 2). The intensity of 2658 cm^{-1}, corresponding to the CH symmetric stretching vibration in fatty acid or lipid acyls, was measured to be statistically significantly increased in hair treated with 30 μg/mL APCPs.

3.3. APCPs enhance keratin expression in the hHFs

After treating hHFs with APCPs (25 or 50 μg/ml) for 7 days, the thickness of the hHFs increased (Fig. 2A). Subsequently, to determine whether APCPs enhance hair elasticity, key regulators of hair elasticity – Keratin type I+II, K17, K85, K31, and K86-were examined in APCPs-treated hHFs. Hair keratin Type I and II are expressed throughout the entire hair. K17 is expressed in the IRS and ORS; K31 is expressed in the cortex; K85 in the cuticle and cortex; and K86 in the cortex (Langbein et al., 2001). The expression of K17, K85, K86, and hair keratin Types I and II increased in response to APCPs treatment. Additionally, in untreated hHFs, K31 was observed in zone 2 above the hair bulb within the cortex and medulla. However, with APCPs treatment, K31 expression was detected starting from zone 1 in the hair bulb, encompassing the cortex, medulla, and cuticle (Fig. 2B–F). These findings suggest that APCPs enhanced HS elasticity by increasing keratin expression.

3.4. APCPs enhance hair surface fatty acids, including 18-MEA, to improve gloss

The outermost layer of the hair cuticle, the epicuticle, was analyzed using ToF-SIMS to quantify the fatty acids comprising the hair surface of APCPs-treated hHFs. The methyleicosanoic acid (18-MEA) content in untreated hair was 19,106 statistically significantly to 28,326 in APCPs-treated hair. Lauric acid (C12:0), oleic acid (C18:1), 11,13-eicosenoic acid, behenic acid (C22:0), 18-MEA and squalene (SQ) statistically significantly increased in APCPs-treated hHFs (Table 3).

3.5. APCPs enhance the surface smoothness of hHF

To assess whether APCPs enhance hair glossiness, the surface

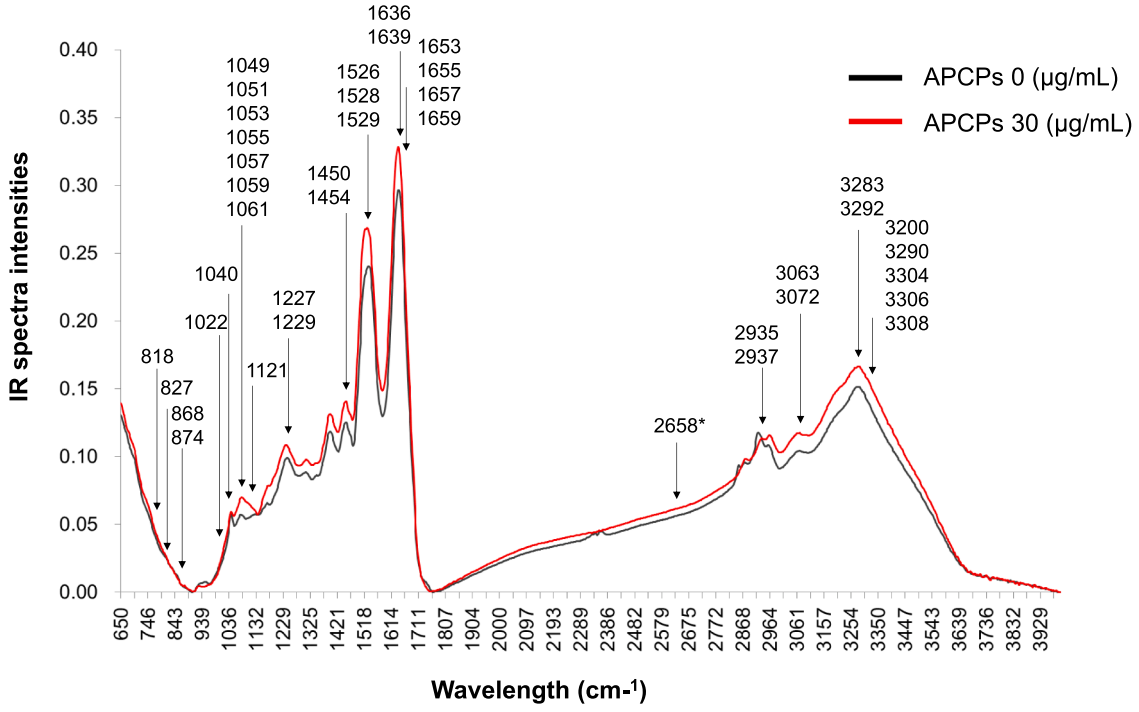

Fig. 1. Chemical bonding changes in hHFs-treated with APCPs. hHFs (10 hair follicles/group) were treated with APCPs (0 or 30 μg/mL) for 10 days. High-resolution FT-IR was used to measure the increase in chemical bonds, specifically within the cortex of hHFs treated with APCPs.

Table 2
Effect of APCPs on chemical bonds between hair constituents in hHFs. High-resolution FT-IR was used to measure chemical bond changes in the hair cortex of hHFs treated with treated APCPs (0 or 30 μg/mL) for 10 days.

Wave number (cm^{-1})	Assignment	APCPs (μg/mL)	
		0	30
1173	C-O, C–C, C-N, stretching C-O–H, C-O-C	0.06596	0.07818
1174	deformation of carbohydrates, C–H and $=CH_2$	0.06575	0.07862
1196	S=O thiosulfate ions	0.07026	0.08489
1188	S=O sulphonate	0.06640	0.08128
1053	Cystine	0.04939	0.05615
1055		0.04895	0.05661
1057		0.04905	0.05738
1059		0.04944	0.05837
1061		0.05020	0.05952
1121	Cystine dioxide	0.05677	0.06186
1227	Amide III, complex vibration contains N–H	0.09162	0.10434
1229	bending, C-N stretching, C=O stretching,	0.09301	0.10542
1238	and O=C=N bending	0.09850	0.10859
1450	CH2, CH3 asymmetric bending modes of	0.12495	0.14052
1454	lipids, proteins	0.12529	0.14071
1526	Amide II, C-N stretching/N–H bending	0.23701	0.26806
1528		0.23849	0.26842
1529	Amide II peak region – protein NH, (C-N), NO_2 bond in nitro compounds, carboxylic acids and derivatives	0.23945	0.26841
1636	H-OH bending mode of water, NO_2 bond in	0.29331	0.32673
1639	nitro compounds, Amide I band components β region pleated structures confirmation of proteins, C=C, C=N, (υC=C), NH_3 in υas and υ	0.29665	0.32824
1653	Amide I, 80 % C=O stretch and a small	0.27212	0.29298
1655	contribution from NH bend	0.25375	0.27476
1657		0.24409	0.26621
1659		0.23623	0.25912
2658	C–H symmetric stretching of CH_2 in fatty acids, symmetric stretching vibrations of lipid acyl CH_2 groups	0.05783	0.063278*
3072	C–H, CH_2, C=C alkenes	0.10444	0.11751
3283	O–H carboxylic acids and derivatives,	0.15142	0.16660
3292	alcohols and phenols	0.14908	0.16400
3063	Amide A, N–H stretching	0.10413	0.11728
3200		0.13028	0.14770
3290		0.14981	0.16478
3304		0.14501	0.16019
3306		0.14411	0.15927
3308		0.14317	0.15848

* $p < 0.05$.

roughness of hair was analyzed using SEM imaging (Fig. 3A). APCPs reduced the average roughness (Ra) of hHFs (Fig. 3B). This indicates that APCPs decreased the unevenness and elevation of the hair cuticle, resulting in a more uniform and smoother surface.

3.6. APCPs increase the content of bonded water in the hHFs

Moisture within hair can be categorized into external and internal water based on its location (cuticle or cortex) and thermodynamic properties (evaporation at 65 °C or transpiration at 180 °C) (Barba, Méndez, Martí, Parra, & Coderch, 2009). External moisture content in hair changes significantly with the relative humidity of the environment, while internal moisture content remains largely unaffected (Robbins & Robbins, 2012). Internal water, hydrogen-bonded with the amine groups of amino acids, is found in the inter-lamellar space between the hair cortex intermediate filaments and contributes to hair softness, flexibility, and elasticity (Pauling, 1945; Wang, Yang, McKittrick, & Meyers, 2016). This internally bonded water within the hair shaft is identified as –OH due to hydrogen bonding with amino acids. This bonding can be quantified either by measuring the center mass at 17.003 using ToF-SIMS analysis or by analyzing the 3400 cm^{-1} wavenumbers through FT-IR analysis (Bantignies et al., 2000; Deb-Choudhury, 2018). In the ToF-SIMS analysis of the hair shaft (Table 4A), the average intensity of bonded water (OH–) in the cortex of the untreated hair group was 136,593, while in the cuticle layer, it averaged 205,172. In contrast, hair shafts from follicles treated with 30 μg/mL of APCPs showed statistically significantly increased average intensities, with the cortex-bonded water measuring 273,165 and the cuticle-bonded water measuring 246,292. These findings suggest that APCPs treatment enhances the amount of bonded water in the hair. Similarly, in the FT-IR experiments conducted on the hHFs (Table 4B), the intensity of the 3400 cm^{-1} wavenumber, indicative of bonded water (–OH), averaged 0.11440 in the cortex and 0.06374 in the cuticle layer of the untreated hair group. Conversely, hair shafts from hHFs treated with 30 μg/mL of APCPs exhibited increased average intensities, with cortex-bonded water measuring 0.11944 and cuticle-bonded water measuring 0.09858. These two results indicate a trend of increasing bonded water especially in hair cortex statistically significantly increased following APCPs treatment. The content of external moisture can be compared by analyzing the 3774 cm^{-1} value in FT-IR experiments (Aziz et al., 2017). In the untreated hHFs, the intensity of 3774 cm^{-1} wavenumbers, indicative of water (H_2O) in the cortex was 0.00974, but it increased to 0.01077 in hHFs treated with 30 μg/mL of APCPs, which is statistically significant (Table 4C).

4. Discussion

The composition of chemical bonds and keratin content are critical factors in regulating the elasticity and glossiness of the human hair shaft (hHS) (Wolfram, 2003). Notably, keratin 86 and keratin-associated proteins, which significantly contribute to hair structure, have been reported to decrease with aging, leading to diminished hair elasticity and gloss (Plott et al., 2020). The decline in these properties is attributed to reductions in hard keratin proteins, cysteic acid, hair surface ceramides, and fatty acids, particularly 18-MEA (Thibaut et al., 2010).

In this study, AP collagen peptides (APCPs) demonstrated a capacity to enhance hair elasticity and gloss by modulating several critical hair components. APCPs significantly increased the content of essential amino acids, such as proline, threonine, cysteine, lysine, phenylalanine, tyrosine, and arginine, within the hair shaft (Table 1). These amino acids are integral to protein structures that influence hair elasticity, highlighting APCPs' potential to counteract age-related declines in hair quality.

The study further revealed that APCPs treatment led to an enhancement in the strength of chemical bonds within the hair, as evidenced by FT-IR analysis (Fig. 1 and Table 2). Specifically, the increased presence of S=O bonds, cysteine, and amide bands (I and III) correlated with stronger disulfide bonds and higher hair tensile strength, factors directly linked to improved hair elasticity (Harland et al., 2022). The statistically significant rise in the intensity of the 2658 cm^{-1} wavelength, associated with CH symmetric stretching vibrations in fatty acid

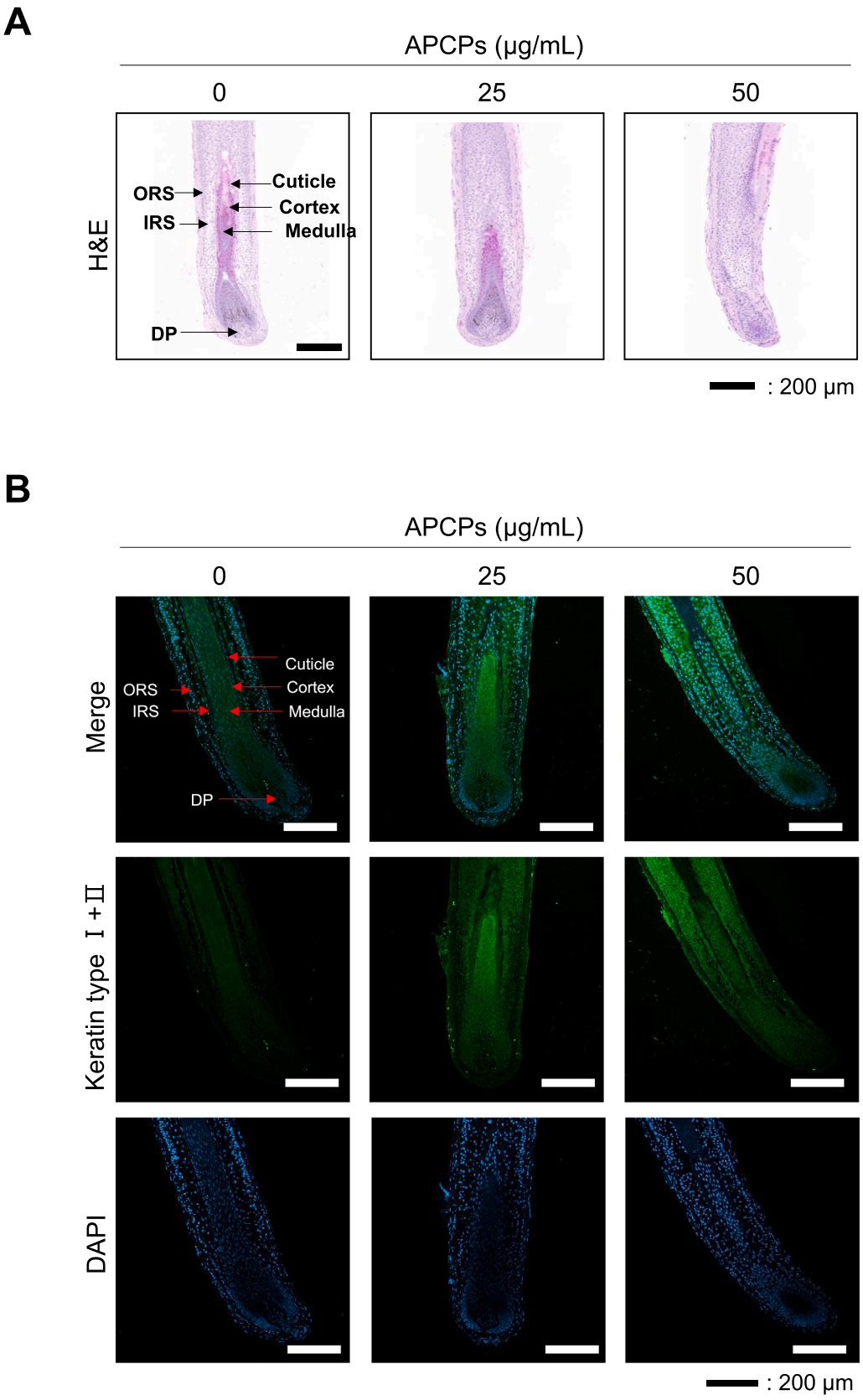

Fig. 2. Effect of APCPs on hair keratin expression in hHFs organ culture model. hHFs (8 hair follicles/group) were treated with APCPs (0, 25, or 50 μg/mL) for 7 days. (A) H&E staining of hHFs. (B-F) IF staining of hHFs for hair keratin Type I and II, K17, K85, K86, and K31 on 7 days. (Scale: 200 μm).

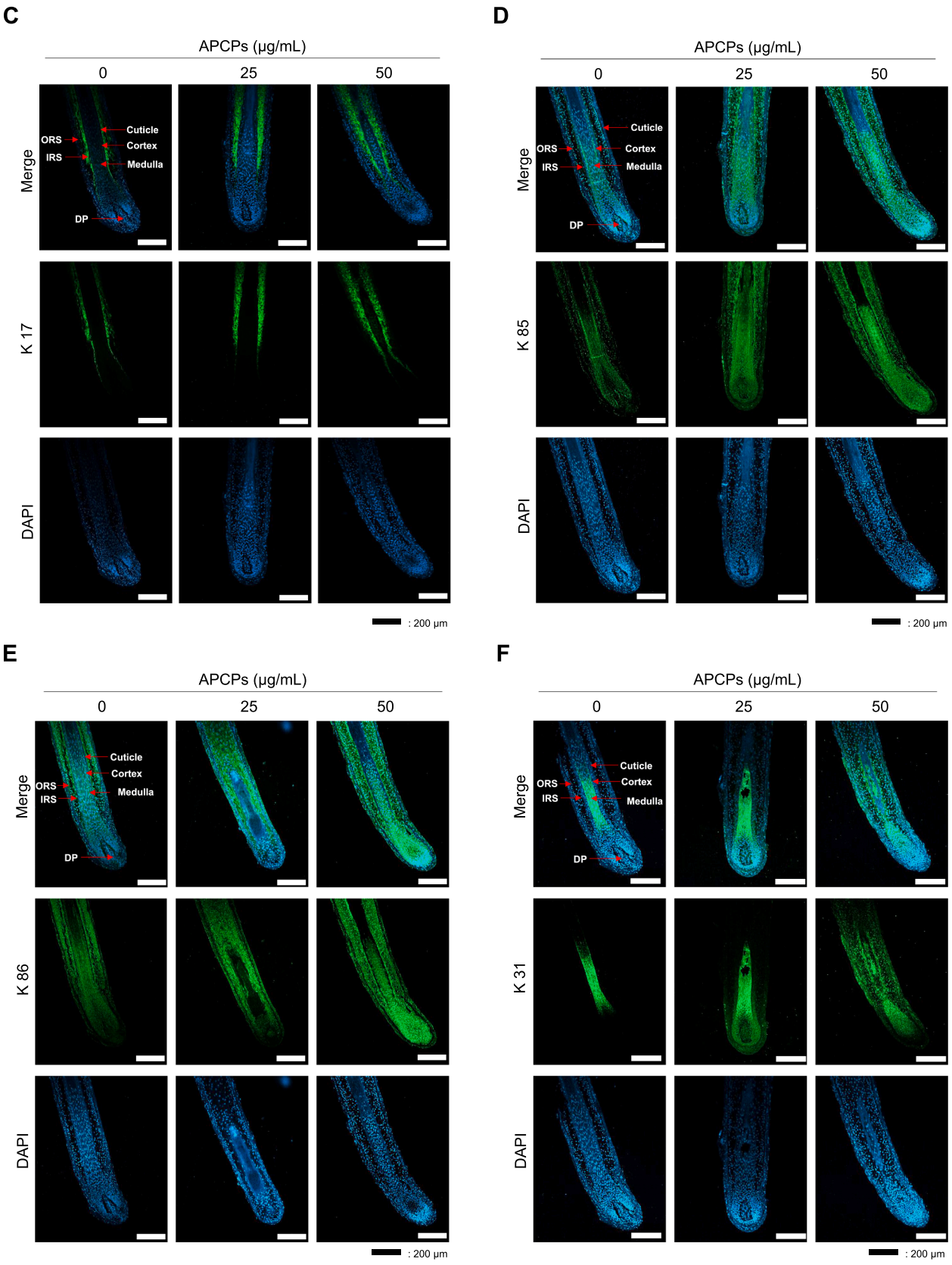

Fig. 2. (*continued*).

or lipid acyls, further supports the positive impact of APCPs on hair structure and function.

Keratin expression is another pivotal factor in hair health. APCPs treatment enhanced the expression of key keratins, including K17, K31, K85, and K86, which are involved in the structural integrity of the IRS, ORS, cortex, and cuticle (Fig. 2). Specifically, through the IF analysis,

Table 3
Effect of APCPs on Fatty acid, 18-MEA, and Squalene content in hair shaft. ToF-SIMS analysis was used to measure the Fatty acid, 18-MEA, and squalene contents in the outer cuticle of hHFs treated with APCPs (0 or 30 μg/mL) for 10 days.

Lipid		Chemical Formulas	Molecular weight (MW)	Peak Assignment	Center mass (u)	APCPs (μg/mL)	
						0	30
Lauric acid	C12:0	$C_{12}H_{24}O_2$	200.32	$C_{12}H_{23}O_2$-	199.12	2,317	4,485**
Palmitoleic acid	C16:1	$C_{16}H_{30}O_2$	254.414	$C_{16}H_{29}O_2$-	253.22	5,638	12,704
Palmitic acid	C16:0	$C_{16}H_{32}O_2$	256.43	$C_{16}H_{33}O_2$-	255.23	14,191	30,136
Linoleic acid	C18:2	$C_{18}H_{32}O_2$	280.452	$C_{18}H_{31}O_2$-	279.24	5,689	6,873
Oleic acid	C18:1	$C_{18}H_{34}O_2$	282.468	$C_{18}H_{33}O_2$-	281.25	11,326	35,348*
17-Hydroxy linolenic acid	C18:3	$C_{18}H_{30}O_3$	294.465	$C_{18}H_{29}O_3$-	293.25	2,044	3,116
11, 13-eicosenoic acid	C20:1	$C_{20}H_{38}O_2$	310.51	$C_{20}H_{37}O_2$-	309.24	2,005	3,198*
Erucic acid	C22:1(n-9)	$C_{22}H_{42}O_2$	338.6	$C_{22}H_{40}O_2$-	337.21	1,497	1,772
Behenic acid, DHA	C22:0	$C_{22}H_{44}O_2$	340.6	$C_{22}H_{43}O_2$-	339.26	1,660	4,762***
Methyleicosanoic acid	18-MEA	$C_{21}H_{42}O_2$	326.6	$C_{21}H_{41}O_3$-	341.24	19,106	28,326*
Tricosanoic acid	C23:0	$C_{23}H_{46}O_2$	354.6	$C_{23}H_{45}O_2$-	353.28	1,527	2,128
Cholesterol	C27-steroid	$C_{27}H_{46}O$	383.3	$C_{27}H_{45}O$-	383.31	1,392	1,895
7-dehydrocholesterol	7-DHC	$C_{27}H_{44}O$	384.6	$C_{27}H_{43}O$-	383.31	1,392	1,895
Squalene	SQ	$C_{30}H_{50}$	410.73	$C_{30}H_{47}$-	407.31	1,344	1,612*
Cholesterol Sulfate	Chol-sulf	$C_{27}H_{46}O_4S$	466.72	$C_{27}H_{45}SO$-	465.31	21,438	25,681
Mycocerosic acid	C31	$C_{31}H_{62}O_2$	466.8	$C_{31}H_{61}O_2$-	465.34	7,208	9,845

** $p < 0.01$, * $p < 0.05$.

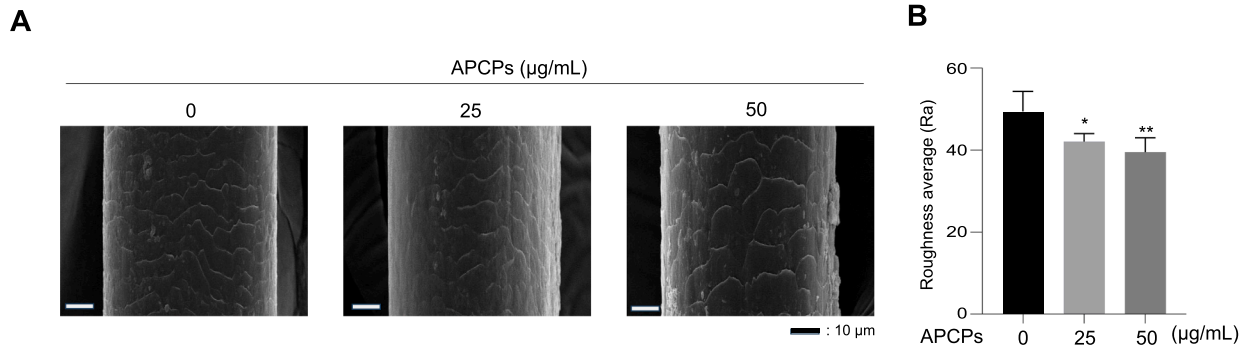

Fig. 3. Effect of APCPs on hair shaft roughness in hHF. hHFs (8 hair follicles/group) were treated with APCPs (0, 25, or 50 μg/mL) for 7 days. SEM image and Ra graph of hHFs on day 7 are shown. Scale: 10 μm.

Table 4A
Effect of APCPs on bonded water in hHFs. ToF-SIMS analysis was used to measure the bonded water content in the cortex and cuticle layers of hHFs treated with treated APCPs (0 or 30 μg/mL) for 10 days.

(A) ToF SIMs Bonded water in the hHFs						
Bonded water	Chemical formula	Molecular weight (MW)	Peak assignment	Center mass (u)	APCPs (μg/mL)	
					0	30
Internal, hair cortex	OH^-	17.007	OH^-	17.00332	136,593	273,165**
Outermost, hair cuticle				17.00332	205,172	246,292

** $p < 0.01$, * $p < 0.05$.

Table 4B
Effect of APCPs on bonded water in hHFs. High-resolution FT-IR analysis was used to measure the bonded water content in the cortex and cuticle layers of. hHFs treated with treated APCPs (0 or 30 μg/mL) for 10 days.

(B) FT-IR Bonded water in the hHFs					
Bonded water	Chemical formula	Wave number (cm^{-1})	Assignment	APCPs (μg/mL)	
				0	30
Internal, hair cortex	–OH	3400	–OH	0.11440	0.11944*
Outermost, hair cuticle				0.06374	0.09858

Table 4C
Effect of APCPs on water in hHFs. High-resolution FT-IR analysis was used to measure the bonded water content in the cortex and cuticle layers of. hHFs treated with treated APCPs (0 or 30 μg/mL) for 10 days.

(C) FT-IR Water in the hHFs					
Water	Chemical formula	Wave number (cm^{-1})	Assignment	APCPs (μg/mL)	
				0	30
Internal, hair cortex	H_2O	3774	H_2O	0.00974	0.01077**
Outermost, hair cuticle				0.01020	0.01118

the alteration in K31 expression, observed in the control group from zone 2 (above the hair bulb) to zone 1 (within the hair bulb) in APCPs-treated hair, suggests that APCPs influence not only the quantity but also the spatial distribution of keratins, potentially contributing to enhanced hair elasticity. This shift indicates a more comprehensive involvement of keratins in the hair's structural layers, enhancing its overall resilience.

Moreover, APCPs were found to significantly impact the regulation of genes involved in hair growth and health. mRNA sequencing and differential expression gene (DEG) analysis revealed upregulation of genes such as PCDHB7, ADAP1, DRICH1, MMP13, KCGRG, PILRB, WNT2B, TAS2R10, FLG, and GSDMB (Supplementary Table 1). These genes are associated with various functions, including sulfotransferase activity, which is crucial for hair shaft formation, and Wnt signaling, which is pivotal for hair follicle development and hair cycle regulation. The observed increase in Wnt family member 2B expression, alongside decreased BMP4 expression, supports the idea that APCPs promote hair growth by modulating these key signaling pathways. The confirmed increase in FGF expression further highlights APCPs' role in enhancing HFs health and growth.

In addition to keratin and amino acid content, hair surface lipids play a crucial role in maintaining hair gloss. The ToF-SIMS analysis indicated that APCPs treatment significantly increased the levels of 18-MEA, lauric acid, oleic acid, and squalene on the hair surface (Table 3). These lipids are vital for preserving the hydrophobic nature of the hair surface, which is essential for maintaining gloss and preventing tangling (Tokunaga et al., 2019). The increased lipid content, particularly 18-MEA, likely contributed to the observed reduction in cuticle surface roughness, as evidenced by SEM imaging (Fig. 3). This smoother surface directly correlates with increased hair gloss. The ability of APCPs to enhance the content of bonded water within the hair shaft further underscores its potential benefits for hair elasticity and gloss. ToF-SIMS and FT-IR analyses both showed significant increases in bonded water content in the cortex and cuticle layers of APCPs-treated hair (Table 4).

The findings from this study suggest that APCPs have a multi-faceted impact on hair health, enhancing both the structural integrity and aesthetic properties of hair through increases in amino acids, keratins, lipids, and bonded water. The improvement in these components supports the hypothesis that APCPs can counteract age-related and chemically induced declines in hair elasticity and gloss. However, while these results are promising, they are derived from ex vivo models, and further in vivo studies are necessary to confirm the clinical relevance of these findings in real-world hair care applications.

5. Conclusion

This study suggests that APCPs may enhance hair shaft elasticity and gloss by increasing the expression of key keratins (17, 31, 85, 86) and essential components like amino acids, bonded water, and lipids. Fluorescent staining showed a rise in major keratins, while ToF-SIMS and FT-IR analyses indicated increases in amino acids and bond strength (S=O, C=O, cystine, C–N, C–H). Additionally, SEM analysis revealed a significant reduction in hair cuticle roughness. By directly examining human hair follicles, these findings indicate the possibility of APCPs playing a role in improving hair texture, which could be explored further in future research and clinical applications.

Availability of data and materials

All hHFs were obtained from Dankook University Hospital (IRB approval numbers: DKUH 2021-12-005-003 and DKUH 2021-12-025), with written informed consent obtained from all patients.

CRediT authorship contribution statement

Ahreum Kim: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Yujin Kim:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Jung Min Lee:** Investigation, Data curation. **Jin-Oh Chung:** Investigation, Data curation. **Jong Hwa Roh:** Investigation, Data curation. **Jung Ok Lee:** Writing – review & editing. **Beom Joon Kim:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106534.

Data availability

All the data generated and analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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