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A novel cell-penetrating peptide supports hair follicle growth through anti-inflammatory and growth factor–associated mechanisms in preclinical models

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Abstract

Background Hair loss remains a prevalent condition for both men and women worldwide. Current therapies for this condition, including minoxidil and finasteride, are limited by inconsistent efficacy, side effects, and dependence on continuous use. Cell-penetrating peptides (CPPs) have emerged as promising bioactive agents in dermatology due to their ability to traverse cell membranes and modulate multiple biological pathways relevant to hair regeneration.

Objective This study aimed to identify and evaluate a novel synthetic CPP, DualPep-ALO, for its potential to promote hair growth by restoring the follicular microenvironment and stimulating key regenerative pathways.

Methods Using the intra-dermal delivery technology platform (REMEDI), over 200 human protein–derived peptides were screened for cell-penetrating potential. The lead candidate, DualPep-ALO, was selected based on its proliferative effect on human follicle dermal papilla cells and upregulation of Wnt/β-catenin and ERK signaling. In vitro and ex vivo assays were conducted to assess antioxidant enzyme activity, inflammatory cytokine suppression, follicle elongation, anagen maintenance, and growth factor expression.

Results DualPep-ALO significantly enhanced human follicle dermal papilla cell proliferation, restored antioxidant enzyme (SOD, CAT) activity, and suppressed pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, PGE-2). In ex vivo human scalp tissue, it promoted hair follicle elongation, maintained the anagen phase, and upregulated VEGF, HGF, and EGF expression, achieving outcomes comparable to minoxidil.

Conclusion DualPep-ALO demonstrates potent antioxidant, anti-inflammatory, and pro-regenerative effects, restoring follicular vitality through multimodal biological pathways. These findings support its potential as a novel CPP-based topical agent for hair loss, warranting further clinical investigation to validate efficacy, safety, and long-term therapeutic potential.

Keywords Cell-penetrating peptide (CPP), DualPep-ALO, Hair loss, Hair follicle regeneration, Antioxidants

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Introduction

Hair loss, or alopecia, is the absence of hair in its designated area, causing significant stress and lowering quality of life for patients. Common forms include androgenetic alopecia, telogen effluvium, and alopecia areata, each with multifactorial pathophysiology [1]. Despite differing etiologies, they share a few pathophysiologic mechanisms such as oxidative stress, hair cycle dysregulation, and impaired follicular keratinocyte and dermal papilla cell proliferation [2–4]. Moreover, excessive reactive oxygen species (ROS) and inflammatory cytokines secreted from macrophages and other immune cells [e.g., interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α)] can arrest the anagen phase, driving premature telogen and catagen phases, inducing apoptosis in the follicular microenvironment, leading to miniaturization and reduced hair density [2, 4, 5].

Current treatments for alopecia aim to restore follicular cycling and promote regrowth. Typically, minoxidil, regarded as the gold standard, enhances scalp microcirculation and vascular endothelial growth factor (VEGF) expression to prolong the anagen phase, while finasteride, a 5α-reductase inhibitor, prevents androgen-driven miniaturization by inhibiting dihydrotestosterone [6, 7]. Despite proven efficacy, both treatments are limited by inconsistent responses, the need for ongoing use, recurrence after discontinuation, and side effects such as scalp irritation and sexual or hormonal issues [6, 7]. These challenges underscore the need for novel, safe, and biologically compatible alternatives for long-term management.

Cell-penetrating peptides (CPPs) are short amino acid sequences that traverse cell membranes to deliver bioactive molecules intracellularly [8–10]. In dermatology, CPPs exhibit therapeutic potential by regulating collagen synthesis, modulating melanogenesis, and alleviating inflammation in atopic dermatitis. Notably, GHK-Cu promotes keratinocyte and fibroblast proliferation while enhancing dermal matrix remodeling [11]; DualPep-Shine, derived from Neurog1, suppresses melanogenesis and reduces melanin accumulation [12]; and RMSP1, a synthetic peptide with strong penetration and anti-inflammatory activity, shows efficacy in atopic dermatitis models [13]. Collectively, these findings highlight CPPs as versatile agents in regenerative medicine and cosmeceuticals, although their role in hair restoration remains largely unexplored.

As hair growth is regulated by follicular cell proliferation, redox balance, and inflammation, biomimetic peptides with high cell permeability targeting these pathways offer a promising therapeutic approach. This study investigates DualPep-ALO, a novel synthetic CPP developed using the intra-dermal delivery technology (IDDT) platform (REMEDI), for its potential to restore hair follicle activity. Human protein–derived peptides with strong membrane-permeating properties were screened, and the candidate demonstrating the greatest proliferative effect on dermal papilla cells and highest induction of hair growth–related proteins was selected. Through in vitro and ex vivo assays, we evaluated its ability to promote cell proliferation, sustain the anagen phase, and upregulate growth factors, supporting its feasibility as a complementary therapy for hair growth.

Materials and methods

In vitro study

Test product

Process screening Peptide candidates used in this study were selected from a human protein–derived peptide library supplied by REMEDI Co., Ltd. (Incheon, Republic of Korea). The biological activities of individual peptides were not previously confirmed and were experimentally evaluated through the screening procedures described in this study. A total of 260 CPPs were synthesized using standard fluorenylmethoxycarbonyl solid-phase peptide synthesis (Fmoc-SPPS) on 2-chloro-trityl chloride resin with protected amino acids. Upon completion of chain assembly, side-chain protecting groups were cleaved using trifluoroacetic acid (TFA), water, and triisopropylsilane (Sigma-Aldrich, Darmstadt, Germany). The crude peptides were subsequently purified by reverse-phase chromatography on a C18 column with a linear gradient of water and acetonitrile containing 0.1% TFA, and their molecular masses were verified by liquid chromatography–mass spectrometry (LC/MS).

To select the optimal CPP, the synthesized candidates were subjected to a stepwise screening process. All peptides were first evaluated for their proliferative effects in human follicle dermal papilla cells (HFDPCs) under identical experimental conditions. Peptides demonstrating proliferative activity exceeding the predefined threshold were selected for secondary validation. Subsequently, activation of β-catenin and phosphorylated ERK signaling pathways was assessed by Western blot analysis to prioritize biologically active candidates. Among these, Peptide No.218 showed the strongest combined proliferative and signaling activation effects and was selected as the lead peptide. This peptide was subsequently designated as 'DualPep-ALO' for all downstream experiments. Additionally, structural prediction analysis was performed using the peptide structure prediction software PEP-FOLD 3.5. DualPep-ALO was then incorporated into a transparent liquid form (Table 1) for subsequent experiments.

Concentrations expressed in ppm were additionally converted to approximate micromolar (μM) values based

Table 1 Ingredients in DualPep-ALO formulation

No.	INGREDIENT NAME
1	Water
2	Sodium phosphate dibasic
3	Sodium phosphate monobasic
4	DualPep-ALO

on the molecular weight of DualPep-ALO (1257 Da) to allow comparison across compounds.

Cell viability assay HFDPCs (8×10^3 cells/well) were seeded in 96-well plates and allowed to adhere for 12 hours (h). Each peptide candidate was added at the same concentration and incubated for 24 h. Epidermal growth factor (EGF, 10 ng/mL) was included on every plate as a positive control for proliferation. Cell viability was assessed using the CCK-8 assay (Dojindo, Japan), and optical density (OD) was measured at 450 nm using a VARIOSKAN LUX reader (Thermo Fisher Scientific, USA). Peptide-induced proliferative activity exceeding the predefined upper threshold (> 120%) was classified as a positive proliferative response.

Western blot HFDPCs (3×10^5 cells/well) were seeded in 6-well plates and allowed to adhere for 12 h. The cells were then treated with each peptide candidate for 6 h. After treatment, the cells were washed with cold PBS and lysed in RIPA buffer (Biosesang, Republic of Korea) supplemented with a protease inhibitor cocktail (Merck, Darmstadt, Germany). The lysates were incubated on ice for 30 min and centrifuged at 13,000 rpm for 15 min to remove debris. Protein concentrations were determined using a Bicinchoninic Acid Protein (BCA) Assay Kit (Thermo Fisher Scientific, USA). Equal amounts of protein (20 μg per sample) were separated by SDS-PAGE and transferred onto PVDF membranes (Merck, Germany). Membranes were blocked in TBS-T containing 5% skim milk for 1 h at room temperature and then incubated with primary antibodies against β-catenin, phosphorylated ERK (p-ERK), and β-actin (all from Santa Cruz Biotechnology, USA). Following extensive washing, the membranes were incubated with HRP-conjugated secondary antibodies (Thermo Fisher Scientific, USA). Protein bands were visualized using an enhanced chemiluminescence (ECL) solution (AbClon, Republic of Korea), and images were acquired with a LuminoGraph II imaging system (ATTO, Republic of Korea).

Penetration assay For quantitative analysis of cellular uptake, HFDPCs were treated with 2.5 μM FITC-conjugated DualPep-ALO or FITC-TAT peptide for 2 h. After incubation, cells were washed three times with PBS and treated with trypsin to remove membrane-bound peptides. Cells were resuspended in FACS buffer and intracellular fluorescence intensity was analyzed using flow cytometry. Mean fluorescence intensity (MFI) values were used to compare uptake efficiency between peptides.

Cell Culture

HFDPCs were cultured in Human Follicle Dermal Papilla Cell Growth Medium (PromoCell, Heidelberg, Germany) supplemented with fetal calf serum, bovine pituitary extract, basic fibroblast growth factor, and insulin. The mouse macrophage cell line (Raw264.7) was cultured in DMEM (Lonza, Basel, Switzerland) supplemented with 10% FBS (Gibco, Waltham, Massachusetts, USA) and 1% Penicillin-Streptomycin (Gibco), under standard conditions of 37 °C and 5% CO2.

Cell viability and proliferation assessment

Mouse macrophages and HFDPCs were seeded in a 96-well plate at a density of 5×10^4 cells/well and cultured until approximately 80% confluence. The test product was then applied at varying concentrations (0.64, 3.2, 16, 80 μM) to mouse macrophages for 24 h, and at concentrations of 3.2, 16, and 80 μM to HFDPCs, followed by incubation for 24, 48, or 72 h. After treatment, the CCK-8 assay was performed as described above. Cell viability was calculated as the percentage relative to the untreated control, where higher optical density values indicated greater cell viability. NG-Metyl-L-arginine acetate salt (L-NMMA; 25 μM; Sigma, M7033) and minoxidil (20μM) were served as positive controls.

Antioxidant enzyme activity evaluation

To assess antioxidant enzyme activity, HFDPCs were seeded at a density of 5×10^4 cells/well in 6-well plates and cultured to approximately 80% confluence. Oxidative stress was induced by treatment with 400 μM H_2O_2 in the presence of the test product (3.2, 16, 80 μM) or L-Ascorbic acid (100 μM), followed by 24 h of incubation. The culture medium was then collected and centrifuged at 2,000 × g for 10 min, and the clarified supernatant was used for analysis. Superoxide Dismutase (SOD) and catalase (CAT) activities were quantified using the OxiTec™ SOD and Catalase Assay Kits (BIOMAX, Gyeonggi-do, Republic of Korea), respectively, according to the manufacturer's instructions. OD was measured with the VARIOSKAN LUX reader, where lower OD values corresponded to higher antioxidant activity. L-Ascorbic acid (Sigma, A4403) was used as a positive control.

Inflammatory protein production evaluation

Mouse macrophages were seeded into 6-well plates at 1×10^6 cells/well. Upon reaching approximately 80% confluence, the cells were treated with lipopolysaccharide (LPS) at a concentration of 1 μg/mL to induce inflammation, along with the test product (3.2, 16, 80 μM) or

L-NMMA (25 μM), and incubated for 24 h. After incubation, the culture medium was collected, centrifuged at 2,000 × g for 10 min to remove cellular debris, and the supernatant was carefully harvested for subsequent cytokine quantification using enzyme-linked immunosorbent assays (ELISA) kits: Mouse TNF alpha SimpleStep ELISA Kit (ab208348; Abcam), Mouse IL-6 SimpleStep ELISA Kit (M6000B; R&D Systems), Mouse IL-1 beta SimpleStep ELISA Kit (ab197742; Abcam), and Mouse PGE-2 ELISA Kit (MBS266212; MyBioSource). The assays were conducted following the provided protocols, and OD was measured using the VARIOSKAN LUX reader. L-NMMA served as a positive control.

Ex vivo study

Human scalp tissue culture

Human scalp tissue was obtained with approval from the Global Medical Research Center Institutional Review Board (IRB No. GIRB-25605-OC) and all experiments were conducted with adherance to the Declaration of Helsinki. The study was conducted using scalp hair follicles obtained from four independent female donors in their 40s and 50s. For each donor, multiple individual hair follicles were microdissected and allocated to 6 experimental groups. The number of hair follicles per group was 20. Tissues were rinsed with PBS to remove residual impurities. Individual hair follicles were isolated and cultured in Williams' Medium E (Sigma-Aldrich), supplemented with 2 mM L-glutamine (Sigma-Aldrich), 10 μg/ml insulin (Sigma-Aldrich), 100 ng/ml hydrocortisone (Sigma-Aldrich), 0.1% fungizone (Gibco), 1% antibiotic-antimycotic (Gibco), and 1% Penicillin-Streptavidin (Gibco) under standard conditions (37 °C, 5% CO2). For protein analysis, follicles were lysed using PRO-PREP™ Protein Extraction Solution (iNtRON), and protein quantification was carried out using the BCA Assay Kit (Sigma-Aldrich).

Hair follicle length evaluation

The test product was diluted in follicle medium to achieve concentrations of 4, 20, and 100 ppm. Isolated follicles were treated with the test product (3.2, 16, 80 μM), caffeine (7.77 μM), or minoxidil (100 μM). Culture medium was replaced every 2–3 days over a 12-day period. To assess hair follicle elongation, follicles were observed and imaged at 2.4× magnification using a stereomicroscope (Stemi 508; Zeiss, Germany). The obtained images were analyzed with ImageJ software (NIH, USA) to determine follicle length. Measurements were performed on days 3, 6, 9, and 12, and the increase in follicle length was calculated relative to day 0. Caffeine and minoxidil were used as positive controls.

Anagen (growth phase) evaluation

Hair follicles were treated with the test product (3.2, 16, 80 μM), caffeine (7.77 μM) or minoxidil (100 μM) and cultured for nine days, with the medium refreshed every 2–3 days. Each follicle was individually imaged under the Stemi 508 stereomicroscope at 2.4× magnification. The images were visually evaluated to determine the percentage of follicles remaining in the anagen phase out of the total follicles per donor. Anagen/catagen classification was performed according to previously established morphological criteria reported in prior human hair follicle organ culture studies [14]: in the fully mature anagen VI stage, terminal hair follicles are characterized by a well-defined, onion-like hair bulb and a slim, elongated dermal papilla; during early catagen, the hair bulb becomes constricted and opens widely at its proximal portion; in mid-catagen, a partially keratinized structure destined to form the club hair appears just above the dermal papilla; by late catagen, the follicle is reduced to a thinner epithelial strand. Importantly, scoring was conducted in a blinded manner by three independent, experienced investigators. Each evaluator assessed the follicles independently, and final classification was determined by consensus. The anagen phase ratio, calculated as the number of hair follicles in the anagen phase out of all follicles, was measured on days 3, 6, and 9 of culture to evaluate the maintenance rate of the growth phase. The 9-day assessment time point was determined based on previous studies using human scalp hair follicle ex vivo organ culture models, which reported that spontaneous catagen development frequently occurs between day 7 and day 10 under culture conditions. For example, Philpott et al. (1990, 1994) established the human hair follicle organ culture model and demonstrated catagen progression within approximately 7–10 days ex vivo [15, 16]. Furthermore, Peters et al. (2005) also described donor-dependent variability in hair cycle progression in ex vivo cultured follicles [17].

Cell proliferation evaluation via immunofluorescence staining

To assess Ki67 expression, a cell proliferation marker, human hair follicles were treated with the test product (80 μM), caffeine (7.77 μM), or minoxidil (100 μM) for 24 h, then processed into frozen blocks. Tissue sections, 5 μm thick, were cut using a Cryostat microtome (Leica Biosystems, Germany) and mounted onto Silane-coated slides. After removing the OCT compound, the sections were incubated with anti-Ki67 antibody (ab15580, dilution, 1:100; Abcam), followed by a secondary antibody (Goat pAb to Rb IgG (H + L): a11012, dilution, 1:200; Invitrogen). The tissue was then mounted with VECTASHIELD® mounting medium containing DAPI (Vector Laboratories) to stain the cell nuclei. Images were captured at 100x magnification using a fluorescence microscope (M2; Zeiss). The percentage of Ki67-positive cells in the hair bulb was quantified based on DAPI-stained nuclei.

Growth factors evaluation

To evaluate hair loss-related factor protein production in hair follicles, the following ELISA kits were utilized: Human VEGF ELISA Kit (ab222510; Abcam), Human HGF ELISA Kit (ab275901; Abcam), and Human EGF ELISA Kit (ab217772; Abcam). The protocol was performed in accordance with the procedures outlined in Sect. "Inflammatory protein production evaluation".

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 27.0, and graphing was conducted using GraphPad Prism version 10.2.2. The significance between the experimental and control groups was assessed with a significance level set at 5% ($p < 0.05$). For parametric data, the independent t-test was used to calculate statistical significance. For non-parametric data, the Mann–Whitney U test was applied to determine statistical significance.

Results

Screening and determining peptides with optimal hair-restoring effects

The IDDT platform developed by REMEDI was used to identify human protein–derived peptides with potential cell-penetrating activity. Using a deep-learning model trained on a high-quality database, the platform generated CPP scores (indicating the cell membrane permeability) [18, 19], from which 260 top candidates were selected. HFDPCs were then treated with each peptide, with EGF serving as a positive control for its known proliferative effect [20]. Cell viability assays identified 22 peptides with the highest proliferative activity (Fig. 1A). Western blot analysis was subsequently performed to assess β-catenin and phosphorylated ERK levels as screening indicators associated with hair follicle proliferation and anagen initiation [21]. Among these, peptide No. 218 induced the strongest upregulation of β-catenin and phosphorylated ERK (p-ERK) (Fig. 1B) and was therefore selected as the lead candidate, designated "DualPep-ALO". The amino acid sequence of DualPep-ALO is VENNSTLEKHS (11 amino acids), with a calculated molecular weight of 1257 Da and a predicted net charge of – 1 at pH 7.0.

To test the cell-penetrating capability of DualPep-ALO, cellular uptake was evaluated using FITC-conjugated peptides followed by flow cytometry analysis. HFDPCs were treated with FITC-DualPep-ALO or FITC-TAT under identical experimental conditions. After extensive washing and trypsin treatment to remove membrane-associated peptides, intracellular fluorescence intensity was quantified. Flow cytometry analysis demonstrated that DualPep-ALO exhibited approximately two-fold higher intracellular fluorescence levels compared with the classical cell-penetrating peptide TAT, indicating efficient cellular internalization (Fig. 1C). These results confirm that DualPep-ALO possesses cell-penetrating capability in dermal papilla cells.

The predicted structure of DualPep-ALO via PEPFOLD 3.5 is depicted in Fig. 2A. The analysis suggests that the peptide predominantly adopts a flexible coil conformation with partial helical propensity toward the C-terminal region (Fig. 2B).

DualPep-ALO demonstrates potent antioxidant and anti-inflammatory properties in in vitro models

Cell viability

DualPep-ALO exhibited no cytotoxicity in mouse macrophages at concentrations up to 80 μM, with cell viability consistently exceeding 95%. Treatment with L-NMMA (25 μM) also did not produce any significant effects (Table S1, Fig. 3A). Additionally, DualPep-ALO promoted the viability of HFDPCs in a dose- and time-dependent manner (Table S2, Fig. 3B). After 48 and 72 h of treatment, cell proliferation significantly exceeded that of the control group at all tested concentrations ($p < 0.05$).

Antioxidant properties of DualPep-ALO on HFDPCs

Exposure to H_2O_2 significantly reduced SOD and CAT activities in HFDPCs ($p < 0.05$). DualPep-ALO treatment (3.2, 16, 80 μM) effectively restored both enzyme activities in a dose-dependent manner, showing levels comparable to those observed with the L-ascorbic acid (100 μM) positive control at 80 μM concentration (Tables S3 & S4, Figs. 4A & B). These findings demonstrate that DualPep-ALO enhances cellular antioxidant defenses against oxidative stress.

Anti-inflammatory properties of DualPep-ALO on mouse macrophages

LPS exposure significantly increased the secretion of TNF-α, IL-6, IL-1β, and PGE-2 in mouse macrophages compared to the negative control group ($p < 0.05$, Tables S5 – S8, Figs. 5A–D). Treatment with DualPep-ALO (3.2, 16, 80 μM) led to a dose-dependent reduction in all four inflammatory mediators. These results demonstrate that DualPep-ALO effectively attenuates LPS-induced inflammatory responses in macrophages.

DualPep-ALO exhibits effective enhancement of hair follicle length, maintenance of anagen phase, cell proliferation, and growth factor production in ex vivo human scalp tissue model

Elongation of hair follicle length in human scalp tissue

Hair follicles treated with DualPep-ALO exhibited visually elongation after 12 days, with a more pronounced increase compared to the negative control group (Fig. 6A). Treatment with DualPep-ALO also induced

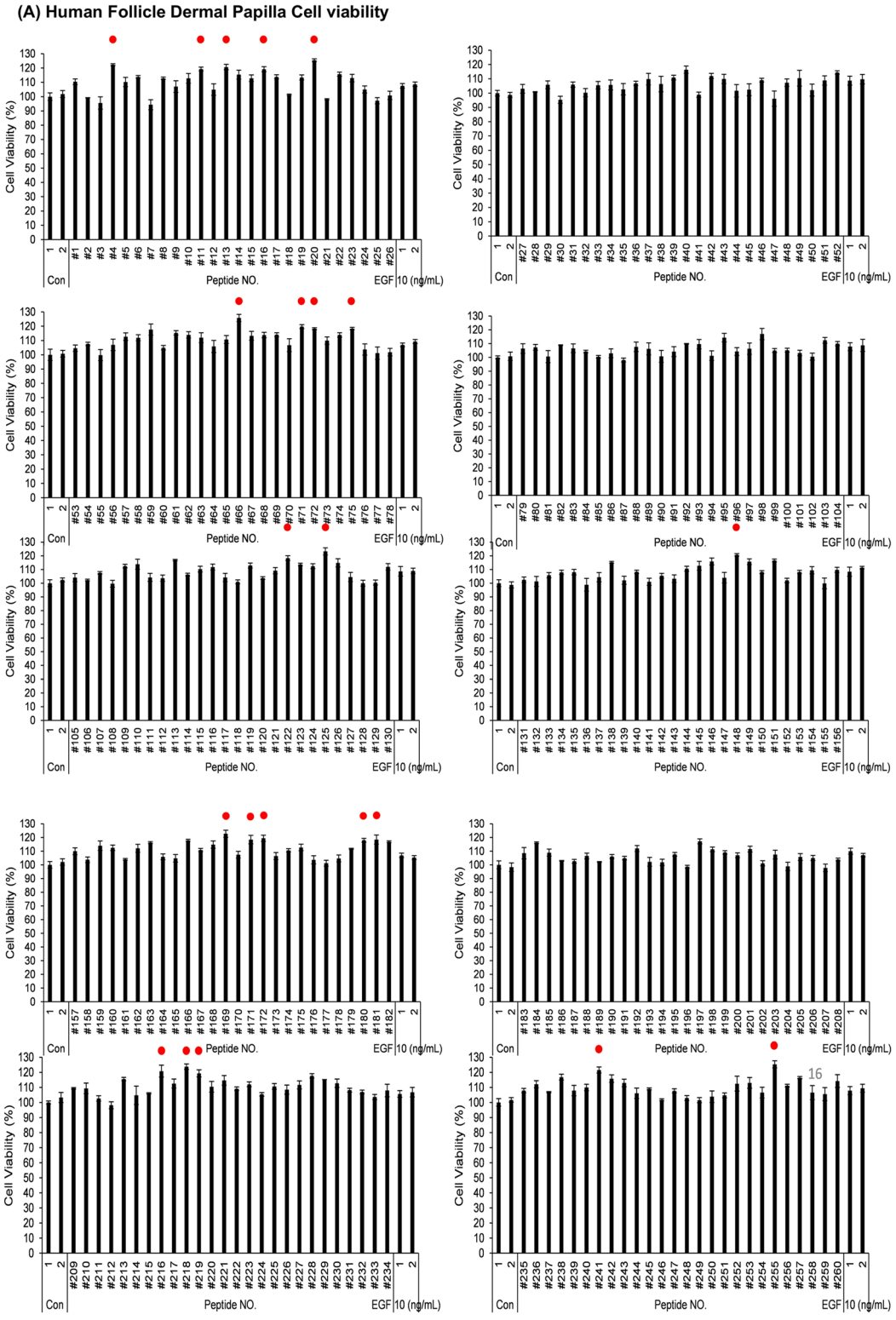
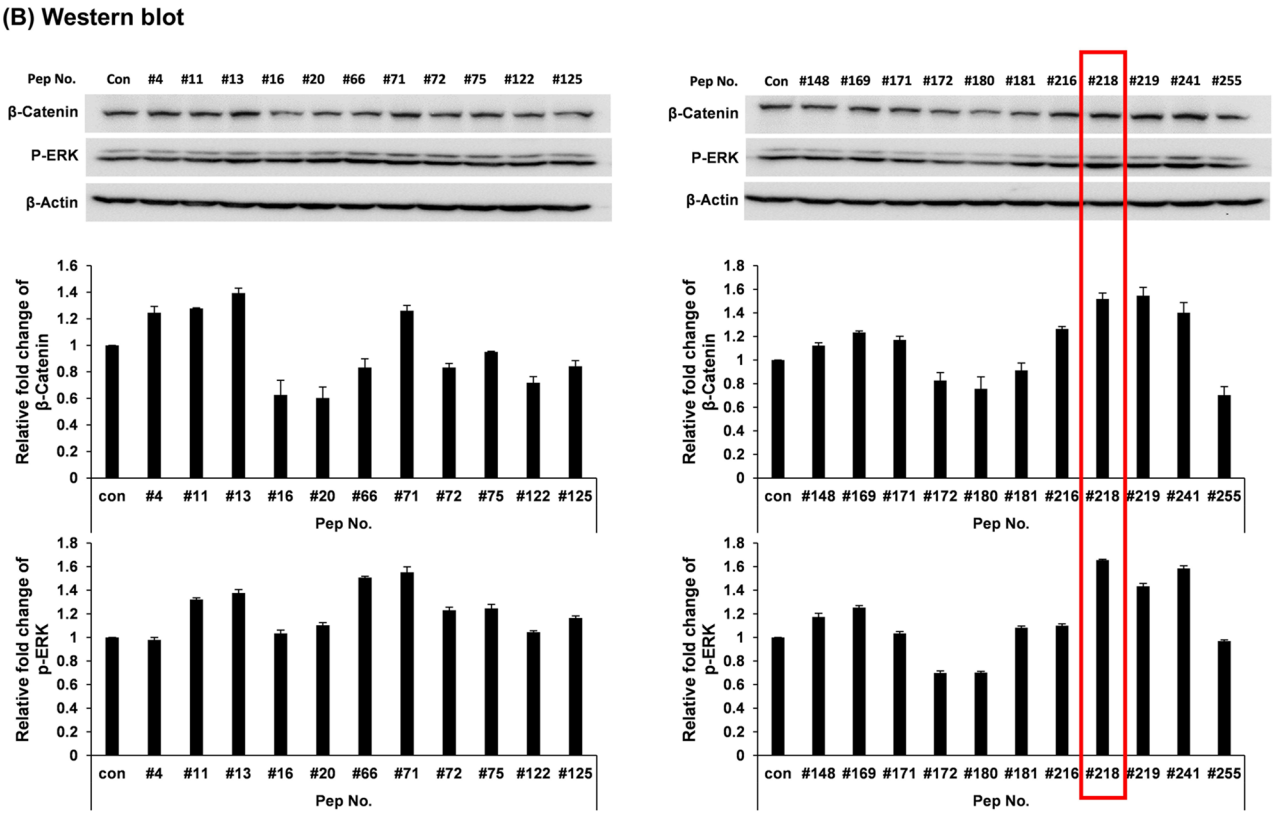


Fig. 1 Screening process to determine peptides with hair-restoring properties. (**A**) Human Follicle Dermal Papilla Cell (HFDPCs) viability of over 200 cell-penetrating peptides, with EGF as the positive control. The red dots mark the peptides with the highest cell viability. (**B**) Western Blot results showing the upregulation of β-catenin and p-ERK proteins of 22 chosen peptides compared to the control. Peptide No.218 (in red rectangle) showed the highest expression level of β-catenin and p-ERK proteins, and was chosen for subsequent analyses. (**C**) Cell-penetrating capability of DualPep-ALO was evaluated using FITC-conjugated peptides in HFDPCs. Cells were treated with FITC-DualPep-ALO or FITC-TAT (2.5 μM, 2 h), followed by extensive washing and trypsinization to remove membrane-bound peptides. Intracellular fluorescence intensity was quantified by flow cytometry. Each experiment was performed three times



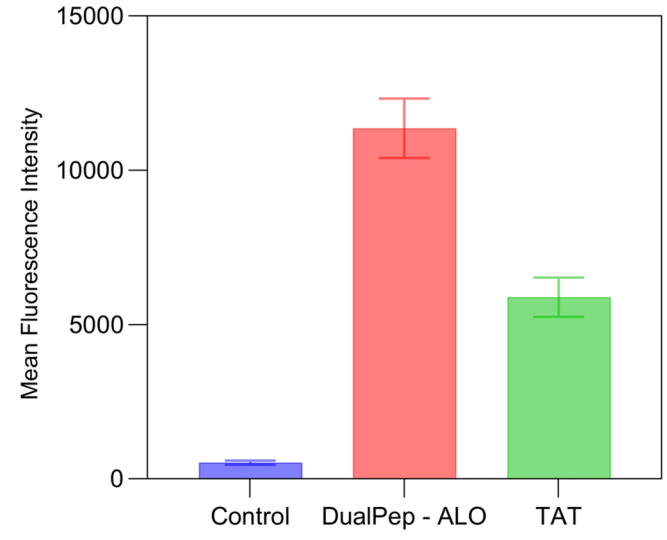


Fig. 1 (continued)

a concentration-dependent increase in follicle length throughout the 12-day culture period. Significant elongation was observed from day 6 onward in the 16 and 80 μM groups, compared to the negative control ($p < 0.05$). By day 12, the growth-promoting effect of DualPep-ALO was comparable to that of caffeine (7.77 μM) and minoxidil (100 μM) (Table S9, Figs. 6B & C). These results suggested that DualPep-ALO effectively stimulates hair follicle elongation.

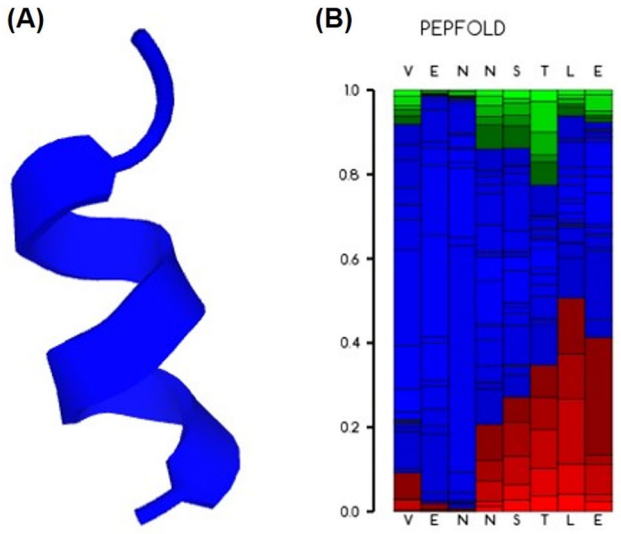


Fig. 2 The three-dimensional structure of the peptide (VENNSTLEKHS) was predicted using the PEP-FOLD 3.5 de novo peptide structure prediction server. (**A**) Representative lowest-energy model generated by PEP-FOLD showing the predicted peptide conformation in ribbon representation. (**B**) Residuewise secondary structure propensity map generated during the simulation. Color coding indicates predicted structural states: red, α-helix; blue, random coil; and green, β-sheet propensity

Maintenance of anagen phase in human scalp hair follicles

Hair follicles treated with DualPep-ALO displayed a darker and thicker appearance compared to the negative control after 9 days of culture, indicating prolonged retention of the anagen phase (Fig. 7A, Figures S1 – S4). DualPep-ALO treatment also exhibited a concentration-dependent effect on the maintenance of the anagen phase (the number of hair follicles in the anagen phase out of all follicles) throughout the culture period. Significant differences were observed on days 6 and 9, with the 16 and 80 μM groups maintaining higher growth phase rates than the negative control ($p < 0.05$, Table S10, Figs. 7B & C). By day 9, the effect of DualPep-ALO was statistically equivalent to that of caffeine (7.77 μM) and minoxidil (100 μM) ($p > 0.05$). These findings demonstrate that DualPep-ALO effectively supports sustained hair follicle activity and delays the transition to the regression phase.

Effect of DualPep-ALO on cell proliferation in human scalp hair follicles

DualPep-ALO (80 μM) markedly increased Ki67-positive cells in the hair bulb compared with the negative control, indicating enhanced cell proliferation. The fluorescence intensity was comparable to that observed with caffeine (7.77 μM) and minoxidil (100 μM) (Fig. 8A). Quantitative analysis confirmed these observations, showing a statistically significant increase in Ki67 expression following DualPep-ALO treatment ($p < 0.05$, Table S11, Fig. 8B). These results demonstrate that DualPep-ALO promotes cellular proliferation within human scalp hair follicles.

Influence of DualPep-ALO on growth factors in human scalp tissue

Treatment with DualPep-ALO significantly increased the expression of hair growth–related factors VEGF, HGF, and EGF in human scalp hair follicles. DualPep-ALO (3.2, 16, 80 μM) elevated the levels of all three proteins in a dose-dependent manner compared with the negative control ($p < 0.05$). At 16 and 80 μM, the concentrations were higher compared to those observed with caffeine (7.77 μM) and minoxidil (100 μM) (Tables S12 – S14,

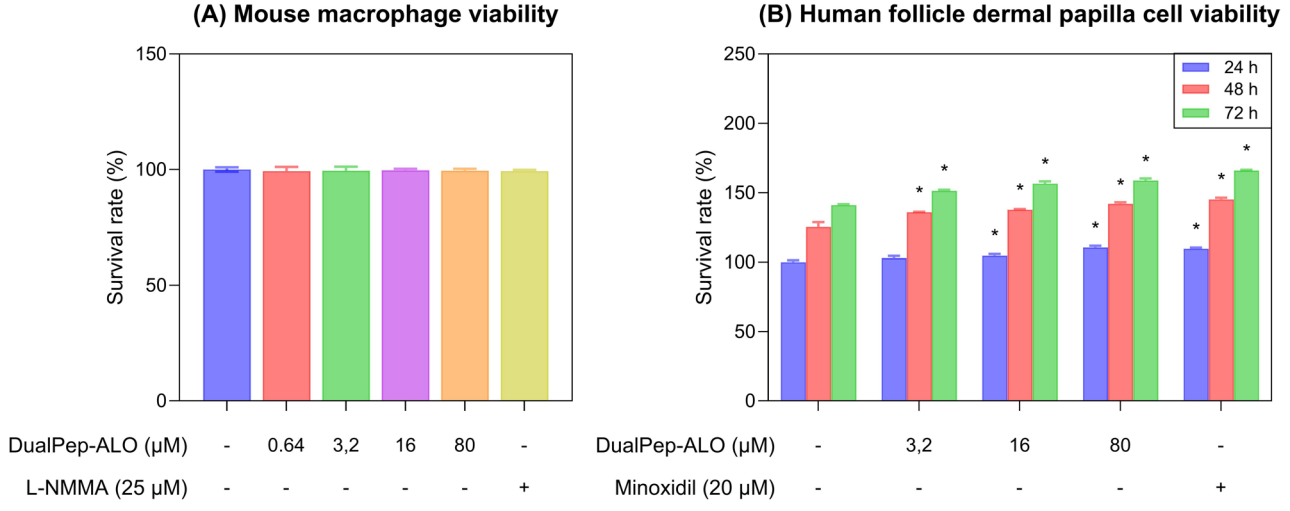


Fig. 3 Effects of DualPep-ALO on cell viability. (**A**) Mouse macrophage viability after 24-hour treatment with various concentrations of DualPep-ALO (0.64, 3.2, 16, 80 μM), compared with L-NMMA (25 μM). (**B**) Human Follicle Dermal Papilla Cells viability after treatment with DualPep-ALO (3.2, 16, 80 μM) for 24, 48, and 72 hours, compared with minoxidil (20 μM). Data are presented as mean ± SD. *$p < 0.05$ vs. negative control at the same time point. Each experiment was performed three times

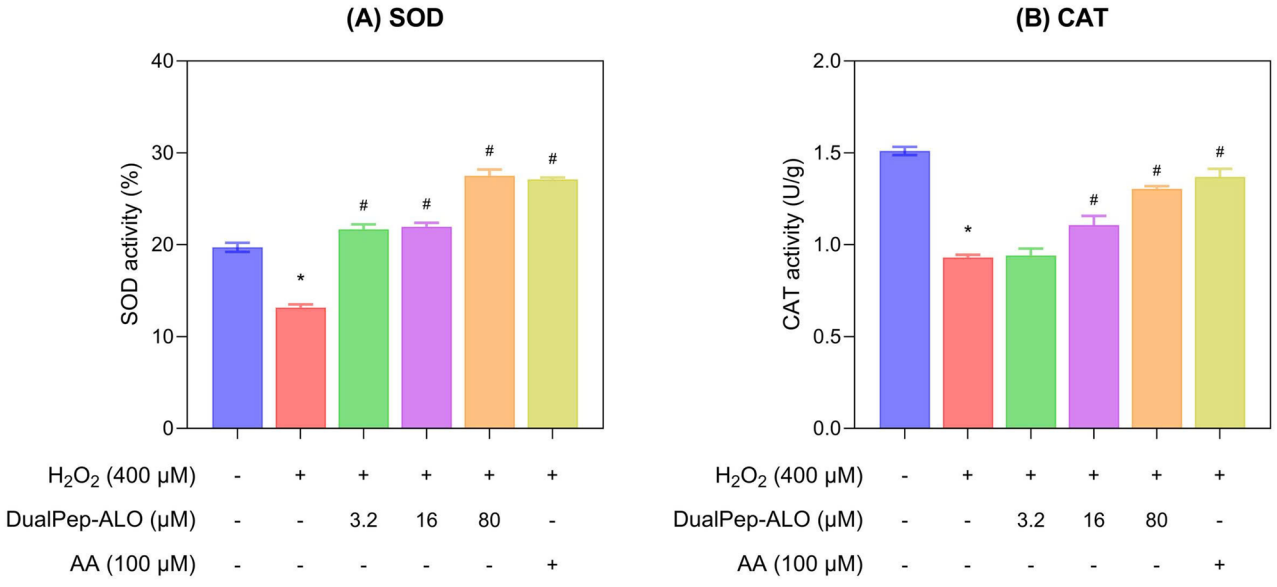


Fig. 4 Antioxidant enzyme activities in Human Follicle Dermal Papilla Cells (HFPDCs) treated with DualPep-ALO. DualPep-ALO (3.2, 16, 80 μM) enhanced the activities of (**A**) Superoxide dismutase (SOD) and (**B**) catalase (CAT) in HFPDCs after oxidative stress induction with H_2O_2 (400 μM). L-Ascorbic acid (100 μM) was used as a positive control. Data are expressed as mean ± SD. *$p < 0.05$ vs. negative control; #$p < 0.05$ vs. H_2O_2-treated group. Each experiment was performed three times

Figs. 9A-C). These findings indicate that DualPep-ALO enhances the production of key growth factors involved in follicular regeneration and hair growth promotion.

Discussion

Numerous bioactive peptides have been explored for dermatologic applications; however, their clinical translation remains limited by poor transdermal permeability, high molecular weight, and potential immunogenicity. CPPs effectively overcome these challenges by enabling efficient intra- and transdermal delivery of therapeutic molecules while maintaining low immunogenicity [12, 22]. In this study, DualPep-ALO was identified as a promising CPP-based candidate for hair follicle growth through its ability to reestablish a favorable follicular microenvironment. A major strength of this work lies in its comprehensive design, which simultaneously evaluates multiple biological parameters using both in vitro and ex vivo models. Furthermore, the inclusion of positive control groups reinforces the reliability and robustness of the findings.

Oxidative stress is a key factor in many forms of hair loss, where excessive ROS damage follicular cells, shorten the anagen phase, and promote follicle miniaturization [2–5]. The test product effectively restores SOD and CAT activities, essential enzymes for cellular antioxidant defense. Specifically, SOD converts superoxide radicals (O_2^-) into H_2O_2, which is then broken down by CAT into water and oxygen, preventing oxidative damage [23]. By enhancing these enzymes, DualPep-ALO preserves the redox balance in the follicular environment, protecting proliferative cells from ROS-induced apoptosis and supporting sustained hair growth.

Chronic inflammation is another important driver of alopecia, leading to premature follicular regression and impaired regeneration [3, 4, 24]. Elevated levels of pro-inflammatory cytokines from macrophages and other immune cells, such as TNF-α, IFN-γ, IL-1β, and IL-6, have been detected in patients with different types of hair loss, where they disrupt the follicular stem cell signaling and the follicle cycle [4, 25, 26]. This study showed that the test product markedly reduced cytokine secretion, suggesting its potential to preserve the immune privilege of hair follicles and prevent inflammatory damage within the perifollicular microenvironment.

A shortened anagen phase and reduced follicular cell proliferation lead to thinner, shorter hair shafts and slower hair growth in alopecia [2, 4, 11]. These changes result from disrupted signaling between dermal papilla cells and matrix keratinocytes, decreased growth factor expression, and reduced proliferation in the follicular bulb [2, 27]. The test product effectively counteracts these processes by maintaining the anagen phase, enhancing Ki67-positive proliferation, and upregulating growth factors. VEGF promotes perifollicular angiogenesis, HGF activates follicular morphogenesis, and EGF supports hair sheath differentiation and morphology [28–30]. By stimulating these pathways, DualPep-ALO sustains follicular vitality, promotes hair shaft elongation, mitigates anagen shortening, and restores the growth potential of the follicle.

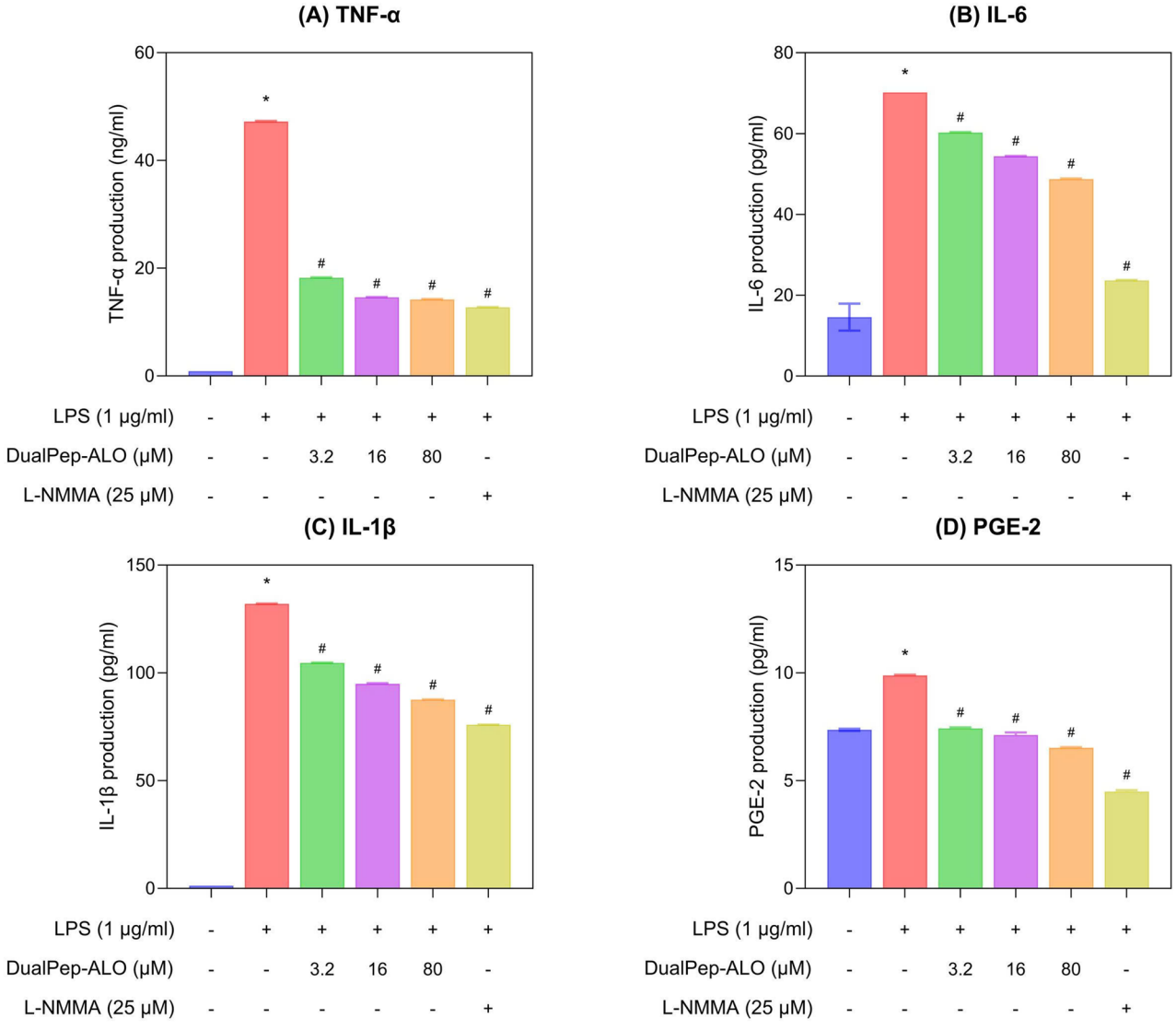


Fig. 5 Anti-inflammatory effects of DualPep-ALO in LPS-treated mouse macrophages. DualPep-ALO (3.2, 16, 80 μM) reduced the production of (**A**) TNF-α, (**B**) IL-6, (**C**) IL-1β, and (**D**) PGE-2 in mouse macrophages treated with LPS (1 μg/mL). L-NMMA (25 μM) was used as a positive control. Data represent mean ± SD. *$p < 0.05$ vs. negative control; #$p < 0.05$ vs. LPS-treated group. Each experiment was performed three times

Notably, DualPep-ALO demonstrated hair growth–promoting effects comparable to minoxidil, as evidenced by similar levels of follicle elongation, anagen maintenance, and proliferative activity. However, the underlying mechanisms differ, as minoxidil acts as a potassium channel opener that enhances scalp microcirculation and prolongs the anagen phase, targeting the vascular and metabolic pathways of androgenetic alopecia [6]. While further studies are warranted to compare their clinical efficacy, the multifunctional actions suggest DualPep-ALO may serve as a promising adjuvant therapy to enhance follicular resilience and treatment outcomes.

The bioactive profile of DualPep-ALO parallels that of several well-characterized synthetic peptides. The copper-binding tripeptide GHK-Cu exhibits strong antioxidant and anti-inflammatory activities by inhibiting TNF-α, suppressing pro-fibrotic TGF-β1 in keloid fibroblasts, and promoting angiogenesis via VEGF and FGF induction [31]. Likewise, the hexapeptide Peptamide-6 (Phe-Val-Ala-Pro-Phe-Pro) enhances growth factor expression, matrix protein synthesis, and heat-shock response, contributing to skin firmness and repair [31].

Although many hair growth–associated pathways are initiated through membrane receptor signaling [32], intracellular states such as oxidative stress and inflammatory signaling critically determine the stability and activity of downstream mediators including β-catenin and ERK in dermal papilla cells [5]. Therefore, therapeutic strategies targeting intracellular regulatory environments may complement receptor-level interventions.

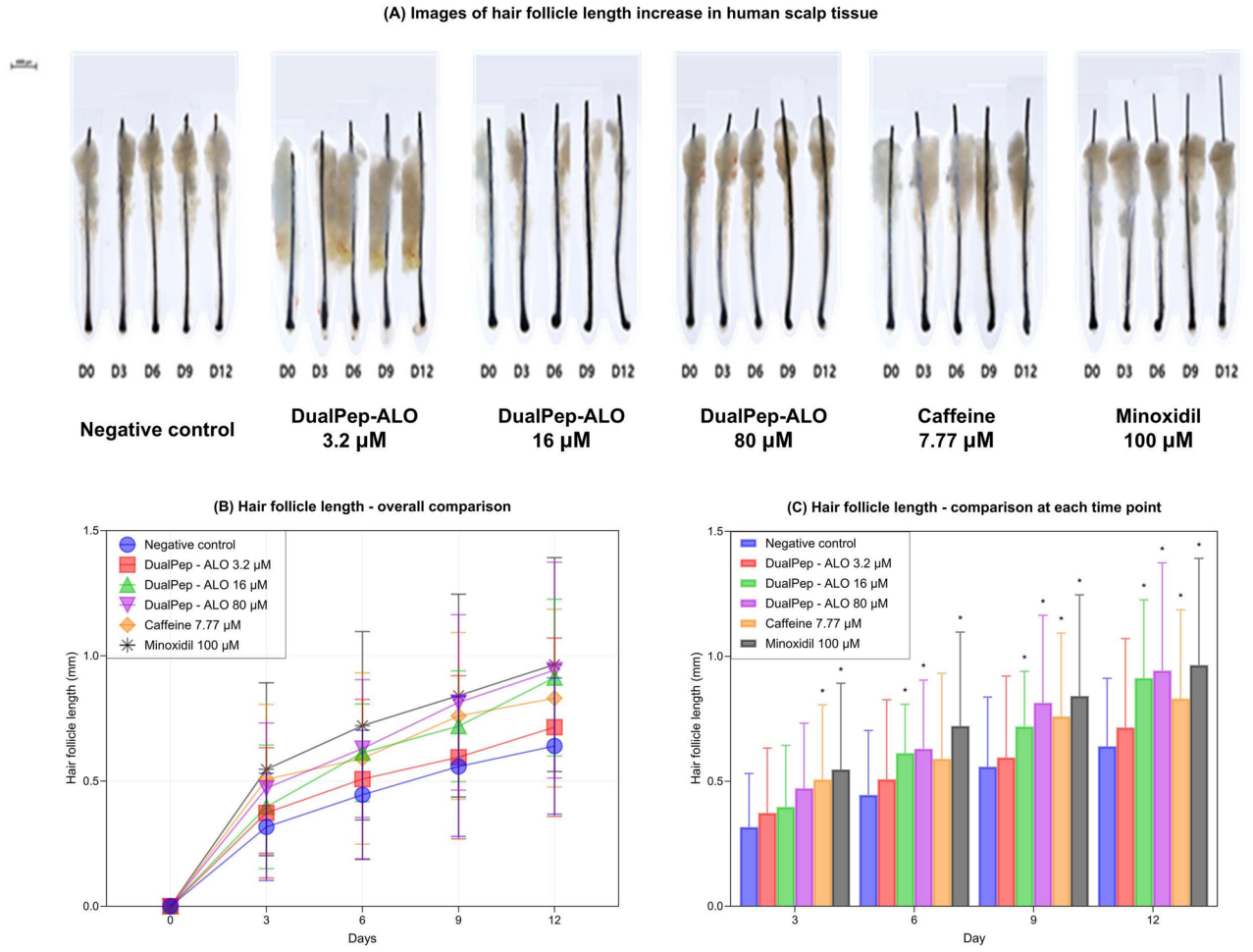


Fig. 6 DualPep-ALO promotes hair follicle elongation in human scalp tissue. (**A**) Representative images showing hair follicle length on days 0, 3, 6, 9, and 12 after treatment with DualPep-ALO (3.2, 16, 80 μM), caffeine (7.77 μM), or minoxidil (100 μM). (**B**) Overall comparison and (**C**) time-dependent changes in human scalp hair follicle length. Data are mean ± SD. *$p < 0.05$ vs. negative control. Each experiment was performed three times

DualPep-ALO was developed not as a receptor ligand mimic but as a bioactive peptide capable of intracellular modulation of follicular stress responses, providing a mechanistic rationale for the use of a cell-penetrating peptide in hair growth regulation.

While the present findings highlight the therapeutic potential of DualPep-ALO in promoting hair growth, several limitations should be considered. First, this study was limited to preclinical experimental models, including oxidative stress (H_2O_2), inflammatory macrophage activation (LPS), and ex vivo human hair follicle culture, which do not completely represent specific disease models of androgenetic alopecia, or telogen effluvium. Therefore, our study only serves to evaluate whether DualPep-ALO could support hair follicle growth under conditions relevant to oxidative and inflammatory stress, which are commonly implicated in various forms of hair loss. Real-world studies are needed to validate its true therapeutic efficacy in specific alopecia subtypes. Moreover, future investigations, including combination studies with established treatments such as minoxidil or finasteride, are needed to confirm its efficacy and define its role as an adjunct or maintenance therapy for hair loss. Additionally, the absence of a scrambled peptide or a length-matched non-CPP control in our study renders the validation of sequence specificity difficult. Finally, we did not perform adjustments for multiple comparisons due to the small sample size of the experiments and the exploratory and hypothesis-generating characteristics of the study.

Conclusion

This preclinical study identified DualPep-ALO, a novel cell-penetrating peptide, as a promising candidate to support hair follicle growth. Through in vitro and ex vivo models, DualPep-ALO promoted hair follicle cell

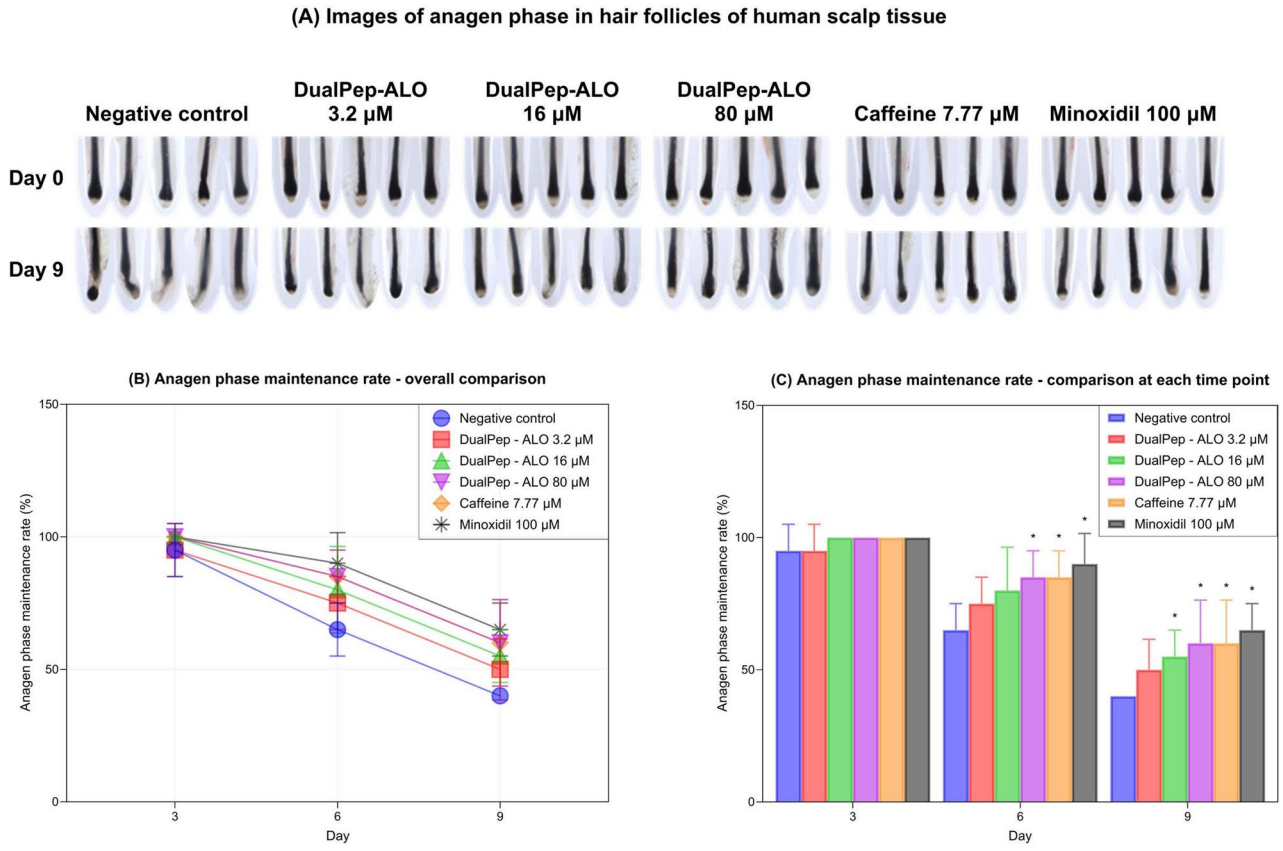


Fig. 7 DualPep-ALO maintains the anagen phase of human scalp hair follicles. (**A**) Representative images of hair follicles on days 0 and 9 after treatment with DualPep-ALO (3.2, 16, 80 μM), caffeine (7.77 μM), or minoxidil (100 μM). (**B**) Overall comparison and (**C**) time-point analysis of growth phase maintenance rate in human scalp hair follicles. Data are expressed as mean ± SD. *$p < 0.05$ vs. negative control. Each experiment was performed three times

proliferation, sustained the anagen phase, and upregulated key growth factors, while enhancing antioxidant defenses and reducing inflammation. These multimodal effects contributed to follicular regeneration and produced outcomes on hair growth comparable to minoxidil. By combining potent biological activity with efficient transdermal delivery, DualPep-ALO offers a feasible topical strategy for hair growth and may complement existing therapies. Further clinical studies are warranted to validate its long-term safety, optimal dosing, and therapeutic efficacy in human subjects.

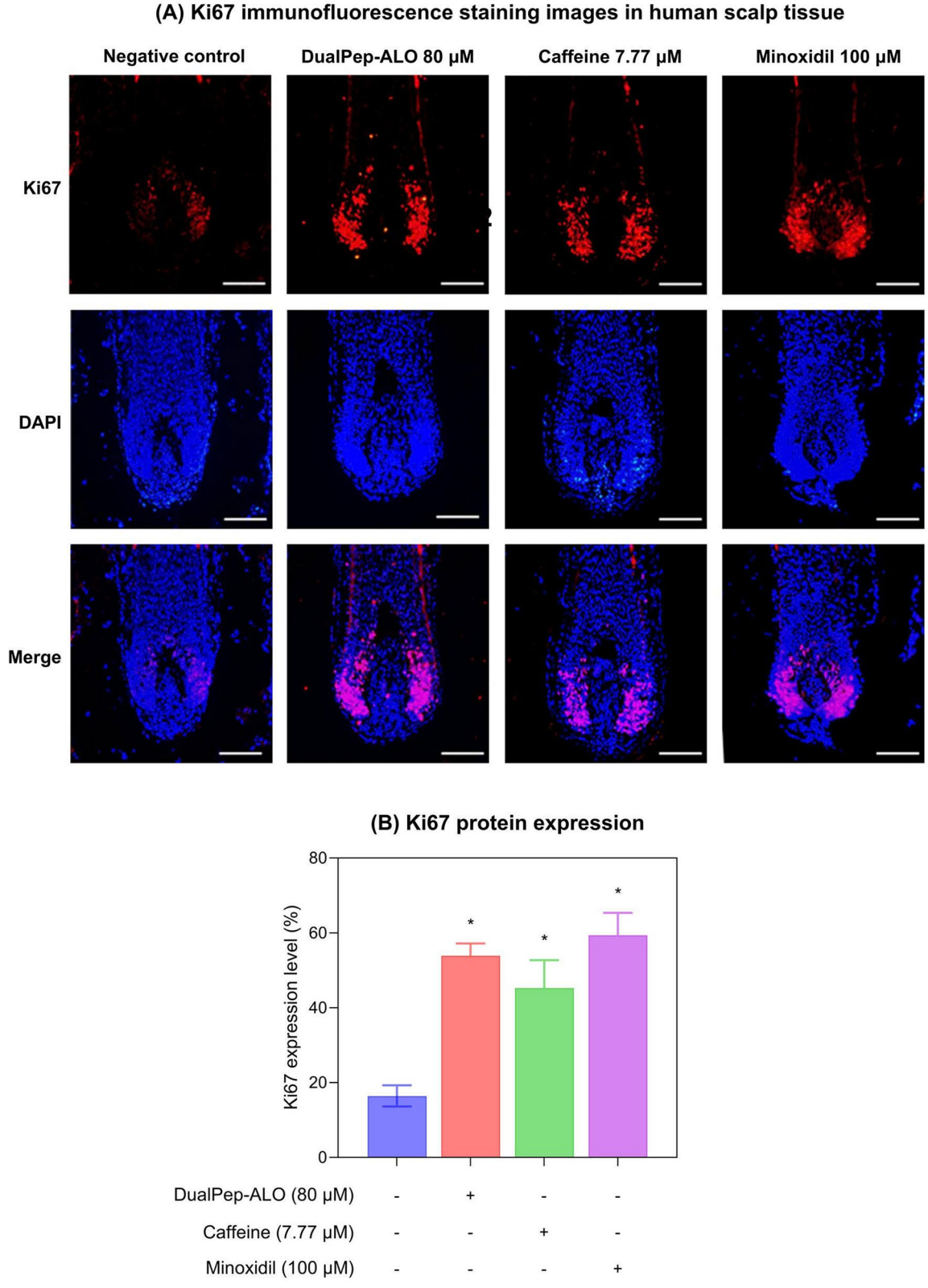


Fig. 8 DualPep-ALO enhances cell proliferation in human scalp hair follicles. (**A**) Representative immunofluorescence images showing Ki67 (red) and DAPI (blue) staining in hair bulb regions after treatment with DualPep-ALO (80 μM), caffeine (7.77 μM), or minoxidil (100 μM). Scale bar = 100 μm. (**B**) Quantification of Ki67 expression levels. Data are mean ± SD. *$p < 0.05$ vs. negative control. Each experiment was performed three times

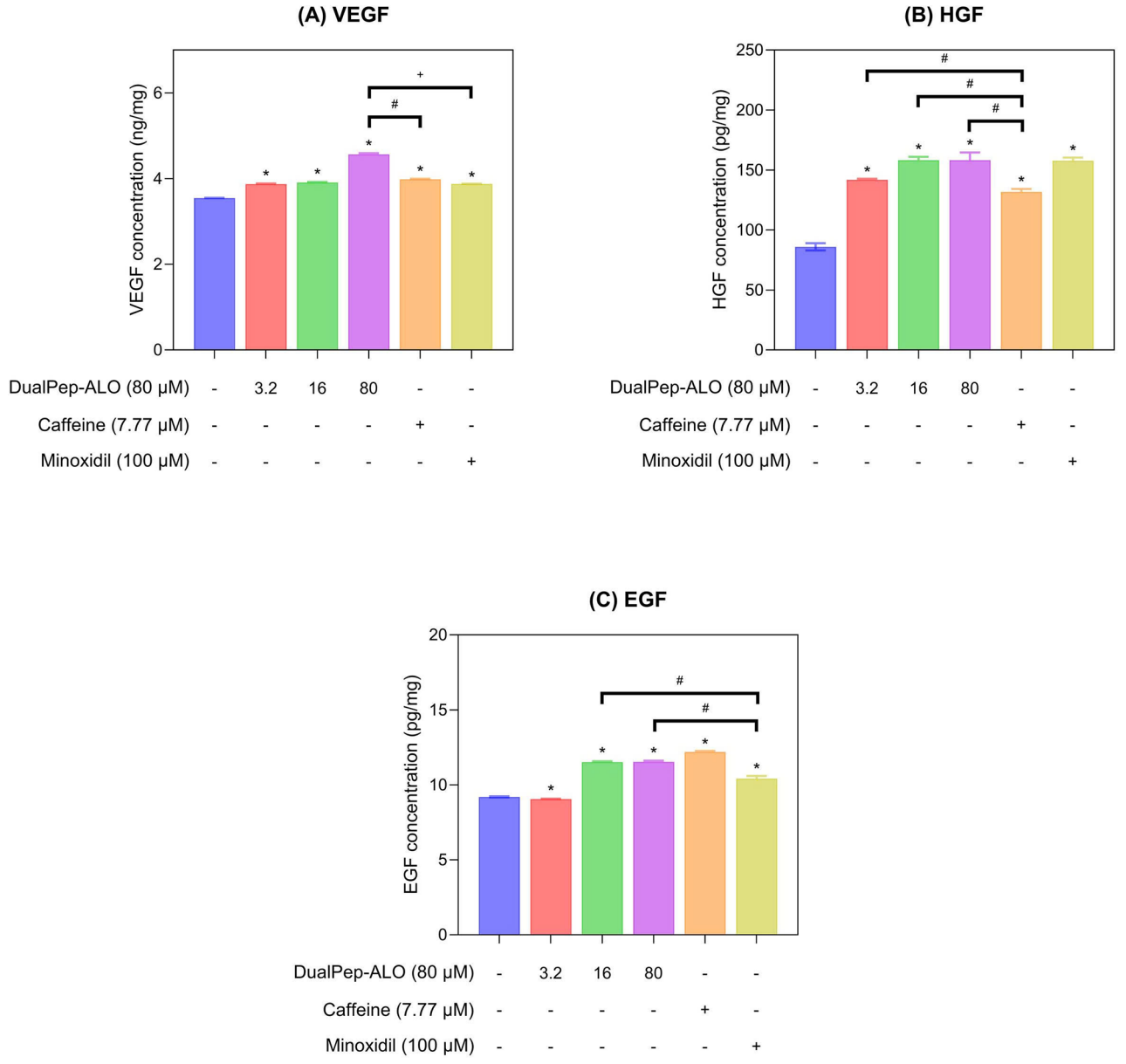


Fig. 9 DualPep-ALO increases growth factor expression in human scalp hair follicles. Protein levels of (**A**) VEGF, (**B**) HGF, and (**C**) EGF were quantified by ELISA after treatment with DualPep-ALO (3.2, 16, 80 μM), caffeine (7.77 μM), or minoxidil (100 μM). Data are presented as mean ± SD. **p* < 0.05 vs. negative control; *#p* < 0.05 between indicated groups. Each experiment was performed three times

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions
Conceptualization, Y.I.L., and J.H.L.; methodology, Y.I.L., W.K. and H.R.; software, A.M., J.J., H.C. and N.H.N.; validation, J.J., H.C., and J.K.H.; formal analysis, Y.I.L., J.K.H. and J.H.P.; data curation, Y.I.L., N.H.N., J.H.P, and I.J.; investigation, Y.I.L., N.H.N., and I.J.; visualization, W.K., H.R. and A.M.; writing – original draft, Y.I.L., W.K., H.R., A.M., J.J., and N.H.N.; writing – review & editing, Y.I.L., H.C., J.K.H., N.H.N., J.H.P., I.J., and J.H.L.; supervision, J.H.L.; project administration, J.H.L. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval
Human scalp tissue was obtained with approval from the Global Medical Research Center Institutional Review Board (IRB No. GIRB-25605-OC) and all experiments were conducted with adherance to the Declaration of Helsinki.

Competing interests
The authors declare no competing interests.

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