

Anti-Aggregation System for the Enhanced Transdermal Delivery of Cell Membrane-Coated Nanoparticles

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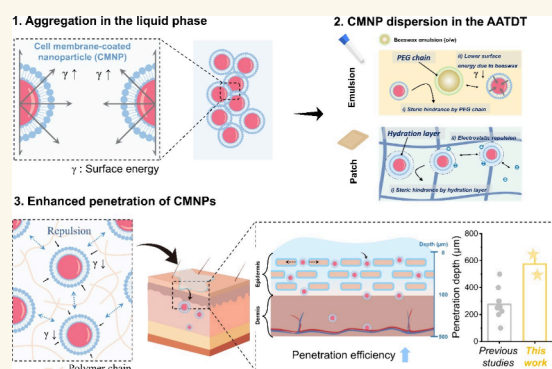
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ABSTRACT: Biomimetic nanoparticles such as cell membrane-coated nanoparticles (CMNPs) are widely used for the *in vivo* treatment of various diseases because they offer a high targeting efficiency, excellent biocompatibility, immune evasion capability, and longer circulation times. However, current noninvasive delivery strategies for CMNPs often face limitations regarding maintaining optimal coating stability and distribution efficiency. In this study, we develop a platform for the transdermal delivery of CMNPs as a noninvasive alternative to injections. Specifically, an antiaggregation transdermal delivery tool (AATDT) was developed using biocompatible beeswax and hyaluronic acid. The proposed system reduced the surface energy of the CMNPs, thus preventing aggregation in the liquid phase and promoting skin permeation via enhanced hydration effects. The CMNPs were delivered to a depth of over 500 μm into the skin tissue without aggregation or systemic toxicity. The proposed AATDT thus offers a versatile, clinically translatable strategy for enhancing the stability and delivery efficiency of CMNPs in noninvasive therapeutic applications.

KEYWORDS: cell membrane-coated nanoparticles, antiaggregation, surface energy, skin penetration, transdermal delivery



1. INTRODUCTION

Cell membrane-coated nanoparticles (CMNPs) designed to mimic the surface properties of living cells have been widely used for *in vivo* biomedical treatment due to their excellent biocompatibility, reduced uptake by macrophages, long circulation lifetime, and effective tumor targeting.^{1,2} These biomimetic nanoparticles adopt a core–shell structure in which the cell membrane uniformly covers the entire nanoparticle surface and can function *in vivo* in a manner similar to living cells due to the presence of membrane proteins (e.g., CD86, CD80, MHC I, and MHC II) on the cell membrane surface.^{3,4} CMNPs are generally delivered through needle injection, which is efficient but has limitations such as patient discomfort, a higher infection risk, and difficulty ensuring controlled and sustained drug release.

In contrast, the transdermal delivery of CMNPs is simple and noninvasive, allowing the nanoparticles to be continuously delivered over a long period with high bioavailability, resulting in few side effects.^{5,6} Despite these potential advantages, few studies have investigated the effective transdermal delivery of CMNPs because the stratum corneum (SC) and epidermis

form a dense barrier that restricts the passage of large molecules and particles,^{7–10} while CMNPs are prone to aggregation due to their high surface energy and hydrophilicity,¹¹ limiting their stability and uniform distribution in topical formulations.^{12,13} In addition, the large molecular weight (>30 kDa), strong negative surface charge (<−30 mV), and hydrophilic nature of CMNPs mean that they are not suitable for passive diffusion through the skin.^{6,14–17} CMNPs can also experience structural destabilization within the skin due to pH fluctuations and enzymatic degradation, reducing the delivery efficiency.^{3,18}

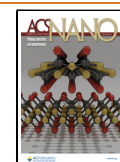
Various delivery platforms, including polymeric or inorganic nanoparticles, have been developed to improve permeation into the skin.¹⁹ In particular, to avoid nanoparticle aggregation,

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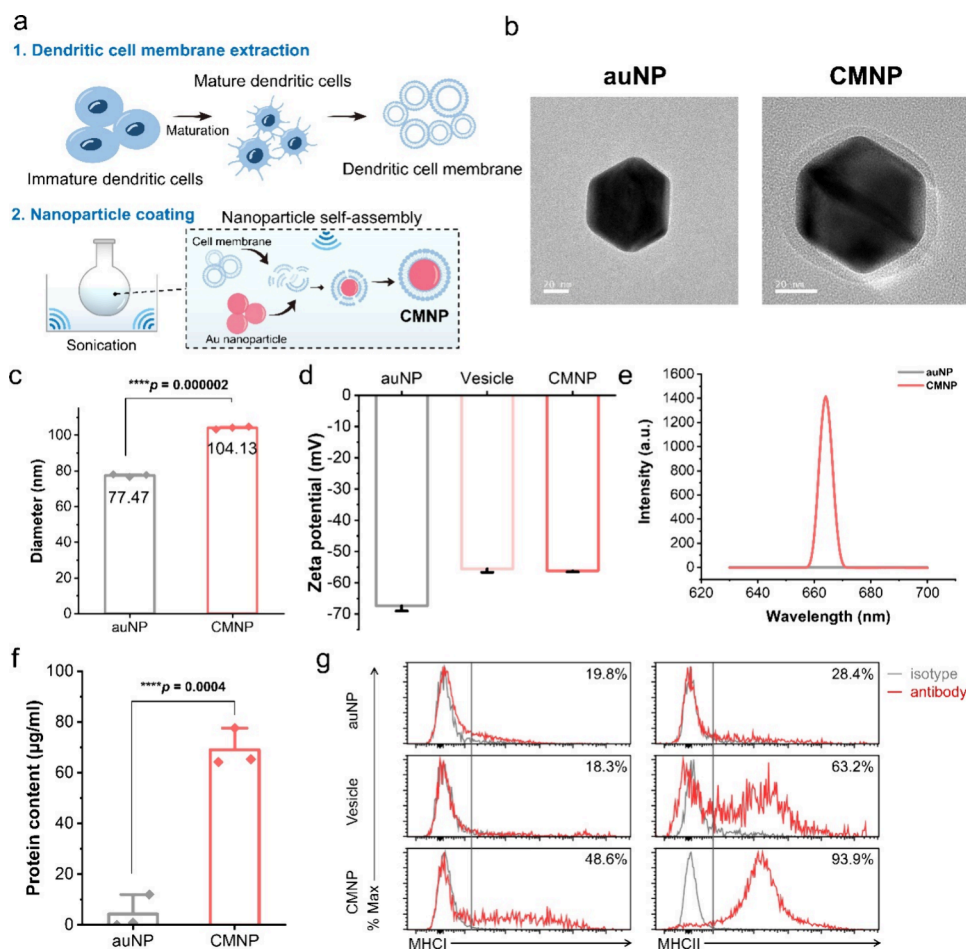


Figure 1. Characterization of CMNPs. (a) Fabrication process for the CMNPs. (b) Representative TEM image of the AuNP and the CMNP. Scale bars: 20 nm. (c) DLS analysis of the AuNPs and CMNPs. (d) Surface zeta potential of the CMNPs. (e) Photoluminescence analysis of the DC membrane coating on the CMNPs. (f) BCA assay results for the protein concentration in the CMNPs. (g) Flow cytometry data showing MHC I and MHC II markers on the CMNPs. Statistical analysis was conducted using one-way ANOVA with Tukey's multiple comparison tests. The data represent the mean $\pm$ SEM and are representative of at least two independent experiments ($n = 3/\text{group}$). $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$.

the size, hydrophobicity, and surface properties of nanoparticles have been modified.^{20,21} Recently, lipid-based systems composed of natural phospholipids or cholesterol have gained attention due to their compatibility with the lipid-rich SC.^{22–25} However, few approaches have simultaneously addressed both nanoparticle aggregation and skin barrier penetration, particularly for fragile, membrane-coated nanostructures such as CMNPs. In addition, the modification of CMNPs for skin penetration is difficult because the bioactive markers present on the surface of CMNPs are essential for their biological function. Therefore, to preserve the biofunctionality of CMNPs while suppressing aggregation and enhancing skin penetration, a suitable strategy is to control the properties of surrounding penetration enhancers such as emulsions or patches, rather than modifying the nanoparticles themselves.

Herein, we designed an antiaggregation transdermal delivery tool (AATDT) for CMNPs. The AATDT supplies moisture to the skin surface, softens the SC, reduces nanoparticle aggregation, improves particle permeability, and controls the hydrophilicity of the particles to improve skin penetration. In this system, biocompatible beeswax (BW) and hyaluronic acid (HA) are employed to enhance penetration by increasing the transport of active ingredients through the SC.²⁶ Emulsions containing BW balance the hydrophilicity and hydrophobicity

of the particles, allowing them to effectively penetrate the skin.^{27,28} In addition, HA has very strong water absorbance, allowing it to hydrate the SC, changing its microstructure and facilitating drug penetration. These changes are reversible, so when the HA is removed, the skin barrier is restored.^{29,30} HA is a hydrophilic polymer, but it contains many hydrophobic domains in its carbon chains, so it can enhance permeability by interacting with components of the SC, including keratin and lipids.^{31,32} Small nanoparticles such as CMNPs tend to reduce their interparticle distance to reduce their surface energy, leading to aggregation, but this can be ameliorated with the use of viscous emulsions or patches.³³ In particular, BW and HA can both create a stabilizing matrix around the nanoparticles to prevent clumping or aggregation by decreasing their surface energy.^{34,35} Based on the dual roles of BW and HA in the AATDT, we successfully delivered CMNPs to a skin penetration depth exceeding 500 μm . This strategy thus represents a clinically translatable, noninvasive platform for the effective delivery of CMNPs by addressing both colloidal instability and physiological barriers in transdermal nanomedicine.

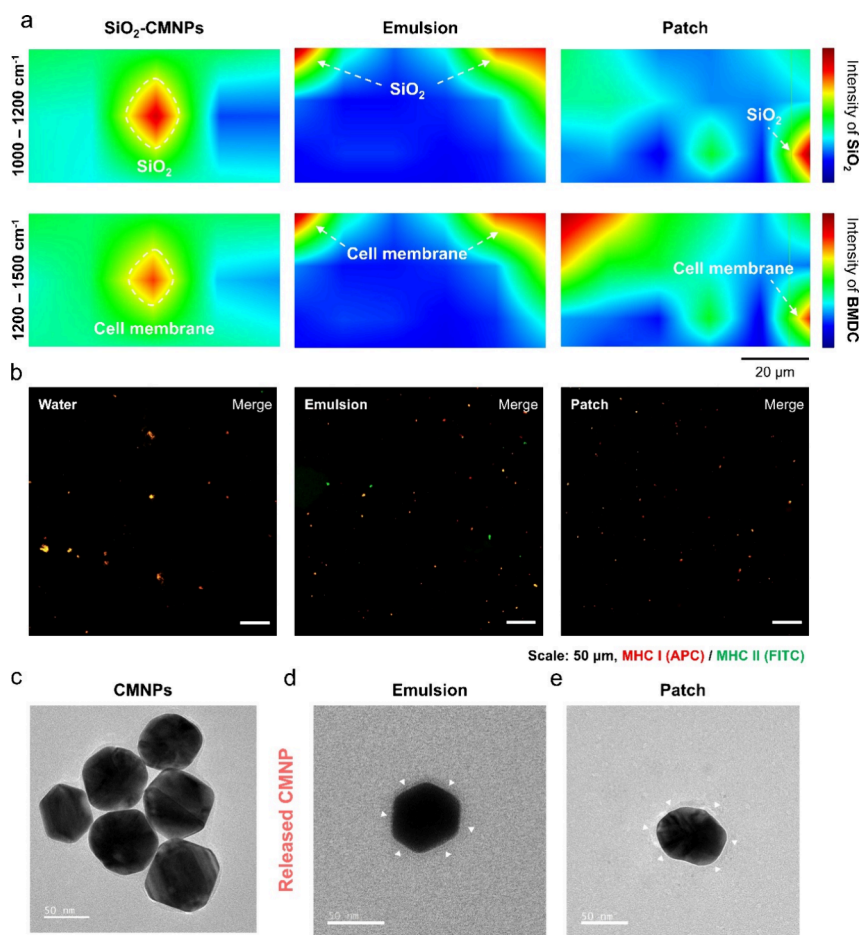


Figure 2. Coating stability of CMNPs in the AATDT. (a) Spatial distribution of SiO₂ and Nile red-stained CMNPs within at a resolution of 1000 to 1200 cm^{−1} and 1200 to 1500 cm^{−1} used to assess the coating stability of the CMNPs in BW and HA. (b) Representative immunostained images of the CMNPs in water, the emulsion, and the patch showing that the cell membrane coating is maintained, with MHC I and MHC II indicated by red and green, respectively. (c) TEM image of pure CMNPs and CMNPs released from the (d) BW emulsion and (e) HA patch. Scale bars: 50 nm.

2. RESULTS AND DISCUSSION

2.1. Characterization of the CMNPs. We used dendritic cells (DCs) differentiated from mouse bone marrow (BM) cells to fabricate the CMNPs. The cell membranes of mature DCs were extracted and sonicated together with Au nanoparticles (AuNPs), causing the cell membrane to degrade and self-assemble on the AuNP surface (Figure 1a). To confirm the successful fabrication of CMNPs, the bare AuNPs and CMNPs were analyzed using transmission electron microscopy (TEM), showing that the cell membranes were completely coated onto the AuNP surface (Figure 1b and Figure S1). The size of the CMNPs was also measured using dynamic light scattering (DLS), with an increase of approximately 27 nm observed after cell membrane coating (Figure 1c). In addition, the surface net charge of the AuNPs, DC membranes, and CMNPs was compared, with the net charge decreasing to approximately −50 mV after coating, similar to that of the DC membranes. After staining the cell membranes with DiD dye, the fluorescence intensity was measured to verify that the cell membranes were completely coated onto the CMNPs.

The presence of the proteins MHC I and MHC II in the DC membranes was confirmed using BCA assays and FACS analysis. When quantitative protein analysis was conducted, almost no protein was detected for AuNPs, compared to 69 μg/mL for the CMNPs (Figure 1f). The percentage values

indicate the proportion of particles showing positive fluorescence signals for each marker. While AuNPs showed small positive peaks with both isotype and antibody staining, likely due to intrinsic autofluorescence,³⁶ CMNPs exhibited significantly higher percentages of MHC I and MHC II than vesicles and AuNPs, suggesting successful incorporation of DC-derived plasma membranes. Unlike vesicles that may include organelle-derived membranes, CMNPs were enriched with DC plasma membranes (Figure 1g). Given that MHC I and MHC II are hallmark features of DC membranes, these findings support that CMNPs are likely well coated with DC membranes.

2.2. Cell Membrane Coating Stability of CMNPs in the AATDT. Raman mapping was used to determine whether the CMNPs maintained their coating within the emulsion and patch in the AATDT. First, the DC membranes were conjugated with Nile red and coated onto the surface of the SiO₂. SiO₂ and Nile Red were utilized as core materials for CMNPs only in Raman spectroscopy analysis, since their Raman peaks did not overlap with those of the sample. The new peak observed between 1200 and 1500 cm^{−1} confirmed the successful coating of the Nile red-stained DC membranes (Figure S2). Raman mapping of the liquid, patch, and emulsion containing the SiO₂–CMNPs was then conducted, with the position of the SiO₂ (1000–1200 cm^{−1}) found to be

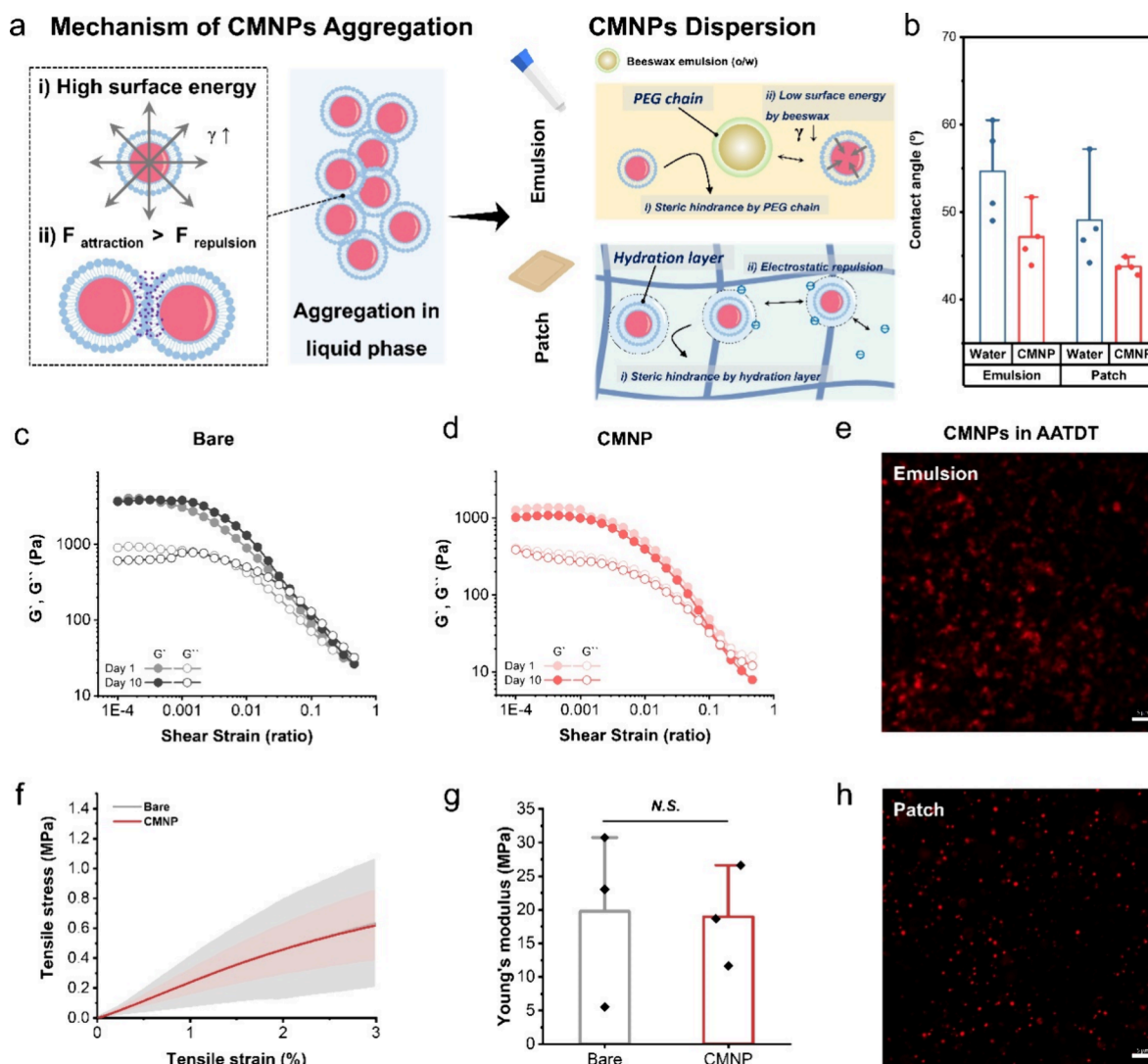


Figure 3. Aggregation prevention using the AATDT. (a) Illustration of the mechanisms associated with CMNP dispersion in the AATDT. (b) Contact angle of water and water mixed with CMNPs measured on the surface of the BW emulsion and HA patch. Storage (G') and loss moduli (G'') of the (c) bare and (d) CMNP-laden BW emulsions. CMNP dispersion in the (e) BW emulsion and (h) HA patch observed using confocal imaging. Scale bars: 5 μm . (f) Tensile stress and (g) Young's modulus for the bare and CMNP HA patches measured using a universal testing machine. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparison tests. The data represent the mean $\pm$ SEM from at least two independent experiments ($n = 3$ or 4/group). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

consistent with the position of the DCs (1200–1500 cm^{-1}), indicating that the coating of DC membranes on the surface of the nanoparticles was stable (Figure 2a). After manufacturing the CMNPs, the membrane proteins MHC I and MHC II were also fluorescently stained with APC and FITC, respectively. When CMNPs were introduced to water, the emulsion, and the patch, these membrane proteins were retained in all cases. Imaging analysis also found that the CMNPs in the AATDT tended to exhibit less aggregation compared to those in water (Figure 2b and Figure S3), with TEM images showing that the CMNP coating was maintained even after release from the emulsion and patch (Figure 2d,e). The thickness of the cell membrane layer on the CMNPs was also confirmed to decrease after storage in the AATDT compared to the CMNPs dispersed in liquid phase (Figure 2c–e and Figure S4).

2.3. Aggregation Prevention and Characterization of the CMNP Emulsion and Patch. Nanoparticles tend to aggregate when they are suspended in liquid due to their high

surface energy, which increases the Van Der Waals forces between the nanoparticles.³⁷ This creates an attraction force that is higher than the repulsion force on the nanoparticle surface and reduces the stability of the nanoparticles in the liquid phase (Figure 3a). However, for effective skin delivery, nanoparticles need to be well dispersed to penetrate the SC. To assist in this, biological waxes such as BW and HA can help reduce the surface energy of nanoparticles while promoting strong electrostatic repulsion and steric hindrance.^{38–41} BW contains fatty acids with long polar chains and hydrophobic properties. When these fatty acids interact with nanoparticles, they can form a low-surface-energy film, weakening the molecular interactions.^{42–44} In contrast, HA contains carboxylate groups that have a negative charge in aqueous environments and can form a hydration layer. It thus contributes to steric and electrostatic stabilization by forming a hydration layer and inducing electrostatic repulsion between negatively charged groups.^{45–47}

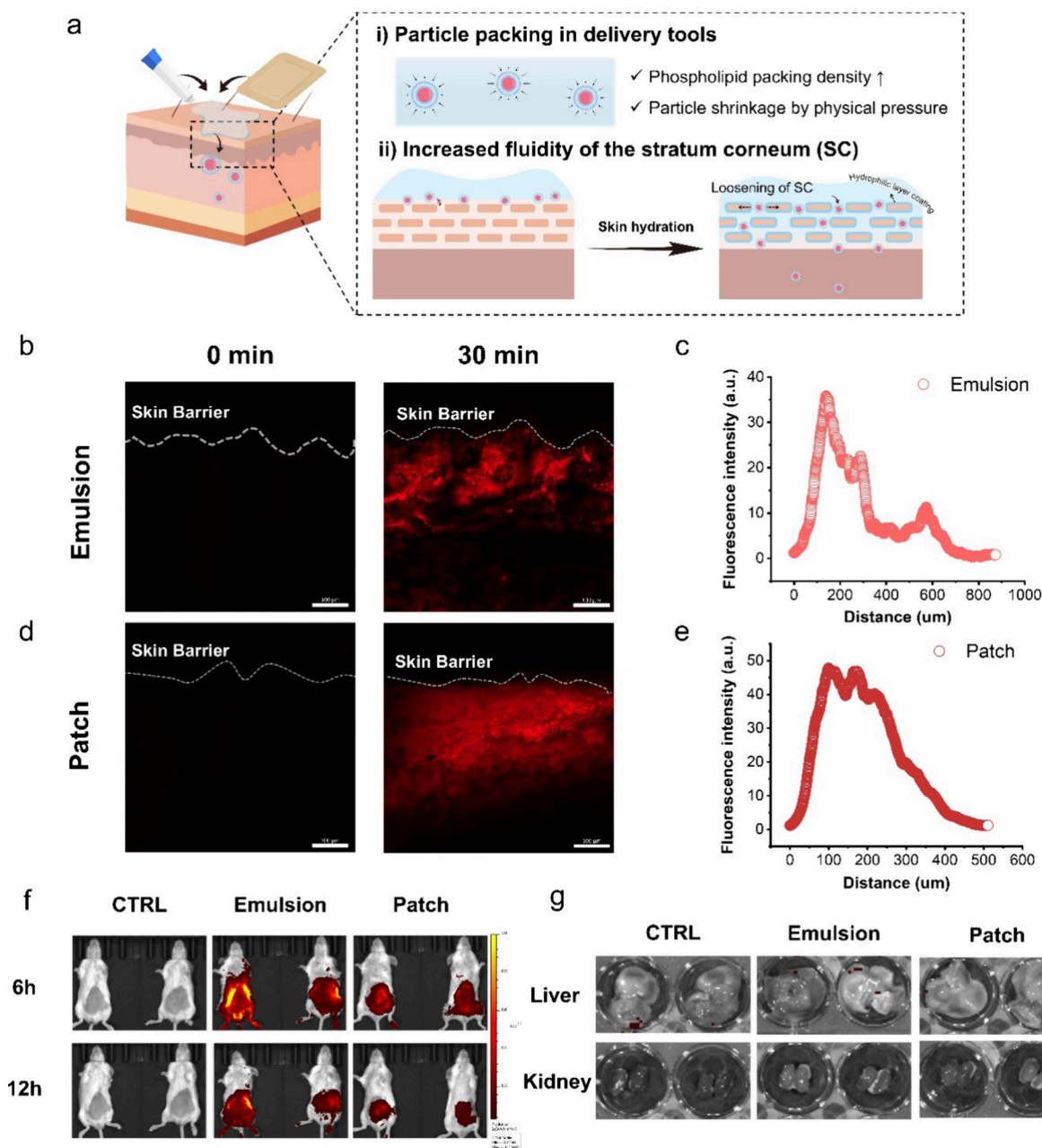


Figure 4. Skin penetration of CMNPs using the AATDT. (a) Representative image of CMNP transdermal delivery using the AATDT. Confocal image of porcine skin for the detection of CMNP penetration using the (b) BW emulsion and (d) HA patch. Scale bars: 100 μm. Penetration depth of CMNPs using the (c) BW emulsion and (e) HA patch measured with ImageJ. (f) NIR fluorescence images of mice 6 and 12 h after using the BW emulsion and HA patch. (g) Quantitative analysis of the fluorescence signal intensity in mouse livers and kidneys.

We prepared a BW-based emulsion and an HA-based patch to deliver dispersed CMNPs into the skin and investigated the change in the surface energy of CMNPs when incorporated into these systems by measuring the contact angle. In particular, we deposited droplets of pure water and CMNP-containing water onto Si wafers coated with either a BW-based emulsion or a HA-based patch and compared the resulting contact angles. According to Young's equation, the interfacial energy between solid and liquid phases can be described as $\gamma_{SL} = \gamma_{SV} - \gamma_{LV} \cos \theta$, where γ , S, L, and V represent the surface free energy, solid, liquid, and vapor, respectively, and θ denotes the contact angle.^{48,49} Based on this equation, when switching from water to the CMNP solution on the same surface, an

increase in θ would indicate an increase in the solid–liquid interfacial energy (γ_{SL}), assuming that the other variables (γ_{SV} and γ_{LV}) remained constant. Conversely, a decrease in θ would suggest a reduction in the interfacial energy. We found that the contact angle decreased from 54.7° to 47.2° in the emulsion system and from 49.1° to 43.8° in the patch system (Figure 3b and Figure S5), suggesting that the AATDT environment effectively lowered the surface energy of the CMNPs, potentially reducing aggregation. The solid–liquid work of adhesion (W_{SL}) can subsequently be determined using the Young–Dupré equation $W_{SL} = \gamma_{LV}(1 + \cos \theta)$. Based on this equation, when the contact angle decreases, the work of adhesion increases, which means that, in the proposed system,

the interaction between the CMNPs and the AATDT increased.^{50,51} An increase in the surface tension of the phospholipid bilayer can reduce the phase transition temperature of phospholipids, promoting the formation of a gel phase in the membrane.^{52,53} As shown in Figure 3b, the interfacial surface energy between the CMNPs and the AATDT decreased, as evidenced by the lower contact angle. This enhanced interfacial interaction likely stabilized the CMNP structure, resulting in an increase in the internal surface tension of the phospholipid bilayer and facilitating the transition to a gel phase.⁵⁴

In the AATDT system, when particles are incorporated into a viscous liquid or solid, agglomeration can lead to inconsistent behavior in properties such as viscosity and the elastic modulus.^{55–57} Therefore, we sought to verify that the particles were evenly dispersed by measuring the viscoelasticity and tensile strength of the emulsion and patch. The storage modulus (G') of the BW-based emulsion was higher than the loss modulus (G''), which indicates resistance to deformation (Figure 3c). The emulsion was subsequently mixed with CMNPs and stored for 10 days, after which the G' and G'' were remeasured. No difference in either modulus from that measured for the pure emulsion was observed, indicating that the CMNPs were stable with no aggregation (Figure 3d). The HA-based patch also maintained its tensile stress and Young's modulus after mixing with CMNPs (Figure 3f,g). To verify that the CMNPs were well dispersed within the AATDT, the cell membranes were stained with DiD dye before the fabrication of the CMNPs and then incorporated into the AATDT. Confocal imaging confirmed that the CMNPs were well dispersed within the emulsion and patch, indicating that the cell membrane coating was maintained (Figure 3e,h). In addition, after mixing in the CMNPs, neither the emulsion nor the patch exhibited a wavelength shift or new peak in the Fourier-transform infrared (FTIR) spectroscopic analysis, suggesting that both platforms could physically stabilize the CMNPs (Figure S6).

2.4. Ex Vivo Skin Penetration Efficiency of the CMNP Patch and Emulsion. As shown in Figure 4a, AATDT offered two major advantages in delivering CMNPs into the skin: decreasing the aggregation of CMNPs, which can reduce skin penetration, and hydrating the skin, thus loosening the SC and enhancing permeability via hydrophobic interactions.

We employed CMNPs stained with DiD in the AATDT and tested their skin penetration *ex vivo* after leaving them on porcine skin for approximately 30 min. Cross-sectional confocal imaging revealed that the CMNPs were able to penetrate the skin in just 30 min (Figure 4b,d), with the majority found at a depth of approximately 200 μm . However, some CMNPs reached a depth of more than 500 μm (Figure 4c,e), which is the thickness of the dermis, indicating that they could potentially enter blood or lymphatic vessels for delivery.^{58–60} These penetration depths were particularly promising compared to previously reported transdermal drug delivery systems (Table S1). Subsequently, we performed *in vivo* tests using the AATDT with CMNPs labeled with near-infrared fluorescent cyanin dye (DiR), showing that they remained *in vivo* circulation for 12 h (Figure 4f). No fluorescence signals were observed in the liver or kidneys, indicating that the CMNPs were not dispersed systemically and thus had a low likelihood of off-target toxicity (Figure 4g). We also assessed the biocompatibility of BW and HA using human dermal fibroblasts (HDFs) as representative skin cells,

with no significant cytotoxicity observed (Figure S7). Given their excellent biocompatibility, BW and HA are thus promising candidates for the transdermal delivery of CMNPs.

3. CONCLUSIONS

In the present study, we developed an AATDT to enhance the skin delivery of CMNPs. By incorporating biocompatible BW and HA, the AATDT formulation effectively prevented nanoparticle aggregation and maintained the stability of the phospholipid membrane coating by reducing the surface energy of the CMNPs. These interactions, in conjunction with the hydration of the SC, enhanced the transdermal penetration of the CMNPs, reaching depths of up to 500 μm . This means that the CMNPs readily penetrated the SC (8–20 μm) and dermis (~600 μm), achieving a promising penetration depth for transdermal drug delivery.⁶¹ Our results demonstrate the feasibility of using the AATDT as a robust strategy for the noninvasive delivery of biomimetic nanoparticles. This work also provides valuable insights into design principles for transdermal systems involving membrane-coated nanoparticles and may pave the way for broader applications in nanomedicine and vaccine delivery.

4. METHODS

4.1. Mice. Six- to eight-week-old specific pathogen-free female C57BL/6 (B6) mice were purchased from Orient Bio. All experiments were conducted with the approval of the International Animal Care and Use Committee at Yonsei University, following the Laboratory Animal Act overseen by the Korean Ministry of Food and Drug Safety. This act aims to improve the ethics and reliability of animal testing by ensuring the appropriate administration of laboratory animals and testing (Permit No. IACUC-A-202404-1841-02).

4.2. DC Differentiation and DC Membrane Derivation. DCs were differentiated from bone marrow cells isolated from the femurs of naive C57BL/6 mice. Red blood cells (RBCs) from the bone marrow were lysed using ACK lysis buffer (Gibco Laboratories). After RBC lysis, the resulting single-cell suspensions were cultured in RPMI medium (Corning) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific), 1% penicillin/streptomycin (P/S) (Thermo Fisher Scientific), and 20 ng/mL recombinant murine GM-CSF (JW Creagene). The culture media were replenished on days 3 and 6. To mature the DCs, 20 ng/mL LPS (Sigma-Aldrich) was added to the culture medium for the final 6 days. Seven days postculture, nonadherent cells were collected and pelleted via centrifugation at 2000 rpm for 5 min at room temperature. The DCs were then resuspended in 1 $\times$ PBS (pH 7.4) containing an EDTA-free Pierce protease inhibitor mini tablet (PIT) (Sigma-Aldrich, 1 tablet per 10 mL of solution).

A 1 mL solution of the DC membranes containing 1 $\times$ 10⁶ cells was disrupted using three freeze–thaw cycles (−70 $^{\circ}\text{C}$ and room temperature, respectively). The solutions were centrifuged at 6000 rpm for 5 min and washed three times with 1 $\times$ PBS containing PIT to obtain pure cell membranes. The DC membranes were then resuspended in deionized water and sonicated at an amplitude of 20% for 60 cycles (3 s of sonication, followed by 2 s on ice) using an ultrasonic processor (VC-505, Sonics & Materials) for a total of 3 min in a glass vial.

4.3. Preparation of the CMNPs. The obtained DC membrane vesicles (1 $\times$ 10⁶) were added to 1 $\times$ 10⁶ AuNPs. The DC membrane and nanoparticle mixture was sonicated in an ice water bath for 3 min (150 W). Uncoated DC membrane fragments and vesicles were eliminated via centrifugation at 10,000 rpm for 5 min, and the CMNPs were stored at 4 $^{\circ}\text{C}$ in deionized water.

4.4. Preparation of the AATDT Emulsion and Patch.
Emulsion. BW (Sigma-Aldrich) was dissolved in olive oil (Daejung) at a concentration of 10% (w/v) at 60 $^{\circ}\text{C}$, and Tween 80 (Sigma-Aldrich) was dissolved in deionized water at a concentration of 1.5%

(w/v). The completely dissolved BW and Tween 80 were added to a vial at a weight ratio of 1:1 and emulsified using a homogenizer (T18 digital ULTRA TURRAX, IKA, Staufen, Germany) at 25,000 rpm for 5 min to prepare the BW emulsion. After homogenization, CMNPs were added to the BW emulsion and stirred vigorously for 10 min.

Patch. HA was dissolved in a 10^{-4} M NaOH solution at a concentration of 3% (w/v) overnight. Following this, 1,4-butanediol diglycidyl ether (BDDE, molecular weight = 202.25 Da, Sigma-Aldrich) was mixed into the HA solution prepared at a concentration of 2% (v/v) and cross-linked at 40 °C for approximately 4 h. The prepared solution was frozen overnight and then lyophilized for 2 days. The CMNP-integrated patch was manufactured by adding CMNPs before freezing the solution.

4.5. Characterization of the CMNPs. The formation of CMNPs was confirmed using scanning TEM (JEM-ARM-200F [NEOARM], JEOL), while the surface zeta potential and hydrodynamic diameter of the particles and vesicles were measured using DLS (SZ-100, Horiba). The cell membrane-coated AuNPs were stained with DiD reagent and the fluorescence intensity was measured based on the photoluminescence (FP-8300, JASCO). The proteins present in the CMNPs were quantified using a bicinchoninic acid assay kit (Thermo Fisher Scientific).

To detect the presence of MHC I and MHC II, the CMNPs were plated in 96-well round-bottom plates in PBS supplemented with 2% FBS and stained with fluorochrome-conjugated antibodies for 20 min at 4 °C. Fluorochrome-conjugated antibodies against MHC I (AF6-88.5, #116517) and MHC II (M5/114.15.2, #107645) were obtained from Biolegend. APC Mouse IgG2a, κ isotype control antibody (Biolegend, #400219) and Brilliant Violet 785 Rat IgG2b, κ isotype control antibody (Biolegend, #400647) were used as isotype controls for MHC I and MHC II, respectively. Due to their small size, two or three CMNPs could aggregate during the staining process. After washing with PBS containing 2% FBS, aggregated CMNPs were detected and gated to assess antibody binding. Stained samples were analyzed on a CytoFLEX LX (Beckman Coulter), and data were processed using FlowJo software (Tree Star).

The DC membranes were also stained with DiD, and images of CMNPs in the AATDT were observed using a confocal laser scanning microscope (LSM 980, Carl Zeiss) with a 10 $\times$ objective. FTIR spectroscopy (VERTEX 70, Bruker) was performed to identify the differences of chemical bonds between the emulsion and patch with and without CMNPs.

4.6. Mechanical Properties of the AATDT. The rheological properties of the emulsion were measured with a rheometer (MCR 302, Anton Paar) using 8 mm-diameter parallel plates, and rheological tests were performed in frequency-sweep mode at a shear strain of 0.1 to 10% to measure the storage and loss moduli of the BW emulsion. The tensile properties of the patch were measured using a universal testing machine (Model 3366, Instron), and samples ($n = 3$) were tensioned at a displacement rate of 5.00 mm/min until failure occurred.

4.7. Effect of the AATDT on Cell Viability. HDF cells were cultured in a 100 mm culture dish (SPL, Pocheon-si, Korea) at 37 °C with 5% CO₂. The culture medium was exchanged every 3 days when a confluency of 95% was reached. The HDF cells were detached using 0.05% trypsin-EDTA for 3 min and centrifuged for collection. The cells were then seeded at a density of 10^4 cells/cm² and treated the next day with either HA or BW for 24 h. Cells were treated with 10% CCK-8 reagent (Dojindo, Kumamoto, Japan), and the absorbance at a visible wavelength of 450 nm was measured using a SpectraMax 340 PC microplate reader (Molecular Devices, San Jose, CA, USA). The cells were treated with 10% DMSO as positive control.

4.8. CMNP Coating Stability Observed Using Raman Spectroscopy. The coating stability of the CMNPs in the AATDT was observed using Raman spectroscopy (XploRA PLUS, HORIBA, France) with a 10 $\times$ objective (NA = 0.25), a 532 nm laser, and an 1800 g mm⁻¹ grating spectrometer. An excitation spot with a diameter of 2.6 μ m was used under a laser intensity of approximately 75 mW. Crystalline silicon, with a peak at 520 cm⁻¹, was employed as the calibration standard. To obtain high-resolution spectra, each specimen

was exposed to the laser for 60 s, and the analysis was repeated three times. Mosaic mapping was performed for the large-area monitoring of AATDT. The unit pixel size was $300 \times 300 \mu\text{m}^2$. The DC membranes were stained with 10 μ M of Nile red and then coated on the surface of the AuNPs. The spectra for the pure CMNPs, CMNPs with BW, and CMNPs with HA were collected at 800–1900 cm⁻¹ and the results summarized as a heatmap.

4.9. Skin Penetration Measurements. Emulsion and patches containing CMNPs dyed with DiD were placed on porcine skin for about 30 min. Prior to this, the patches were moistened with deionized water. A cross-section of the porcine skin was then removed, and the penetration of the CMNPs was observed using a confocal laser scanning microscope (LSM 900, Carl Zeiss) with a 10 $\times$ objective, with the penetration depth measured using ImageJ software.

In vivo skin penetration imaging was conducted using the IVIS Spectrum imaging system (PerkinElmer). Emulsion and patches containing CMNPs dyed with DiD were placed on mouse skin for 2 h. After the removal of the patches and emulsion, the mice were returned to their cages. Six or 12 hours after the application, the mice were anesthetized with isoflurane and placed in an imaging chamber in a supine position. The number of photons was quantified using the IVIS system with an exposure time of 1–3 min.

4.10. Quantification and Statistical Analysis. Statistical analysis was performed using Origin 2018, and comparisons between multiple groups were conducted using one-way ANOVA with Tukey's posthoc tests.

ASSOCIATED CONTENT

Data Availability Statement

Data supporting the findings in the present study are available in the article or its Supporting Information.

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsnano.5c15139>.

TEM images of aggregated CMNPs, Raman spectra of nanoparticles and CMNPs, confocal images showing cell membrane coating, CMNP diameter measured by ImageJ, contact angle data showing surface energy, FTIR spectra of emulsion and patch, cell viability of BW and HA, and comparison table of penetration depths (PDF)

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Author Contributions

¹B.C., Y.L., and D.K. contributed equally to this work as cofirst authors. B.C., Y.L., and D.K. contributed to designing the study and performing experiments as well as writing the manuscript under the guidance of S.L., S.-J.H., and J.H. T.K., S.J., and W.C. contributed to the *in vitro* experiments and analyzed the data. W.-G.K. provided resources. All authors have reviewed and approved the manuscript.

Notes

The authors declare no competing financial interest.

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