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Development of a Triple Permeation System Integrated with Nanocarrier, Atomization and Electro-Enhanced Permeation Technologies for Efficient Skin-Whitening, Repairing and Percu-taneous Penetration

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1. Introduction

Although medical aesthetics like laser and chemical peels can improve appearance, they may cause chronic or non-chronic wounds, leading to skin damage if not properly cared for. Symptoms include redness, swelling, and heat, linked to damaged skin cells and impaired microcirculation. Decreased anti-inflammatory effects and impaired barrier function make the skin susceptible to infection, potentially causing prolonged inflammatory erythema, pigmentation spots (PIH), and dryness, itching, and increased sensitivity after treatments [1, 2].

Current treatments focus on preventing inflammation and promoting tissue repair, but they have limited efficacy in skin whitening [3]. Most post-procedure whitening methods use skincare products or sunscreen, but many active molecules struggle to penetrate the stratum corneum, resulting in weak effects and potential irritation[4-6].

Nanocarrier technology, especially liposomes, can solve these problems. Liposomes exhibit superb biocompatibility and negligible toxicity, improving solubility, stability, skin permeability, and cellular uptake of active ingredients, while reducing side effects[7-9].

To enhance transdermal delivery, combining nanocarriers with atomization and electro-enhanced permeation technologies is promising[10, 11]. Atomization enhances skin penetration, and electro-enhanced permeation, including iontophoresis and electroporation, creates pathways for active ingredients[12, 13].

This article developed a triple permeation system integrating nanocarrier, atomization, and electro-enhanced permeation technologies. Glabridin [14] and nicotinamide [15] have a skin lightening effect, Nonapeptide-1 [16] and palmitoyl tripeptide-8 [17] inhibit melanin production. Acetyl dipeptide-1 cetyl ester reduces inflammation. By co-encapsulating these active ingredients (Free Active Ingredients, FAI) into liposomes to develop a composite nanocarrier named PRO-Peptide311. Co-encapsulating active ingredients into liposomes overcomes stability and penetration challenges, and the combination with atomization and electro-enhanced permeation

facilitates deeper skin penetration. This system shows promise for post-medical aesthetic procedures and skin-lightening cosmetics.

2. Materials and Methods

2.1 Materials

Reagents were sourced as follows: glabridin (Xinjiang Longhuiyuan Pharmaceutical), nicotinamide (LONZA), palmitoyl tripeptide-8 and acetyl dipeptide-1 cetyl ester (Nanjing Leon Biotechnology), PEG-40 hydrogenated castor oil (BASF), 1,3-propanediol and glycerol (Sinopharm), cell culture media/supplements (Gibco), L-DOPA/tyrosine/TritonX-100/α-MSH (Sigma-Aldrich), BCA Protein Assay Kit (Beyotime Biotechnology).

2.2 Biological Samples

Fertilized chicken eggs (Wuhan Guorun Animal Husbandry) were incubated to embryonic day 8. Bama porcine skin was obtained from Zhifu District Yourong Biological Studio. Cell lines (HaCaT, HSF, B16F10) were procured from Kunming Cell Bank. A 3D melanin skin model (MelaKutis® MS240803) was provided by Guangdong Boxi Biotechnology.

2.3 PRO-Peptide311 Synthesis

Formulated via two-phase mixing (deionized water/glycerol-water), heated, homogenized, and ultracentrifuged. Physicochemical properties (size, PDI, zeta potential, morphology) were characterized by DLS/TEM. Stability and permeation-enhanced formulations were evaluated.

2.4 Encapsulation Parameters

Encapsulation efficiency (EE) and drug loading efficiency (DLE) were determined by ultrafiltration-HPLC using wavelength-specific gradients (280 nm for glabridin/nicotinamide; 210 nm for peptides). Calculated as:

$$\text{DLE}\ (\%)=\frac{W_T-W_F}{W_L}\times 100$$

$$\text{EE}\ (\%)=\frac{W_T-W_F}{W_T}\times 100$$

W_T: the mass of active ingredients encapsulated in the nanocarrier, W_F: the mass of the free active ingredients not encapsulated, W_L: the mass of the nanocarrier.

2.5 HET-CAM chicken embryo allantoic membrane assay

Day-8 chick embryos received 200 µL samples. Irritation scores (IS) calculated as:

$$IS = [(301-secH)\times 5 + (301-secL)\times 7 + (301-secC)\times 9] / 300$$

Where secH is the initial time of surface hyperemia (in seconds), secL is the initial time of surface hemorrhage (in seconds), and secC is the initial time of surface coagulation (in seconds).

Classified as non-irritant (0-0.9), mild (1.0-4.9), moderate (5.0-8.9), or severe (9-21.0).

2.6 Cytotoxicity Testing

HaCaT (1.5×10^4 cells/well) and HSF (8×10^3 cells/well) viability post-treatment was assessed via CCK-8 after 24 h exposure to PRO-Peptide311 (1.25-20.0 µg/mL glabridin) or free active ingredients (FAI).

2.7 Cellular Uptake Analysis

RhoB-labeled formulations were incubated with B16F10 cells (2/4 h). Uptake was quantified by confocal microscopy (405 nm excitation/561 nm emission) and flow cytometry (561 nm excitation).

2.8 Tyrosinase Activity

α-MSH-stimulated B16F10 cells were treated with FAI/PRO-Peptide311 (2.5-10.0 μg/mL glabridin). Enzymatic activity was measured at 490 nm post L-DOPA incubation.

2.9 Melanin Synthesis

α-MSH-induced B16F10 cells were treated as above. Melanin content was quantified at 405 nm after NaOH/DMSO lysis.

2.10 Antioxidant Assay

H_2O_2-stressed HaCaT cells were treated with FAI/PRO-Peptide311 (2.5-10.0 μg/mL). Oxidative stress was evaluated via DCFH-DA fluorescence.

2.11 Anti-Inflammatory Study

TNF-α/IFN-γ-stimulated HaCaT cells were treated with formulations. Cytokine levels were measured by ELISA.

2.12 Skin Whitening Model

UVB-irradiated MelaKutis® models were treated with PRO-Peptide311 (10.0 μg/mL), FAI, or VC (70.0 μg/mL). Brightening efficacy was assessed via colorimetry and melanin quantification.

2.13 Skin Penetration

Franz cells with porcine skin evaluated permeation/retention of FAI vs PRO-Peptide311 formulations (±atomization/electro-permeation). RhoB-labeled samples were visualized by confocal microscopy.

2.14 Statistics

Data are mean ± SD. ANOVA with $p<0.05$ denoted significance (SPSS 20.0).

3. Results

3.1 PRO-Peptide311 characterization

The PRO-Peptide311 suspension was transparent and pale yellow, with a mean particle size of 34.1 ± 1.42 nm, PDI of 0.185 ± 0.012, and zeta potential of −28.6 ± 2.1 mV. HPLC analysis showed encapsulation efficiencies of 98.6% for glabridin, 54.2% for niacinamide, 88.3% for nonapeptide-1, 99.5% for acetyl dipeptide-1 cetyl ester, and 98.6% for palmitoyl tripeptide-8. Particle size and PDI remained stable after atomization or electronic enhancement, with all PDI values below 0.3, indicating good dispersion. Storage stability tests showed no significant changes over 60 days at various temperatures and sunlight conditions.

3.2 HET-CAM chicken embryo allantoic membrane test

The HET-CAM irritation results are depicted in Figure 1. After 300 seconds of treatment with PRO-Peptide311, no hemorrhage, vascular lysis, or coagulation in the capillaries was

observed, with an ISHET score of 0.07. Upon administration of FAI with an equivalent active ingredients concentration, the CAM exhibited minor hemorrhagic spots, with an ISHET score of 1.28. This observation suggests that PRO-Peptide311 demonstrate a favorable safety profile.

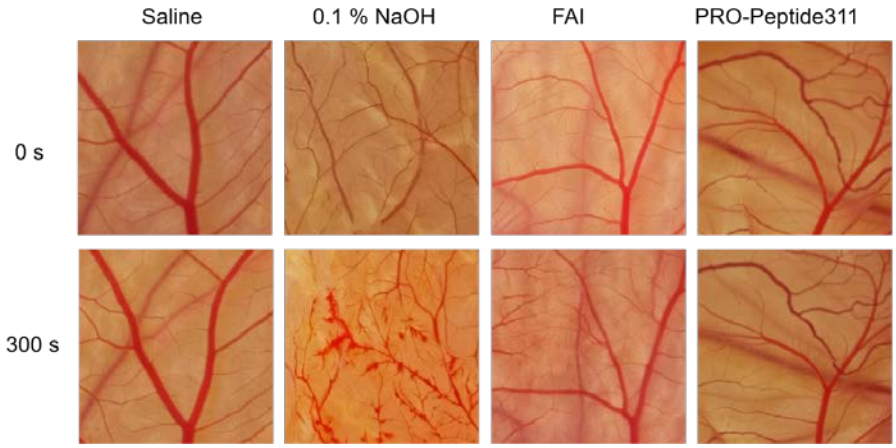

Figure 1. Images of HET-CAM stimulation evaluation results.

3.3 In vitro cytotoxicity

Figure 2 shows the In vitro cytotoxicity of HaCaT/HSF cells treated with FAI or PRO-Peptide311 for 24 h. Both formulations non-toxic at 1.25–10 μg/mL glabridin; at 20 μg/mL, cell viability significantly decreased ($p<0.01$ vs. control). PRO-Peptide311 enhanced HaCaT survival vs. FAI at 20 μg/mL ($p<0.05$), highlighting reduced cytotoxicity via liposomal encapsulation.

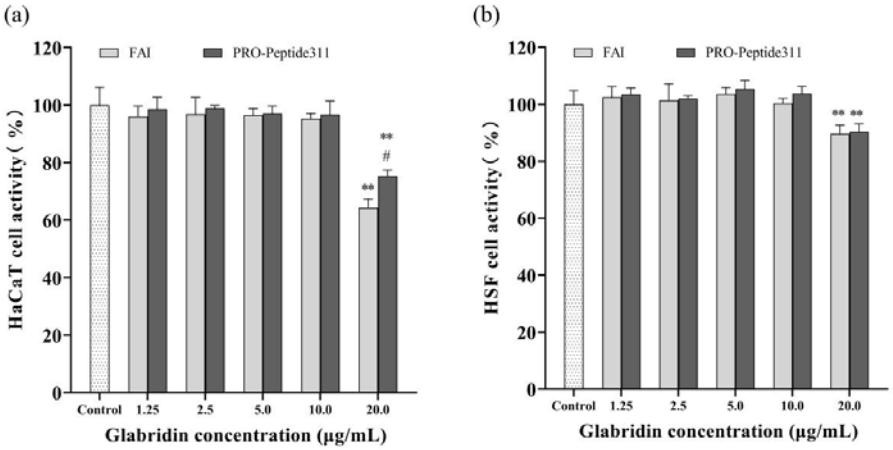

Figure 2. In vitro cytotoxicity of (a) HaCaT cells and (b) HSF cells treated with FAI and PRO-Peptide 311 at various concentrations for 24 h. Compared to the control group, **p < 0.01; compared to FAI at the same concentration, #p < 0.05.

3.4 Cellular uptake

Laser scanning confocal microscopy revealed that B16F10 cells exhibited increased intracellular fluorescence intensity over time when treated with FAI+RhoB and PRO-Peptide311+RhoB (Figure 3a). After 2 h, the PRO-Peptide311+RhoB group showed stronger fluorescence than the FAI+RhoB group. Extending incubation to 4 h further enhanced fluorescence in the PRO-Peptide311+RhoB group, indicating higher cellular uptake. Flow cytometry confirmed that PRO-Peptide311+RhoB increased mean fluorescence intensity by 140.3% and

122.3% after 2 h and 4 h, respectively (Figure 3b). These results demonstrate that PRO-Peptide311 significantly enhances cellular uptake and accumulation of functional ingredients by B16F10 cells.

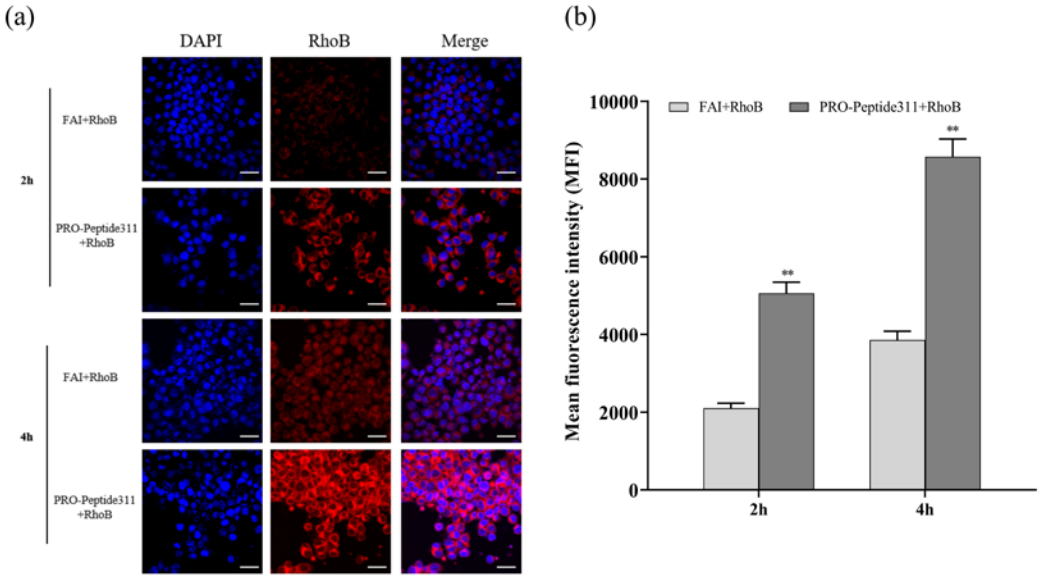

Figure 3. Cellular uptake results. (a) Visualization of cellular uptake of RhoB by B16F10 using a CLSM after incubation for 2 and 4 h. (b) Flow cytometry analysis of relative quantification in B16F10 cells treated with FAI+RhoB and PRO-Peptide311+RhoB. **p < 0.01 vs FAI+RhoB. Mean ± SD, n=5. Scale bar: 50 μm.

3.5 Cellular whitening and antioxidation effect

Figure4a shows that both FAI and PRO-Peptide311 significantly reduced tyrosinase production in B16F10 cells compared to the model group ($p < 0.01$). PRO-Peptide311 at 5.0 μg/mL and 10.0 μg/mL increased tyrosinase inhibition rates more than FAI ($p < 0.01$ or $p < 0.05$), suggesting effective nanocarrier encapsulation. Figure 4b demonstrates that both treatments decreased melanin production ($p < 0.01$), with PRO-Peptide311 showing greater suppression at 5.0 μg/mL and 10.0 μg/mL ($p < 0.01$). Figure 4c confirms that 10.0 μg/mL PRO-Peptide311 exhibited a stronger antioxidant effect than FAI by reducing ROS fluorescent intensity ($p < 0.05$).

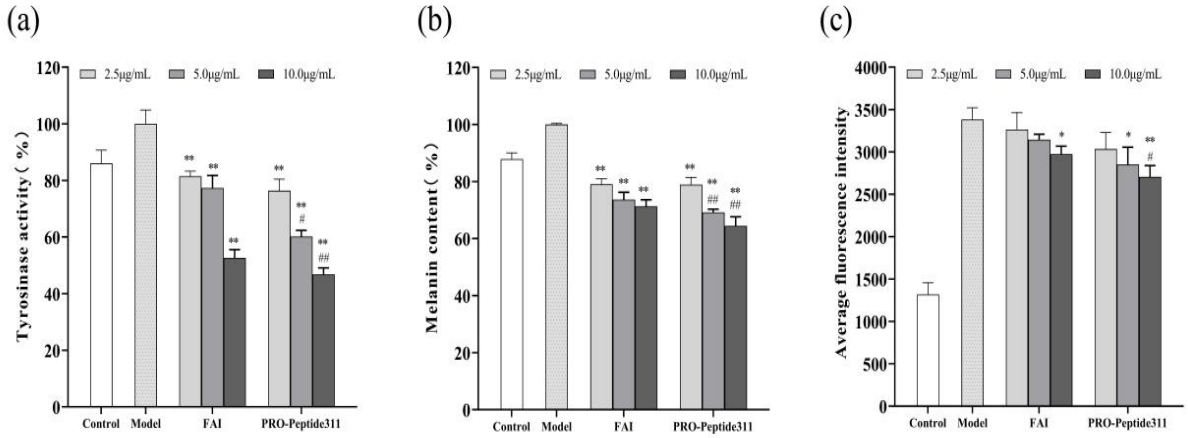

Figure 4. PRO-Peptide311 inhibited B16F10 (a) tyrosinase activity, (b) melanin synthesis, and (c) reduced HaCaT ROS vs. model/FAI (*p<0.05, p<0.01).

3.6 Cellular anti-allergy and anti-inflammatory effect

The results of the effects of FAI (with the same concentration of active ingredients as in the PRO-Peptide311 group) and PRO-Peptide311 on anti-allergy and anti-inflammatory activities are shown in Figure 5. PRO-Peptide311 considerably inhibited the expression of IL-1β (Figure 5a), TNF-α (Figure 5b), TRPV-1 (Figure 5c), and TLSP (Figure 5d) in HaCaT cells, demonstrating better anti-allergic and anti-inflammatory effects than the FAI group at the same concentration.

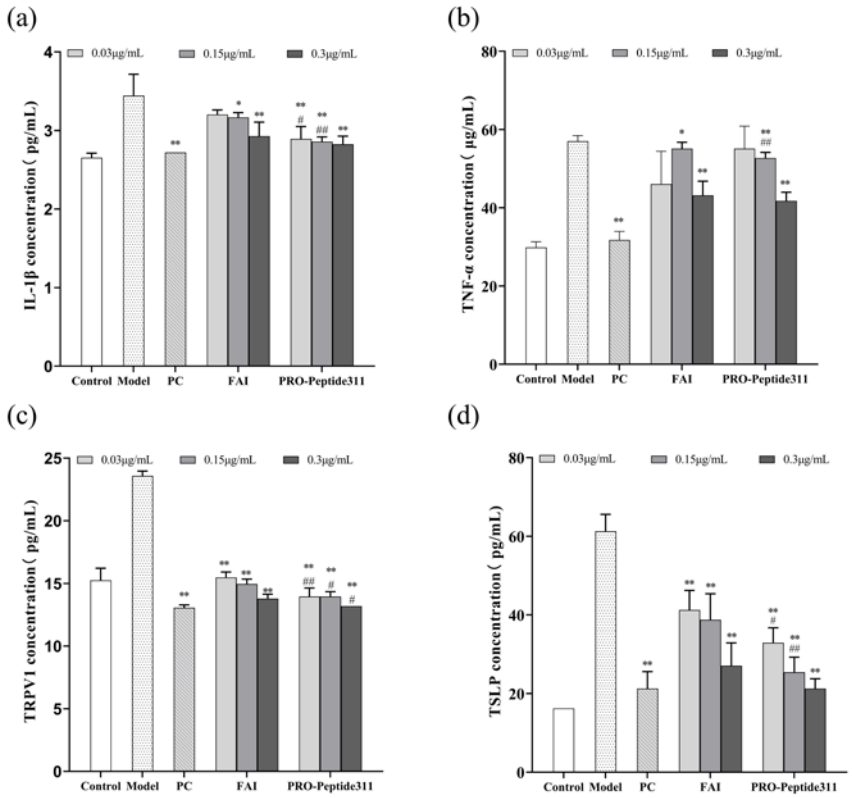

Figure 5. Cellular anti-allergy/inflammatory effects of PRO-Peptide311 vs. FAI on HaCaT cytokines ((a) IL-1β, (b) TNF-α, (c) TRPV1, (d) TLSP) post TNF-α/IFN-γ stimulation (*p<0.05, **p<0.01 vs. model; #p<0.05, ##p<0.01 vs. FAI).

3.7 Whitening efficacy of 3D skin model

The whitening efficacy of PRO-Peptide311 was assessed in a 3D skin model (MelaKutis®) via UVB irradiation. Compared to the NC group, the 5% PRO-Peptide311 sample exhibited significant improvements in apparent chroma (Figure 6a) and a 19.98% increase in apparent luminance (L* value, Figure 6b). Melanin content was reduced by 34.66% (Figure 6c). These results demonstrate that 5% PRO-Peptide311 enhances chroma, luminance, and inhibits melanin synthesis, surpassing the efficacy of the 5% FAI and 7% VC groups. This indicates a stronger whitening effect post-nano-encapsulation.

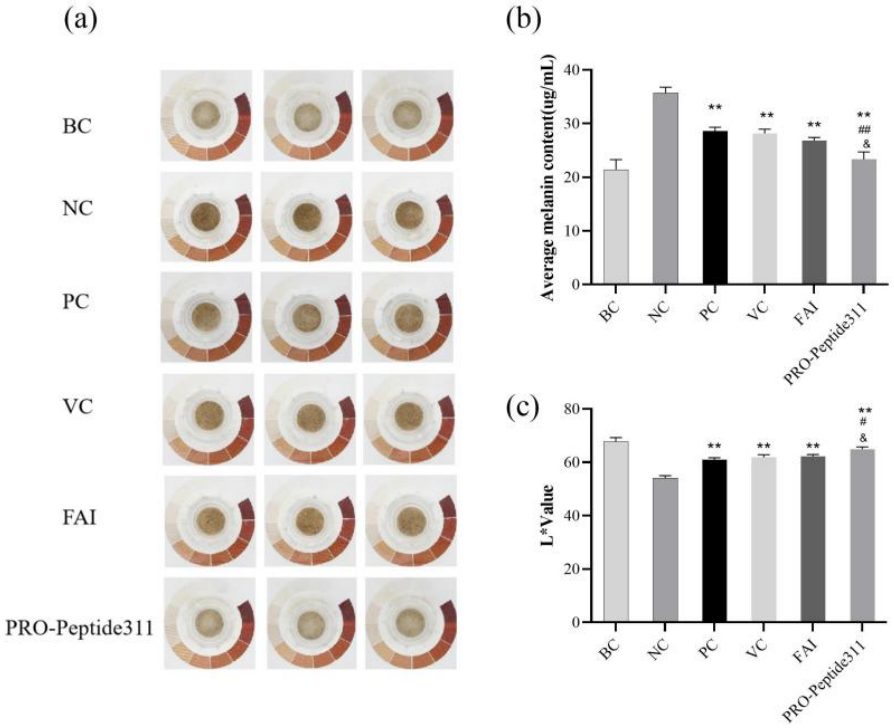

Figure 6. Whitening efficacy of 3D skin models: (a) Apparent chroma results; (b) Apparent luminance (L* value) test results; (c) Melanin content test results. Compared to the NC group, *p < 0.05, **p < 0.01; compared to the 5% FAI group, $^{\#}$p < 0.05, $^{\#\#}$p < 0.01; compared to the 7% VC group, $^{\&}$p < 0.05.

3.8 In vitro skin permeation and retention

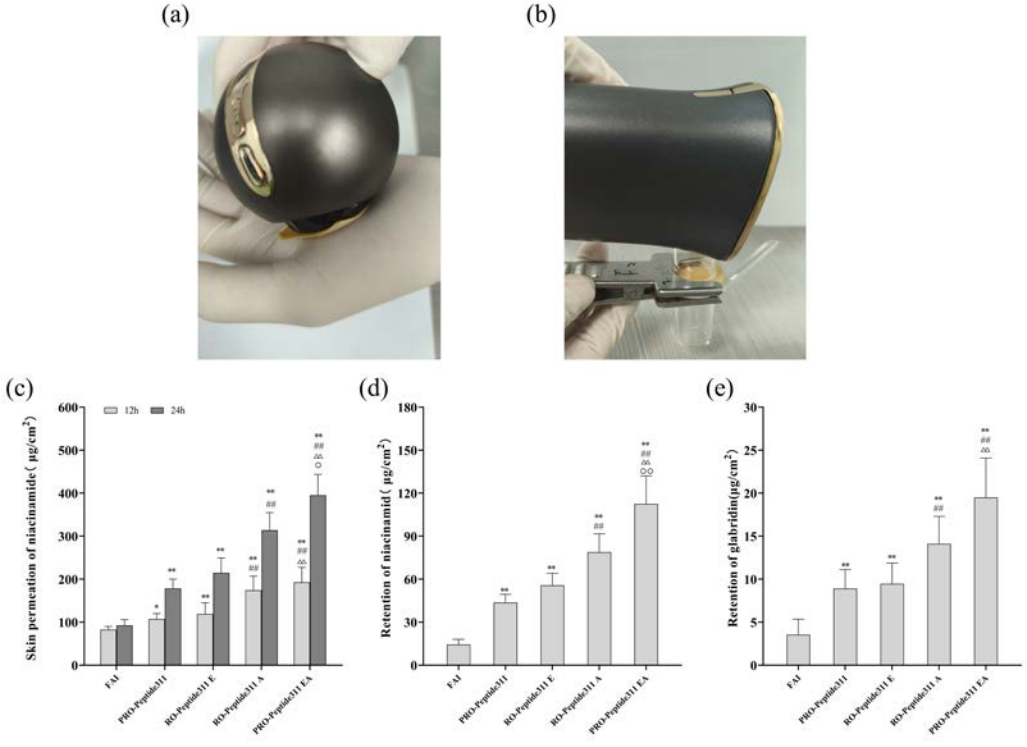

Figure 7. In vitro cumulative skin permeation and retention. (a) Appearance of PRO-Peptide311 combined with electro-enhanced permeation applied on porcine skin for in vitro experiment. (b) Image shows PRO-Peptide311 combined with atomization for in vitro experiment using Franz diffusion cell. Skin permeation of (c) niacinamide and skin retention of (d) niacinamid and (e) glabridin. Compared to FAI, $^{*}p < 0.05$, $^{**}p < 0.01$; compared to PRO-Peptide311, $^{\#\#}p < 0.01$; compared to PRO-Peptide311 E, $^{\triangle\triangle}p < 0.01$; compared to PRO-Peptide311 A, $^{\circ}p < 0.05$.

Figure 7 shows that Franz diffusion assays showed PRO-Peptide311 combinations (especially PRO-Peptide311EA) significantly enhanced niacinamide permeation/retention and glabridin retention vs. FAI ($p<0.01$). PRO-Peptide311EA achieved 450.65% higher glabridin retention, confirming synergistic effects of nanocarriers and physical osmosis for improved drug delivery.

Confocal microscopy with RhoB tracer [18] showed PRO-Peptide311 combinations (especially PRO-Peptide311EA) significantly enhanced skin penetration vs. FAI, with 136-fold higher dermal fluorescence after 4h ($p<0.05$). Depth and intensity increased with treatment time, confirming TPS-enhanced delivery.

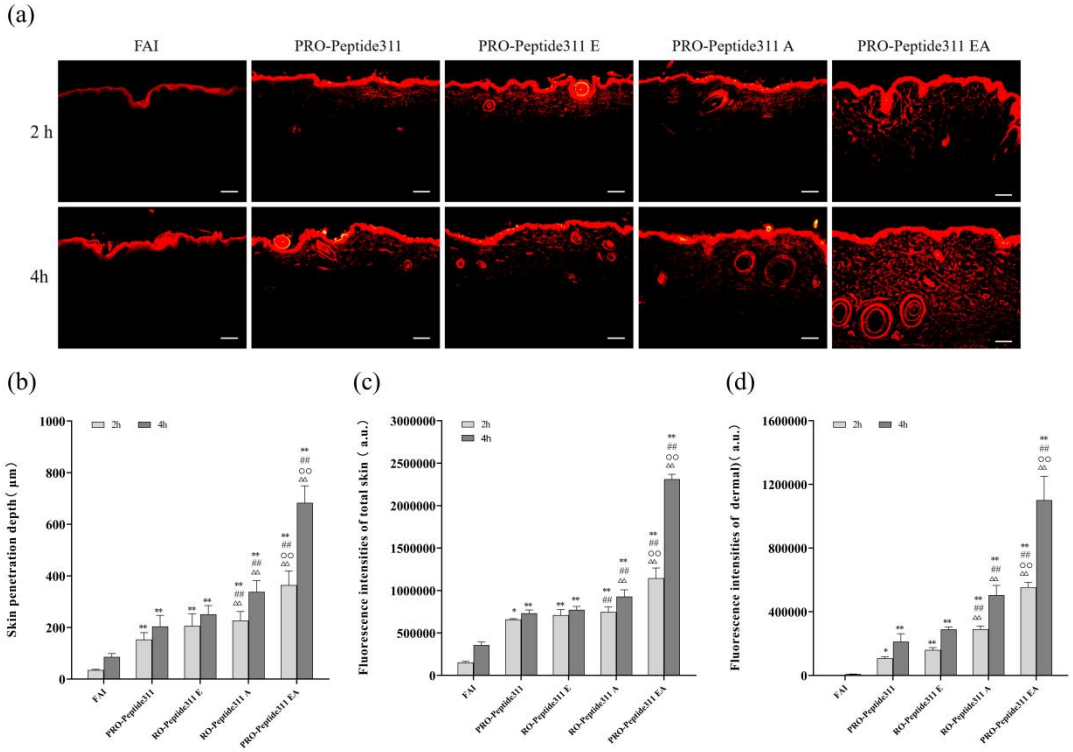

Figure 8. Results of skin penetration biopsy study: (a) Fluorescence distribution in skin tissue; (b) Skin penetration depth; (c) Fluorescence intensities of total skin; (d) Fluorescence intensities of dermal. Compared to FAI, $^{**}p < 0.01$; compared to PRO-Peptide311, $^{\#\#}p < 0.01$; compared to PRO-Peptide311 E, $^{\triangle\triangle}p < 0.01$; compared to PRO-Peptide311 A, $^{\circ\circ}p < 0.01$. Scale bar: 100 μm.

4. Discussion

Nanocarrier drug delivery systems enhance active ingredient permeation through the stratum corneum, improving solubility, stability, and reducing irritation, while enabling sustained

release and synergistic co-delivery of functional ingredients [19-21]. In this study, PRO-Peptide311 formulated with nanocarrier technology significantly inhibited tyrosinase activity, reduced melanin, and enhanced antioxidant, anti-allergy, and anti-inflammatory effects, outperforming an equivalent FAI solution. Atomization increases molecular kinetic energy and skin hydration by transforming liquid medications into aerosol form [22]. Combining nanocarrier technology with physical methods enhances transdermal permeation synergistically [23]. Our study found that PRO-Peptide311 with electro-enhanced permeation and atomization showed superior transdermal permeation, validating TPS technology's effectiveness in enhancing functional ingredient delivery.

5. Conclusion

This study developed a triple permeation system combining nanocarrier, atomization, and electro-enhanced permeation technologies to enhance skin-whitening, repair, and penetration. PRO-Peptide311, encapsulating five active ingredients via liposomal technology, demonstrated ideal stability and low toxicity. Treated cells showed significantly enhanced cellular uptake, reduced tyrosinase activity, melanin expression, ROS levels, and decreased secretion of inflammatory markers, confirming its efficacy in promoting whitening, antioxidation, and anti-inflammatory activities at the cellular level. The whitening effect was validated in a 3D skin model. The TPS system notably improved FAI skin permeation. Further research is needed to explore mechanisms and assess long-term safety and efficacy in clinical trials.

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