

ROS-scavenging microneedles loaded with *Portulaca oleracea* L.-derived exosomes for atopic dermatitis therapy

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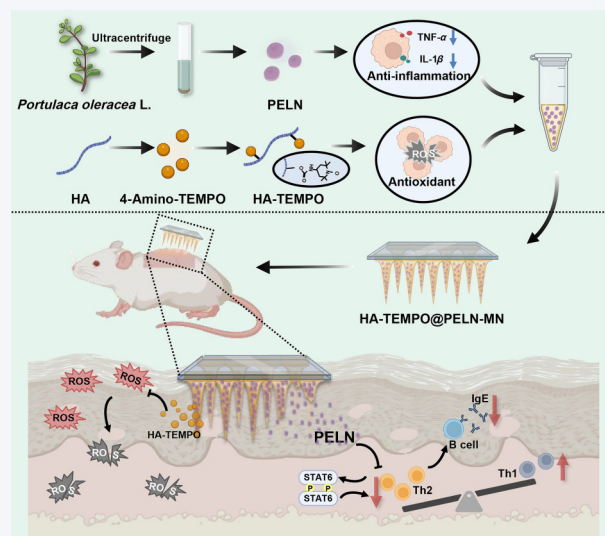
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ABSTRACT: Atopic dermatitis (AD), a chronic inflammatory skin disorder, remains a major therapeutic challenge in clinical practice. Its pathogenesis is primarily driven by dysregulated T helper 2 (Th2) immune responses and pathological accumulation of reactive oxygen species (ROS). Here, we demonstrated that *Portulaca oleracea* L.-derived exosome-like nanoparticles (PELNs) exerted potent anti-inflammatory effects on skin-associated cells. However, PELNs have limited antioxidant activity and poor skin barrier penetration. To address these limitations, we developed a dissolvable microneedle (MN) patch incorporating 4-amino-2,2,6,6-tetramethylpiperidinyloxy (4-amino-TEMPO) conjugated with hyaluronic acid (HA-TEMPO) as both an ROS-scavenging agent and a needle-forming matrix, combined with PELN for synergistic therapy. *In vivo* studies showed that this MN system (designated HA-TEMPO@PELN-MN) effectively attenuated cutaneous oxidative stress and suppressed pathological skin crusting. Notably, the HA-TEMPO@PELN-MN restored Th1/Th2 immune balance. Overall, the MN patch combines excellent biocompatibility, rapid drug release, and efficient delivery, offering a safe and controlled strategy for AD treatment.

KEYWORDS: atopic dermatitis, *Portulaca oleracea* L., exosomes, microneedles, reactive oxygen species



1 Introduction

Atopic dermatitis (AD), also referred to as eczema, affects 15%–20% of children and up to 10% of adults [1, 2]. AD is a chronic inflammatory skin disorder arising from a complex

interplay of immune dysregulation, cutaneous oxidative stress, and epidermal barrier dysfunction. The acute phase is characterized by T helper 2 (Th2) polarization with elevated IL-4 and IL-13 secretion, which activates signal transducer and activator of transcription 6 (STAT6) signaling, driving IgE class switching in B cells and the production of antigen-specific IgE [3]. In contrast, chronic AD lesions display Th1-skewed immunity with marked overexpression of interferon- γ (IFN- γ) [4]. Excessive accumulation of reactive oxygen species (ROS) further contributes to disease progression [5]. Pathological scratching in acute AD exacerbates oxidative stress, inducing cellular mechanical sensitization and amplifying inflammation via ROS-mediated pathways [6]. Consequently, therapies targeting inflammation alone may be

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insufficient to address AD's multifactorial pathology [7]. Thus, interrupting the cycle between oxidative stress and inflammation via ROS scavenging represents a promising therapeutic strategy [8].

Current clinical management of AD primarily relies on glucocorticoids, immunosuppressants, and targeted small-molecule agents, including the IL-4 receptor antagonist dupilumab [9], the IL-31 receptor-blocking monoclonal antibody nemolizumab [10], and upadacitinib, an orally administered Janus kinase 1 (JAK1)-selective inhibitor [11]. Although these therapies form the cornerstone of AD treatment, they are often limited by suboptimal efficacy, safety concerns, or inadequate long-term disease control [12], highlighting the need for complementary or alternative strategies. *Portulaca oleracea* L., a medicinal plant with well-documented nutritional and pharmacological properties [13], has shown therapeutic potential in AD through anti-inflammatory effects and immune cell regulation [14–16]. *Portulaca oleracea* L.-derived exosome-like nanoparticles (PELNs) have demonstrated favorable biocompatibility and bioactivity due to their distinctive physicochemical properties [17]. With intrinsic anti-inflammatory and immunomodulatory functions, PELNs represent a novel complementary and alternative nanotherapeutic strategy for the treatment of inflammatory skin disorders.

Pathological damage in AD predominantly occurs at the epidermal-dermal junction, while the stratum corneum generally permits penetration of molecules smaller than 300 Da [18]. PELNs exhibit limited transdermal delivery due to their large size, low lipophilicity, and poor deformability [19]. Dissolving microneedles (MNs) offer an effective transdermal delivery strategy, enabling painless, minimally invasive penetration of the stratum corneum [20]. Upon insertion, the MNs dissolve, releasing encapsulated therapeutic agents directly into the epidermal and dermal layers. Thus, dissolvable MNs provide an efficient platform for delivering

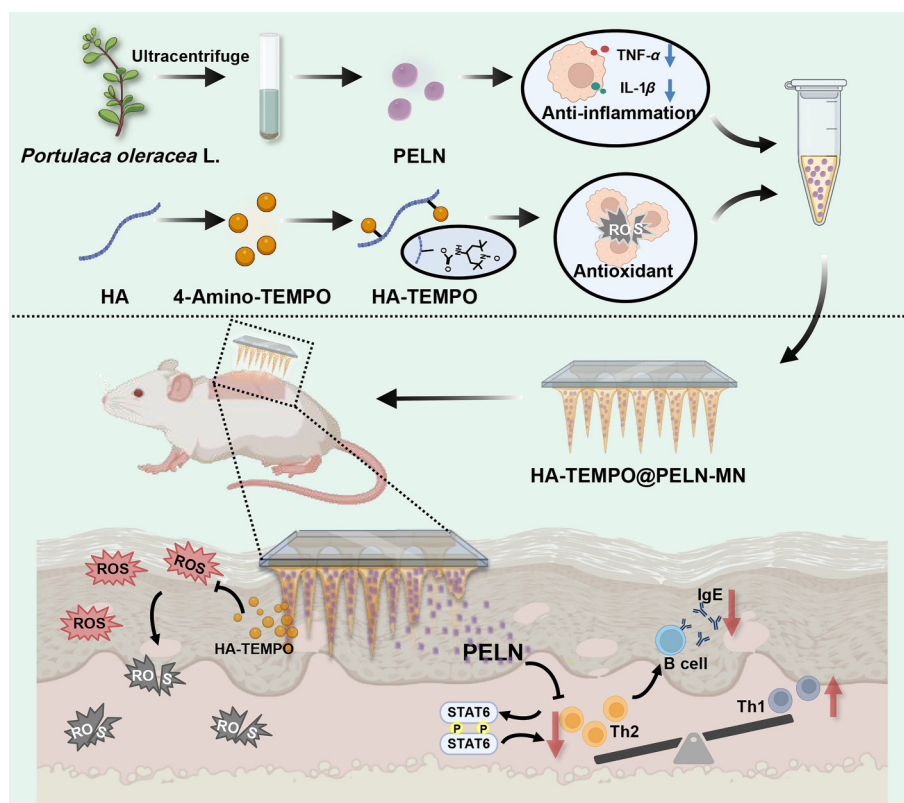
PELNs to both skin layers. Hyaluronic acid (HA), a naturally derived polysaccharide approved by the U.S. Food and Drug Administration for dermal filler applications, has emerged as a promising material for dissolvable MN fabrication owing to its favorable biocompatibility, biodegradability, low immunogenicity, and mechanical robustness.

Thus, we developed a therapeutic system, termed HA-TEMPO@PELN-MN, comprising 4-amino-TEMPO conjugated with HA (HA-TEMPO) as both an ROS scavenger and PELN carrier, and PELNs as synergistic microenvironment regulators. The nitroxide radical compound 2,2,6,6-tetramethylpiperidinyloxy (TEMPO) efficiently scavenges ROS through stable adduct formation with free radicals [21], and effectively protects zebrafish embryos, larvae, and rat models from peroxidative damage and inflammatory cascades (Scheme 1) [22–24]. The MN degradation facilitates the release of PELNs into the epidermal and dermal layers, reducing skin crusting and modulating Th2 cell ratios *in vivo*. Furthermore, HA-TEMPO@PELN-MN treatment significantly modulated the expression of phosphorylated-STAT6 (p-STAT6). Collectively, these findings suggest that HA-TEMPO@PELN-MN constitutes an effective and safe therapeutic approach for AD management.

2 Experimental

2.1 Materials and animals

4-Amino-TEMPO and phenyl chloroformate were obtained from Aladdin Industrial Corporation (China). HA (Mw < 10 kDa) was obtained from Bloomage biotechnology Co., Ltd. (China). Polyvinyl pyrrolidone K90 (PVP-K90) was purchased from BASF (Germany). IL-4, IL-13, IFN- γ , IgE enzyme-linked immunosorbent



Scheme 1 Schematic illustration of preparation and application of HA-TEMPO@PELN-MN.

assay (ELISA) kits were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. (China). High glucose Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS) were obtained from Gibco BRL (USA). Cell counting kit-8 (CCK-8) and bicinchoninic acid (BCA) protein assay kit were obtained from BOSTER Biological Technology Co., Ltd (China). The radio immunoprecipitation assay (RIPA) buffer, ROS assay kit, and paraformaldehyde were purchased from Beyotime Biotechnology (China). 2,4-Dinitrochlorobenzene (DNCB) was obtained from Sigma (USA). Total RNA isolation kit, ChamQ blue universal SYBR qPCR master mix, HiScript IV RT supermix for quantitative polymerase chain reaction (qPCR) were obtained from Vazyme biotechnology (China). The p-STAT6 antibody (#AF3301) and IL-4 antibody (#AF5142) were purchased from Affinity (China). The STAT6 antibody (#66717-1-Ig), β -actin antibody (#66009-1-Ig) were purchased from Proteintech (China). 4-HNE antibody (#bs-6313R), anti-8-OHdG antibody (#bs-1278R), anti-IFN- γ antibody (#bs-0480R) were purchased from Bioss (China). FITC Rat Anti-Mouse CD4 (#553046), APC Rat Anti-Mouse IFN- γ (#562018), and PE Rat Anti-Mouse IL-4 (#562044) were purchased from BD Biosciences (USA). PerCP/Cyanine5.5 anti-mouse CD3 (#100327) was purchased from Biolegend (USA).

Female Balb/c mice (weighing 15–17 g for *in vivo* therapeutic studies) were purchased from SLAC Laboratory Animal Co., Ltd. (Shanghai, China). All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Zhejiang Chinese Medical University and approved by the Animal Ethics Committee of Zhejiang Chinese Medical University (IACUC-20221121-01).

2.2 Isolation of PELN

All *Portulaca oleracea* L. were bought from Ningbo Mingbei Traditional Chinese Medicine Co., Ltd. (China). The purslane samples were thoroughly rinsed under running water and then soaked in 4-Morpholineethanesulfonic acid (MES) buffer overnight. Subsequently, the purslane samples were homogenized using a blender. To eliminate plant fibers and large particles, sequential centrifugation was performed at low speeds (1000 g for 10 min followed by 3000 g for 30 min). The supernatant was then subjected to high-speed centrifugation (10,000 g for 1 h) to remove larger debris and intact organelles. Finally, ultracentrifugation (100,000 g for 1 h) yielded a pellet containing PELN. The pellet was resuspended in sterile PBS and purified using Exo-spin™ Midi Columns (#EX04-20, Cell Guidance Systems, UK). Fractions 7–12 were collected and pooled according to the Exo-spin™ Midi Columns instruction manual, yielding approximately 3 mL of highly purified PELNs.

2.3 Characterization of PELN

The structural morphology of PELN was examined by transmission electron microscopy (TEM; Hitachi, Japan) following standard protocols. Briefly, 10 μ L of the isolated PELN suspension was applied to formvar/carbon-coated copper grids (200 mesh), incubated for 3 min at room temperature, and negatively stained with 2% uranyl acetate. Grids were air-dried prior to TEM imaging at 80 kV. For nanoparticle size distribution analysis, samples were diluted in PBS and measured using nanoparticle tracking analysis (NTA) on a Nano particle tracking analyzer (Particle Metrix).

Lipidomic, proteomic, and miRNA sequencing analyses of PELN were conducted by Beijing Biotech Pack Scientific Co., Ltd. (China). Briefly, ultra-performance liquid chromatography-mass

spectrometry/mass spectrometry (UPLC-MS/MS) analyses were performed using a Thermo Scientific U3000 system equipped with a Sepax GP-C18 column (2.1 mm $\times$ 150 mm, 1.8 μ m) coupled to a Q Exactive mass spectrometer. Lipid identification was performed using Progenesis QI software for initial annotation, followed by spectral matching against the Lipid Metabolites and Pathways Strategy Database (LIPID) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database. For proteomics, proteins were extracted with RIPA Lysis Buffer, followed by reduction/alkylation, tryptic digestion, and solid phase 2 (SP2) desalting prior to LC-MS/MS analysis. Data processing utilized MaxQuant (v2.1.4.0) with the UniProt database. Small RNAs were isolated using the NEB Next Multiplex Small RNA Library Prep Set for Illumina (New England Biolabs Inc, USA), quantified by NanoDrop 2000 (Thermo Scientific, USA), and sequenced with miRBase22.0 for plant miRNA annotation.

2.4 In vitro cellular internalization of PELN

The cellular uptake of PELN-DiD was quantitatively evaluated using flow cytometry. Immortalized keratinocytes (HaCaT) and primary dermal fibroblasts (HSF) cells were inoculated into 12-well plates at a density of 5×10^4 cells/well and cultured for 24 h prior to treatment. The cells were then incubated with 50 μ g/well of DiD-labeled PELN for 2, 4, or 8 h. Following incubation, cells were washed, harvested, and analyzed for fluorescence intensity using flow cytometry. Data processing was performed using FlowJo V10 software.

For oxidative stress conditions, HaCaT (1 mM H₂O₂) and HSF (500 μ M H₂O₂) cells were pretreated for 4 h before adding 50 μ g DiD-PELN. After 4 h incubation, cells were processed for flow cytometric quantification of uptake.

2.5 Synthesis of HA-TEMPO

The synthesis of HA-TEMPO was performed according to our previous study [24] with a few modifications. HA (3.79 g) was dissolved in ultrapure water under magnetic stirring until complete dissolution. Subsequently, 84 mg sodium acetate was added to the solution, followed by dropwise addition of 172 mg phenyl chloroformate under vigorous stirring to activate the hydroxyl groups. The reaction was allowed to proceed at room temperature for 24 h. Next, 172 mg 4-amino-TEMPO was introduced into the mixture, and the pH was adjusted to 7.8–8.5 using 1 M NaOH to initiate the conjugation reaction, which continued for 24 h at room temperature. Finally, the synthesized HA-TEMPO conjugate was purified by dialysis against ultrapure water for 72 h and lyophilized to obtain the product.

2.6 Preparation and morphology characterization of microneedle

The microneedles were prepared via two-step centrifugation in polydimethylsiloxane (PDMS) micromolds (12 $\times$ 12 array; 300 μ m base diameter $\times$ 600 μ m depth; Micropoint Technologies Pte Ltd., Singapore). The HA-TEMPO@PELN-MN formulation was prepared by first dissolving PELNs at a concentration of 25 μ g/ μ L in an aqueous solution of 60% (w/v) HA-TEMPO, followed by centrifugation at 4000 rpm for 5 min to infiltrate the matrix solution into the mold cavity. The excess solution was removed by scraping with a clean blade, and the mold was then dried overnight at room temperature. Subsequently, the patch backing was fabricated by sequential addition of 40% (w/v) PVP-K90 solution

followed by centrifugation at 4000 rpm for 3 min, with final overnight desiccation in a silica gel-containing desiccator. Finally, HA-TEMPO@PELN-MN was obtained after carefully demolding them. For HA-TEMPO-MN, PELN were omitted from the HA-TEMPO aqueous solution, whereas, for DiD-HA-TEMPO-MN, PELNs were first labeled by incubation with 10 μ M DiD for 30 min, followed by ultracentrifugation at 110,000g for 70 min to remove unbound dye. The supernatant was discarded, and the labeled PELNs were subsequently incorporated into the 60% (w/v) HA-TEMPO aqueous solution. All other fabrication steps remained identical to the HA-TEMPO@PELN-MN preparation protocol.

MN morphology was characterized using scanning electron microscopy (SEM; Thermo FEI, Czech Republic) and confocal laser scanning microscopy (CLSM; Zeiss, Germany).

2.7 Properties of microneedle

MN mechanical strength was assessed using a universal testing machine (Zwick/Roell Z200, Germany). Patches were affixed needle-up on a platform and compressed at 1 mm/min until a 400 μ m displacement. For penetration analysis, MN patches were applied to rat skin for 5 min, with subsequent micropore visualization by stereomicroscopy (Nikon, Japan). To determine insertion depth, DiD-PELN-loaded MN was applied, followed by skin cryosectioning and confocal imaging (Zeiss, Germany).

2.8 *In vitro* release of PELNs from HA-TEMPO@PELN-MN

To evaluate the *in vitro* release profile of HA-TEMPO@PELN-MN, patches were immersed in 2 mL of PBS (pH 7.2) for predetermined intervals (5, 30, 60, 120, 180, and 300 s) at room temperature (25 °C). At each time point, 100 μ L of the solution was collected with gentle agitation and immediately replaced with an equal volume of fresh PBS to maintain sink conditions, followed by PELN quantification using a BCA protein assay (BioTek, USA).

2.9 *In vitro* biocompatibility of HA-TEMPO@PELN-MN

HaCaT cells (human immortalized keratinocyte cell line) were cultured in DMEM supplemented with 10% (v/v) FBS, 100 μ g/mL Streptomycin, and 100 U/mL Penicillin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO₂. HaCaT cells were seeded in 96-well plates at a density of 6×10^3 cells/well. After 24 h of incubation, one HA-TEMPO@PELN-MN patch was dissolved in 1 mL complete culture medium, and 100 μ L of the resulting leachate was added to each well. Cell viability was assessed 12 h later using the CCK-8 assay.

2.10 *In vitro* anti-inflammatory assay

HaCaT cells were seeded in 12-well plates at a density of 6×10^4 cells/well and allowed to adhere overnight. To induce inflammation, cells were stimulated with a combination of tumor necrosis factor- α (TNF- α) and IFN- γ (10 ng/mL each) for 24 h, after which the medium was replaced with either PBS-supplemented medium (control), PELN-supplemented medium (25 or 50 μ g/mL), or HA-TEMPO@PELN-MN leachate (generated by dissolving one microneedle patch in 1 mL medium). Following 12 h of treatment, total RNA was isolated and reverse-transcribed into complementary DNA. Real-time PCR was performed in a Mastercycler (Roche, Switzerland). The primers (Sangon, China) are shown as follows:

TNF- α Forward: CCTCTCTCTAATCAGCCCTCTG

TNF- α Reverse: GAGGACCTGGGAGTAGATGAG

IL-1 β Forward: ATGATGGCTTATTACAGTGGCAA

IL-1 β Reverse: GTCGGAGATTTCGTAGCTGGA

IL-6 Forward: ACTCACCTCTTCAGAACGAATTG

IL-6 Reverse: CCATCTTTGGAAGGTTCAAGTTG

GAPDH (glyceraldehyde-3-phosphate dehydrogenase) Forward: GGAGCGAGATCCCTCCAAAAT

GAPDH Reverse: GGCTGTTGTCTACTTCTCATGG

2.11 *In vitro* antioxidant assay

HaCaT cells were seeded in 12-well plates at a density of 6×10^4 cells/well and allowed to adhere overnight. The medium was replaced with either 1 mM H₂O₂-containing medium, PELN-supplemented medium (25 or 50 μ g/mL in 1 mM H₂O₂-containing medium), HA-TEMPO solution (dissolved in 1 mM H₂O₂-containing medium) or MN leachate (prepared by dissolving one microneedle patch in 1 mL of 1 mM H₂O₂). After 4 h of treatment, intracellular ROS levels were quantified by flow cytometry using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) (10 μ M), with results normalized to the control group.

2.12 *In vivo* experiment on AD treatment

Female BALB/c mice (15–17 g) were depilated on the dorsal skin using a commercial depilatory cream 24 h prior to experiment initiation. The animals were randomly assigned to five experimental groups: (1) control group, (2) model group, (3) HA-TEMPO-MN treatment group, (4) PELN intradermal injection (PELN) group, and (5) HA-TEMPO@PELN-MN treatment group. AD induction was performed according to an established protocol with modifications [25]. Briefly, all groups except the control received daily topical applications of 1% DNCB dissolved in acetone/olive oil (4:1, v/v) on the depilated area for three consecutive days. Following a 4-day rest period, mice received morning topical application of 4% sodium dodecyl sulfate (SDS) solution on day 8, followed by an afternoon topical application of 0.5% DNCB. From day 10 onward, 0.375% DNCB was applied daily for 11 consecutive days. Therapeutic interventions (MN application or 50 μ g PELN intradermal injection) were administered on days 11, 14, 17, and 20. Clinical assessments were conducted 24 h after each treatment (days 12, 15, 18, and 21), including photographic documentation of skin lesions, body weight monitoring, and quantitative analysis of scab formation. The scab area percentage was calculated using ImageJ according to the formula: (scabbed area/total depilated area) $\times$ 100%.

2.13 Histological analysis

The AD lesional skin samples were collected at day 21. Skin samples were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned into 5 μ m thick slices. For histological evaluation, the paraffin-embedded sections were stained with hematoxylin and eosin (H&E) and toluidine blue. For immunofluorescence evaluation, the slices were stained with anti-4-HNE antibody, anti-8-OHdG antibody, anti-IL-4 antibody, anti-IFN- γ antibody, followed by staining with the corresponding fluorescence-labeled secondary antibodies and 4',6-diamidino-2-phenylindole (DAPI). Immunofluorescence, H&E and toluidine blue staining results were observed using a slide scanner (VS200, Olympus). The results were evaluated using the ImageJ software.

2.14 Analysis of inflammatory cytokines

Following euthanasia, the collected skin tissues were homogenized in ice-cold PBS and centrifuged at 5000 g for 10 min at 4 °C. The resulting supernatant was carefully collected, and the concentrations of inflammatory mediators including IFN- γ , IL-4, IL-13, and IgE were quantified using commercial ELISA kits according to the manufacturers' protocols.

2.15 Flow cytometric analysis of Th cell subsets

Skin tissues were digested in complete digestion buffer (10% FBS, 0.8 mg/mL collagenase IV, 0.05 mg/mL DNase I, 0.05 mg/mL CaCl_2 in RPMI-1640) for 1 h at 37 °C. After 70- μm filtration, single cells were surface-stained with anti-CD3 and anti-CD4 antibodies (1 h, 4 °C, dark), then fixed/permeabilized for intracellular cytokine staining (anti-IL-4 for Th2 cells, anti-IFN- γ for Th1 cells; 1 h, 4 °C, dark). Flow cytometry was performed with the following gating strategy: $\text{CD3}^+\text{CD4}^+$ T cells $\rightarrow$ IL-4 $^+$ (Th2) or IFN- γ^+ (Th1) subsets.

2.16 Western blot analysis

Skin samples from treated areas were collected for protein extraction. Total protein was quantified using a BCA assay kit. For immunoblotting, proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were incubated overnight at 4 °C with primary antibodies against p-STAT6, STAT6, and β -actin, followed by a 2 h incubation with secondary antibodies at room temperature. Protein bands were visualized and quantified using ImageJ software.

2.17 In vivo safety evaluation

Female BALB/c mice (15–17 g) were randomly assigned to two groups ($n = 5$ per group): a control group and an HA-TEMPO@PELN-MN treated group. Mice in the treated group received an application of HA-TEMPO@PELN-MN on days 1, 4, 7, and 10. Body weight and erythema scores were recorded daily. Changes in transepidermal water loss (TEWL) were measured using a vapometer (Delfin SWL5636, Finland), and skin thickness was measured with a vernier caliper. On day 11, blood samples were collected for routine blood tests and assessment of liver and kidney function indicators. Tissue samples from the heart, liver, spleen, lungs, kidneys, and skin were harvested for H&E staining to evaluate histopathological changes.

2.18 Statistical analysis

Results are expressed as mean $\pm$ standard error of the mean (s.e.m.). Statistical analyses were performed using GraphPad Prism 9.0, with Student's t-test for two-group comparisons and one-way ANOVA for multi-group analyses, P value of < 0.05 was considered statistically significant.

3 Results

3.1 Preparation and characterization of PELN

PELNs were isolated and purified as previously described (Fig. 1(a)). These cup-shaped vesicles showed an average hydrodynamic diameter of 230.3 ± 4.1 nm (Figs. 1(b) and 1(c)), consistent with the reported size range of plant-derived exosome-like nanoparticles [26–28]. Zeta potential measurements demonstrated a negative surface charge of -11.3 ± 1.5 mV, supporting the stability of

PELNs [29]. Plant-derived exosome-like nanoparticles are known to carry proteins, nucleic acids, and lipids from their source plants [30, 31]. PELNs predominantly consist of fatty acyls ($\sim 40.9\%$), glycerolipids ($\sim 11.4\%$), and glycerophospholipids ($\sim 11.0\%$) (Fig. S1 in the Electronic Supplementary Material (ESM)). Proteomic profiling identified 12 protein components, all below 60 kDa (Table S1 in the ESM), while miRNA sequencing detected 115 miRNAs, including 47 conserved miRNAs and 68 undefined miRNAs (Table S2 in the ESM).

To investigate the therapeutic potential of PELNs in AD, we evaluated their cellular uptake kinetics using two established human skin cell lines: HaCaT and HSF, representing epidermal and dermal compartments, respectively [32]. DiD-labeled PELNs displayed time-dependent uptake in both cell types (Fig. 1(d)), reaching $41.37\% \pm 1.40\%$ in HaCaT cells and $25.23\% \pm 1.07\%$ in HSF cells after 8 h (Fig. S2 in the ESM), highlighting their potential as carriers for cutaneous therapies. Considering the documented anti-inflammatory and antioxidant properties of *Portulaca oleracea* L., we further investigated whether PELNs retain these bioactivities. HaCaT cells, as primary epidermal targets in AD pathogenesis [33], were treated with 50 $\mu\text{g/mL}$ PELNs for 12 h. This intervention significantly reduced TNF- α and IL-1 β mRNA levels in the inflammatory cells stimulated by TNF- α /IFN- γ , confirming the anti-inflammatory activity of PELNs (Fig. 1(e)). However, neither 25 nor 50 $\mu\text{g/mL}$ PELNs significantly mitigated ROS levels under H_2O_2 -induced oxidative stress (Fig. 1(f)). Importantly, elevated ROS not only exacerbate the progression of AD [34], but also affect the cellular uptake of PELNs, with oxidative stress reducing internalization by 32.1% in HaCaT cells and 74.06% in HSF cells (Figs. 1(g) and 1(h)).

In summary, PELNs exhibit potent anti-inflammatory activity, yet their therapeutic efficacy is limited by insufficient ROS-scavenging capacity. Effective ROS clearance is therefore essential to enhance cellular uptake and restore immune-inflammatory homeostasis in AD.

3.2 Fabrication and characterization of HA-TEMPO@PELN-MN

Given that elevated ROS levels contribute to tissue damage in AD pathology [7], HA-TEMPO was used as an ROS scavenger to complement the limited antioxidant capacity of PELNs. The TEMPO-modified HA conjugate was synthesized through a two-step process (Fig. S3 in the ESM). HA was first activated with benzyl chloroformate in the presence of sodium acetate, generating nucleophilic substitution-labile carbonate ester intermediates. These activated intermediates then underwent amine-selective nucleophilic substitution with 4-amino-TEMPO, avoiding the use of toxic coupling agents such as dicyclohexylcarbodiimide (DCC). Successful TEMPO functionalization was confirmed by ^1H nuclear magnetic resonance (NMR), which displayed characteristic methyl proton signals at δ 1.2 ppm, and the conjugation efficiency was determined to be 15.3% (Fig. S4 in the ESM). SEM imaging revealed an irregular lamellar morphology of HA-TEMPO (Fig. 2(a)). Functional assays demonstrated that HA-TEMPO effectively reduced ROS in HaCaT cells under oxidative stress (Fig. 2(b)), while control experiments confirmed that HA alone had no significant effect (Fig. S5 in the ESM). These results collectively demonstrate that the HA material has been successfully endowed with antioxidant capabilities.

The HA-TEMPO@PELN-MN system was fabricated via a two-

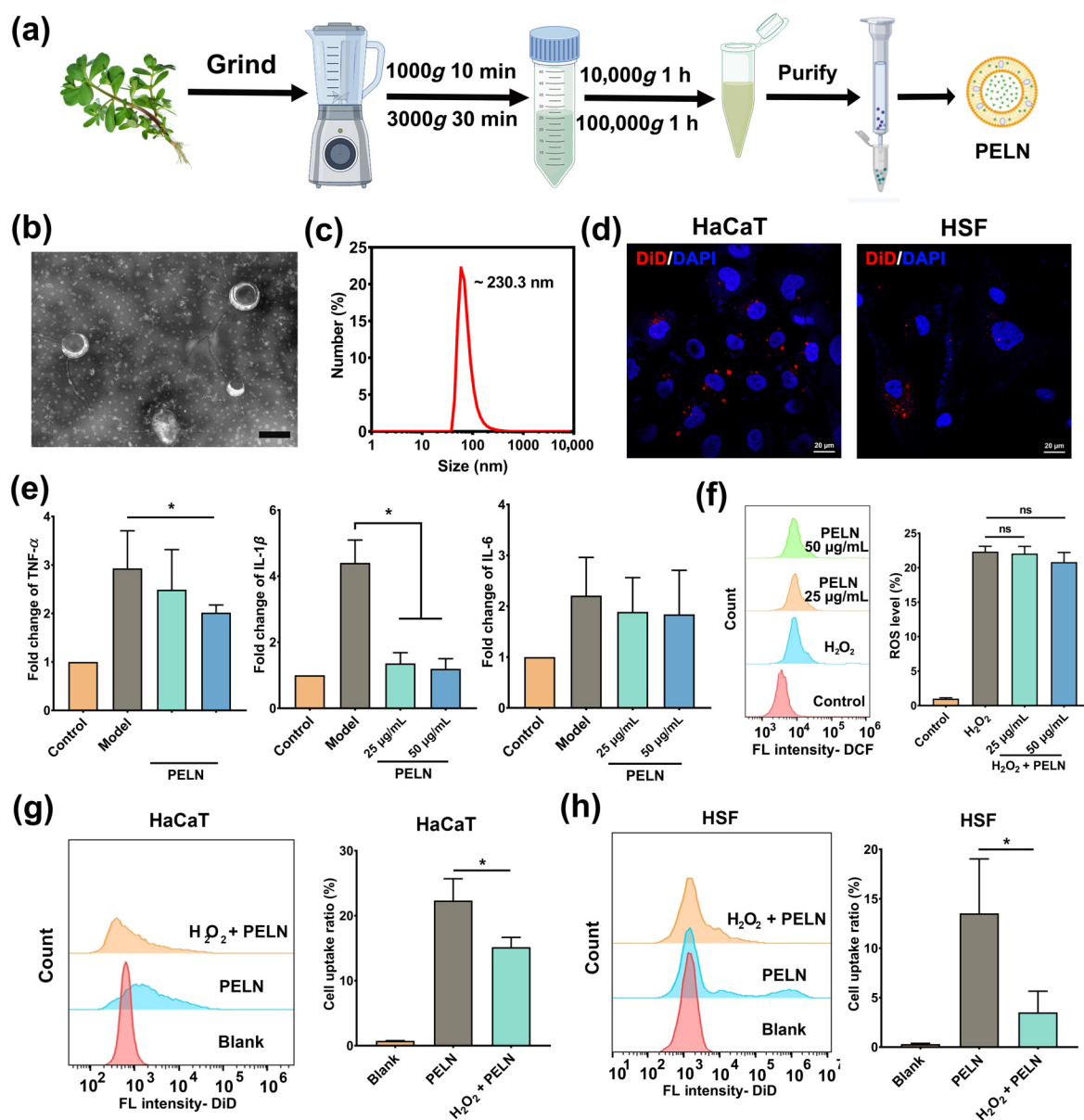


Figure 1 Preparation, characterization, and bioactivity of PELN. (a) Schematic illustration of PELN extraction and purification. (b) Representative TEM image showing PELN morphology. Scale bar: 250 nm. (c) Size distribution and particle concentration of isolated PELN ($n = 3$). (d) Confocal fluorescence microscopy analysis of cellular uptake of DiD-labeled PELN in HaCaT and HSF cells after 8 h of incubation. Scale bars: 20 μ m. (e) TNF- α , IL-1 β , and IL-6 mRNA expression levels in cytokine-stimulated HaCaT cells ($n = 3$). (f) Intracellular ROS levels in H_2O_2 -treated HaCaT cells ($n = 3$). (g) Flow cytometric quantification of PELN uptake efficiency in (g) HaCaT and (h) HSF cells exposed to H_2O_2 . Data are presented with mean $\pm$ s.e.m. ($n = 3$).

step centrifugal casting method under mild conditions (Fig. 2(c)). PVP-K90 served as the backing layer matrix to maintain separation from the MN structures due to its slower dissolution relative to HA [35]. The HA-TEMPO@PELN-MN formed uniform pyramidal arrays with a 1×1 cm 2 base, 600 μ m needle height, 300 μ m tip diameter, and a 12×12 configuration (144 needles/patch, Figs. 2(d) and 2(e)).

SEM analysis revealed distinct structural features between the MN types. Cross-sections of HA-TEMPO-MN displayed compact porous networks, while HA-TEMPO@PELN-MN showed PELN particles embedded within the matrix, some retaining the characteristic cup-shaped morphology of exosomes (Fig. S6 in the ESM). Notably, HA-TEMPO@PELN-MN exhibited expanded pore structures relative to HA-TEMPO-MN, suggesting potential

microstructural reorganization mediated by PELN incorporation (Fig. S6 in the ESM). PELNs were preferentially localized at the needle tips (Fig. 2(f)), and upon dissolution in PBS, were released while preserving their native exosomal morphology and structural integrity (Fig. S7 in the ESM). *In vitro* release studies demonstrated rapid and nearly complete PELN release: $4.2\% \pm 5.8\%$ at 5 s, $68.5\% \pm 3.9\%$ at 120 s, and $97.4\% \pm 4.7\%$ at 300 s (Fig. 2(g)), with a loading capacity of 58.6 ± 6.4 μ g per patch. These results demonstrated efficient encapsulation of PELN while maintaining their structural fidelity.

Mechanical integrity is critical for effective transdermal delivery of dissolving MNs [36]. Continuous force-displacement curves for both HA-TEMPO-MN and HA-TEMPO@PELN-MN within the 0–400 μ m displacement range showed no structural fracture at

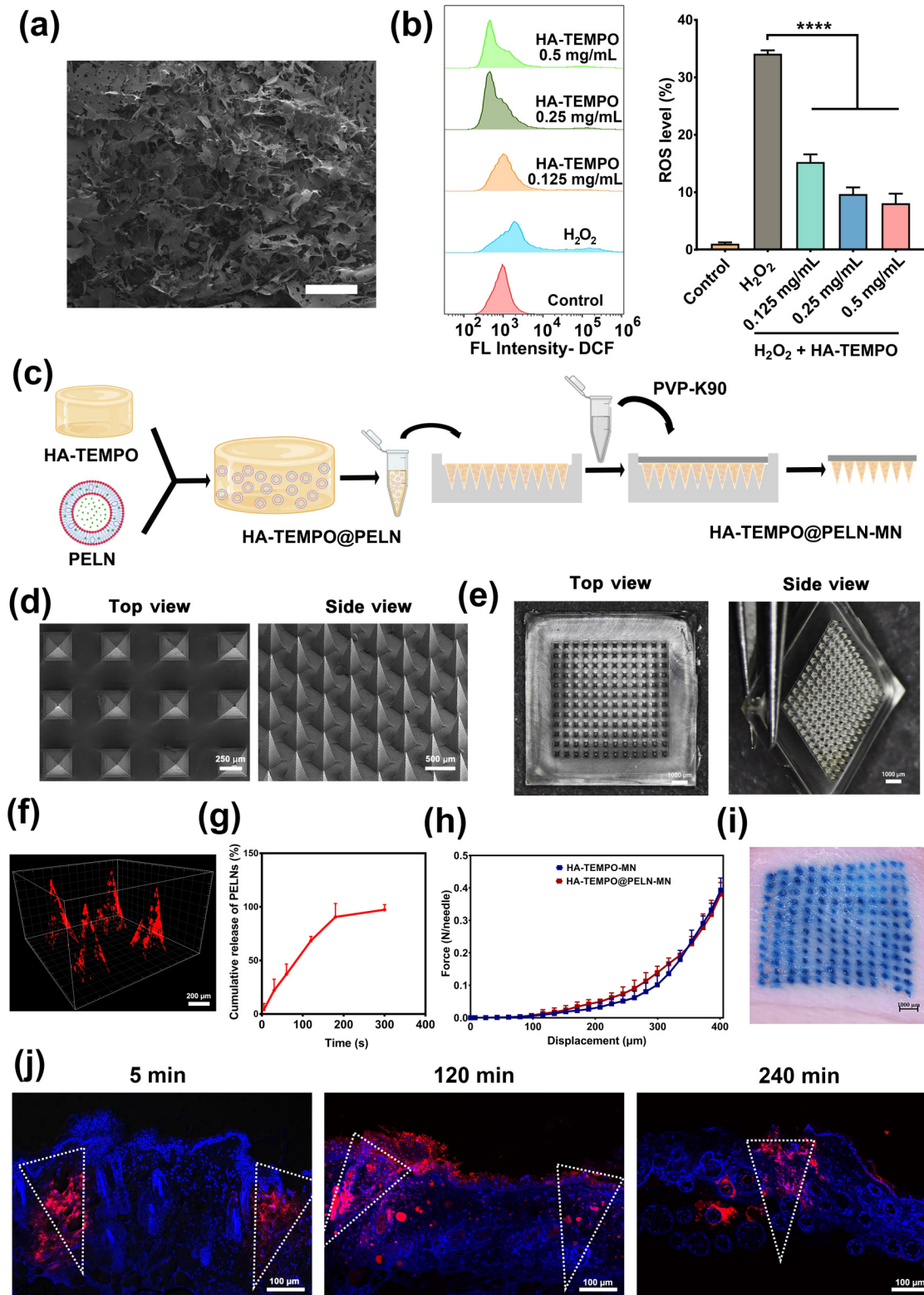


Figure 2 Morphological and physicochemical characterization of HA-TEMPO and HA-TEMPO@PELN-MN, along with *in vitro* PELN release. (a) Representative SEM image of HA-TEMPO morphology. Scale bar: 500 μm . (b) Intracellular ROS levels in H_2O_2 -treated HaCaT cells after HA-TEMPO treatment ($n = 3$). (c) Schematic of the fabrication process for HA-TEMPO@PELN-MN. (d) SEM image of the HA-TEMPO@PELN-MN patch. Scale bars: 250 μm (left), 500 μm (right). (e) Morphological characterization of HA-TEMPO@PELN-MN under stereomicroscopic observation. Scale bar: 1000 μm . (f) Fluorescence microscopy image of HA-TEMPO@PELN-MN loaded with DiD-labeled PELN. Scale bar: 200 μm . (g) PELN release profile from HA-TEMPO@PELN-MN in PBS ($n = 3$). (h) Force-displacement curves for HA-TEMPO-MN and HA-TEMPO@PELN-MN ($n = 3$). (i) Stereoscopic photomicrographs of rat skin after insertion of methylene blue-loaded HA-TEMPO@PELN-MN *in vitro*. Blue dots indicate MN penetration sites. Scale bar: 1000 μm . (j) A cross-sectional confocal image of mouse skin treated with DiD-labeled HA-TEMPO@PELN-MN for 5, 120, 240 min. Scale bars: 100 μm . Data are presented with mean $\pm$ s.e.m.

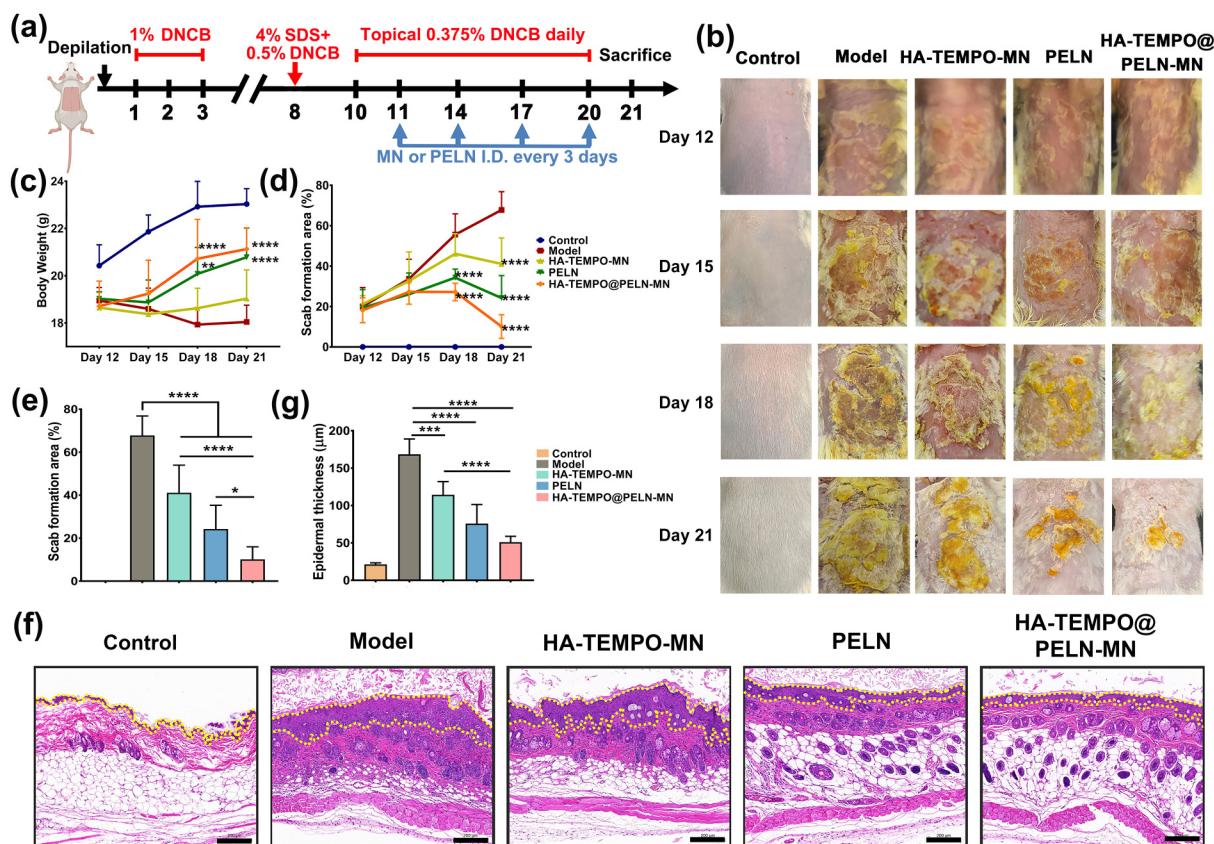


Figure 3 *In vivo* therapeutic evaluation of HA-TEMPO@PELN-MN in DNCB-induced AD mice. (a) Schematic diagram of AD model establishment and treatment protocol. (b) Representative photographs of treated skin areas on days 12, 15, 18, and 21 post-depilation. (c) Body weight changes across treatment groups ($n = 8$). (d) Dynamic changes in the percentage of crusted area ($n = 8$). (e) Quantification of crusted area percentage on day 21 post-depilation ($n = 8$). (f) Representative H&E-stained micrographs of depilated skin on day 21 post-depilation, the yellow dashed line indicates the position of the epidermis. Scale bars: 200 μm . (g) Epidermal thickness measurements on day 21 post-depilation ($n = 5$). Data are presented with mean $\pm$ s.e.m.

maximal deformation [37]. At the 400 μm displacement endpoint, compressive strengths were 0.39 ± 0.04 and 0.38 ± 0.04 N/needle for HA-TEMPO-MN and HA-TEMPO@PELN-MN, respectively (Fig. 2(h)), both significantly exceeded the skin penetration threshold of 0.058 N/needle [38], confirming adequate mechanical robustness for transdermal application.

The transdermal performance was evaluated using methylene blue staining and DiD fluorescence labeling in *ex vivo* skin models [39]. Both HA-TEMPO-MN and HA-TEMPO@PELN-MN efficiently penetrated the epidermis, producing characteristic blue micropores corresponding to the MN array on the skin surface. Quantitative analysis revealed insertion efficiencies of $97.21\% \pm 0.69\%$ and $97.68\% \pm 1.45\%$ for HA-TEMPO-MN and HA-TEMPO@PELN-MN, respectively, with no statistically significant difference (Fig. 2(i)). Confocal imaging further confirmed delivery of PELNs to dermal depths of 100–300 μm . Furthermore, the diffusion of PELNs within the skin was observed at 2 and 4 h after MN application (Fig. 2(j)). These findings confirm that HA-TEMPO@PELN-MN maintains mechanical integrity while achieving reliable transdermal delivery.

Next, we assessed whether HA-TEMPO@PELN-MN preserved the bioactivity of both PELN and HA-TEMPO. *In vitro* inflammation models revealed that extracts from HA-TEMPO@PELN-MN significantly downregulated IL-1 β and IL-6 mRNA expression, confirming retention of PELN's anti-inflammatory activity (Figs. S8(a)–S8(c) in the ESM). Under H_2O_2 -

induced oxidative stress, extracts from both HA-TEMPO-MN and HA-TEMPO@PELN-MN significantly reduced ROS levels, whereas free PELN showed no significant effect, indicating that the MN system effectively retained the antioxidant activity of HA-TEMPO (Fig. S8(d) in the ESM). Cytotoxicity testing demonstrated excellent biocompatibility of HA-TEMPO@PELN-MN (Fig. S8(e) in the ESM). To further resolve the anti-inflammatory and antioxidant capacity of the individual components in HA-TEMPO@PELN-MN, we assessed the combined effect of HA-TEMPO and PELNs on HaCaT cells. The combined treatment significantly suppressed the mRNA expression of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) and reduced H_2O_2 -induced intracellular ROS levels (Fig. S9 in the ESM). These data align with the microneedle extracts results, collectively demonstrating that the functional activities of HA-TEMPO and PELNs were maintained in the MN formulation.

3.3 Therapeutic effects of HA-TEMPO@PELN-MN in an AD mouse model

To recapitulate human AD pathogenesis, we established an AD mouse model (Fig. 3(a)). This model successfully reproduced hallmark dermatological features, including scaling, lichenification, and multiple crusted lesions by the study endpoint. Both intradermal PELN injection and HA-TEMPO@PELN-MN application showed significant therapeutic efficacy from day 18 post-intervention (Fig. 3(b)). These treatments mitigated progressive

weight loss and reduced the area of crusted skin lesions compared with untreated controls (Figs. 3(c) and 3(d)). By day 21, softening and detachment of scabs resulted in a significant reduction in crusted lesion area across all treatment groups (Fig. 3(e)). The HA-TEMPO-MN group also demonstrated significant therapeutic efficacy, indicating that ROS-scavenging contributes critically to regulating crust formation in AD. Importantly, HA-TEMPO@PELN-MN achieved superior lesion improvement compared to PELN alone, indicating that HA-TEMPO functionalization potentiates the therapeutic effects of PELNs. Overall, HA-TEMPO@PELN-MN effectively ameliorated AD-like skin lesions and attenuated disease-associated weight loss.

To delineate the contribution of TEMPO, we compared HA-TEMPO@PELN-MN with HA@PELN-MN (PELN-loaded HA MNs without TEMPO). By day 21, HA-TEMPO@PELN-MN showed a statistically significant reduction in crusted lesion area compared with HA@PELN-MN, confirming that TEMPO functionalization enhances the therapeutic efficacy of PELNs (Fig. S10 in the ESM).

To assess whether the amelioration of AD-like symptoms following HA-TEMPO@PELN-MN treatment corresponded with histopathological improvements, H&E staining of lesional skin sections was performed. Histopathological analysis revealed distinct tissue morphology between groups: control mice exhibited normal epidermal architecture, whereas AD model mice displayed marked epidermal hyperkeratosis, hyperplasia, and significantly increased epidermal thickness. All treatment groups (HA-TEMPO-MN, PELN, and HA-TEMPO@PELN-MN) significantly attenuated epidermal hyperplasia compared with the model group, with HA-TEMPO@PELN-MN showing superior efficacy in reducing epidermal thickness relative to monotherapies (Figs. 3(f) and 3(g)). These findings suggest that the synergistic interaction between the ROS-scavenging capacity of HA-TEMPO and the anti-inflammatory properties of PELN facilitated by the MN delivery system, collectively suppressing pathological epidermal proliferation.

Cutaneous oxidative stress was further evaluated by monitoring dynamic changes in 4-HNE (4-hydroxy-2-nonenal, a lipid peroxidation marker) and 8-OHdG (8-hydroxy-2'-deoxyguanosine, a marker of DNA oxidative damage) [40]. Compared with the model group, both HA-TEMPO-MN and HA-TEMPO@PELN-MN treatments significantly reduced 4-HNE and 8-OHdG-positive signals, while the PELN monotherapy showed no significant effect (Fig. S11 in the ESM). These results confirmed that the antioxidant activity of HA-TEMPO was preserved within the MN delivery system, effectively mitigating oxidative damage through free radical scavenging in the skin.

3.4 Effects and mechanism of HA-TEMPO@PELN-MN on immune responses in an AD mouse model

The pathogenesis of AD is critically associated with Th1/Th2 immune imbalance, with Th2 polarization representing a key pathological hallmark that drives excessive IgE production through IL-4R α /STAT6-mediated B cell activation [41–43]. Quantitative ELISA revealed that both HA-TEMPO@PELN-MN treatment and intradermal PELN injection significantly suppressed Th2-associated cytokines (IL-4, IL-13) and IgE levels compared with the model group, while the PELN group significantly upregulated IFN- γ expression. Importantly, HA-TEMPO@PELN-MN showed superior regulation of IL-4 and IgE compared to HA-TEMPO-MN,

suggesting that PELN delivered via MNs selectively regulates Th2-polarized immune responses and mitigates pathological IgE overproduction (Fig. 4(a)).

Mast cells, as key effectors in AD pathophysiology, are activated through both IgE-dependent and IgE-independent mechanisms, releasing histamine, proteases, and Th2 cytokines that amplify inflammatory cascades [44, 45]. Toluidine blue staining revealed significant mast cell infiltration in AD model skin (Figs. 4(b) and 4(c)). Both PELN and HA-TEMPO@PELN-MN treatments significantly reduced mast cell numbers compared to the model group, while HA-TEMPO-MN showed no inhibitory effect. HA-TEMPO@PELN-MN exhibited superior mast cell modulation compared to HA-TEMPO-MN, suggesting that PELN delivered via the MN system effectively modulates mast cell activation pathways.

Building on cytokine profiling, flow cytometry was used to assess dynamic changes in cutaneous Th cell subsets (Figs. 4(d)–4(f)). Skin cells isolated via mechanical-enzymatic digestion were sorted for CD3⁺/CD4⁺ T helper populations, with intracellular cytokine staining distinguishing Th1 (IFN- γ ⁺) and Th2 (IL-4⁺) subsets. Both PELN and HA-TEMPO@PELN-MN treatments significantly increased Th1 cell proportions while reducing Th2 cell proportions compared to the model group. Importantly, HA-TEMPO@PELN-MN achieved a greater reduction in Th2 cells compared with HA-TEMPO-MN, indicating that PELN is the principal component in the MN system responsible for restoring cutaneous immune homeostasis. Furthermore, immunofluorescence analysis of IL-4 and IFN- γ expression in skin tissues corroborated these findings (Fig. S12 in the ESM).

STAT6 signaling regulates inflammatory cytokine expression and T cell responses in AD [46]. Western blot analysis revealed that HA-TEMPO@PELN-MN treatment significantly reduced p-STAT6 levels, confirming effective modulation of the IL-4R α /STAT6 axis (Figs. 4(g) and 4(h)).

3.5 Preliminary safety assessment of HA-TEMPO@PELN-MN

Finally, the *in vivo* safety profile of HA-TEMPO@PELN-MN was assessed. Throughout the experimental period, no significant differences in body weight were observed between treated and control groups (Fig. 5(a)). Hematological parameters, including white blood cell (WBC), red blood cell (RBC), and platelet (PLT) counts, remained within normal physiological ranges across all groups (Fig. 5(b)). Similarly, hepatic and renal function biomarkers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (CR), and uric acid (UA), showed no abnormal fluctuations, indicating the absence of hepatorenal toxicity (Fig. 5(c)). Histopathological examination of major organs (heart, liver, spleen, lungs, kidneys) and skin at the application site revealed no structural alterations or inflammatory cell infiltration, collectively confirming the excellent biocompatibility and safety of the HA-TEMPO@PELN-MN system (Fig. 5(d)).

Finally, potential adverse reactions at the application site were further investigated. Although transepidermal water loss (TEWL) values increased on the day of MN application due to temporary pore opening, they returned to baseline before the next application (Fig. 5(e)). Similarly, skin thickness increased during HA-TEMPO@PELN-MN treatment but normalized after the treatment concluded (Fig. 5(f)). Furthermore, no significant differences in skin erythema were observed between the HA-TEMPO@PELN-

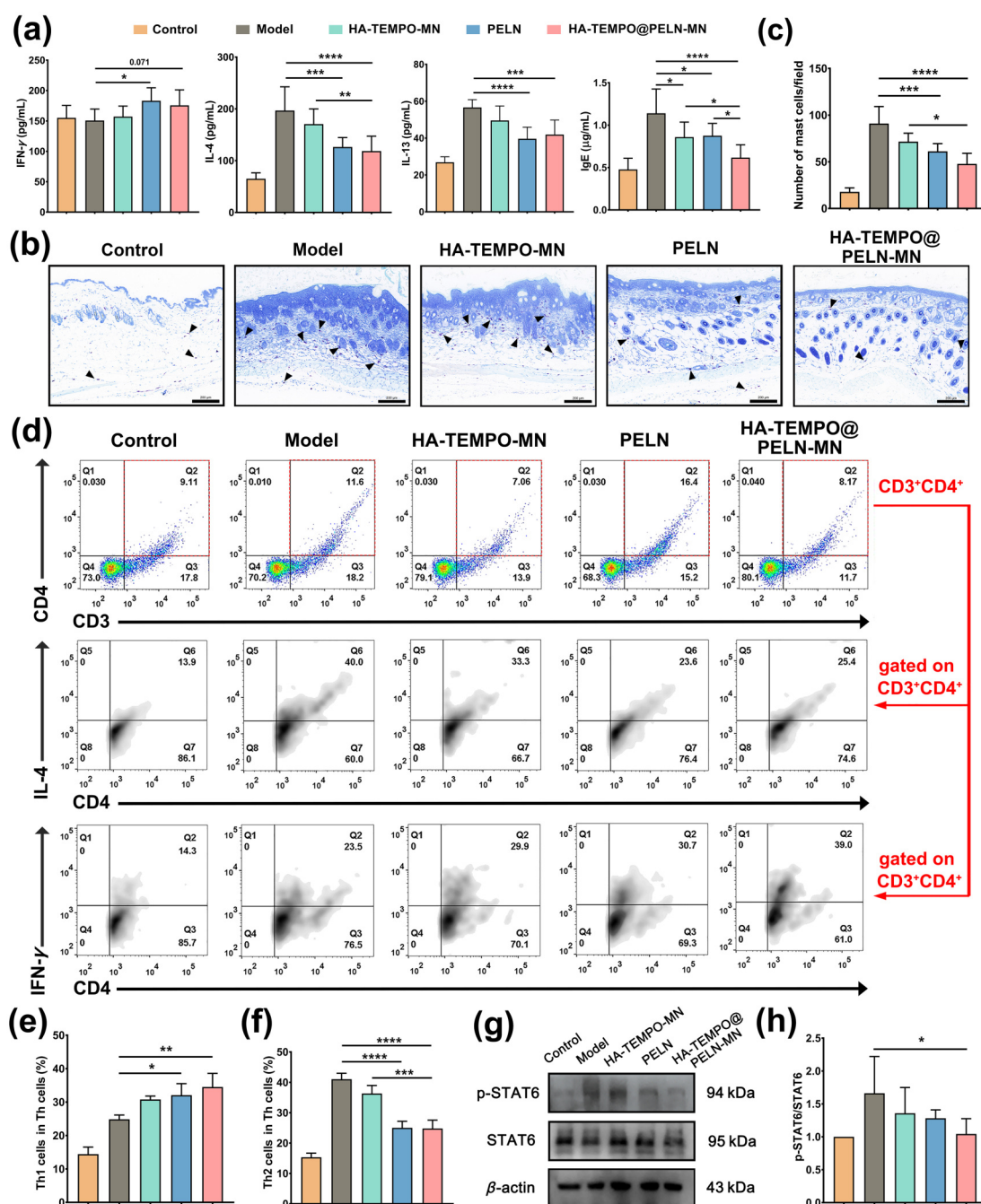


Figure 4 *In vivo* effects and mechanistic evaluation of HA-TEMPO@PELN-MN on immune responses in DNCB-induced AD mice. (a) ELISA analysis of IFN- γ , IL-4, IL-13, and IgE levels in skin tissues. (b) Representative toluidine blue-stained micrographs of depilated skin on day 21 post-depilation. Scale bars: 200 μ m. (c) Quantification of mast cell numbers on day 21 post-depilation ($n = 5$). (d) Flow cytometric analysis of Th1/Th2 cell ratios in skin-derived lymphocytes. (e) Percentage of Th1 cells among total T helper cells ($n = 3$). (f) Percentage of Th2 cells among total T helper cells ($n = 3$). (g) Representative Western blot images. (h) Quantification of p-STAT6 protein expression levels ($n = 3$). Data are presented with mean $\pm$ s.e.m.

MN-treated and normal groups at the endpoint (Fig. 5(g)). In summary, continuous HA-TEMPO@PELN-MN application did not induce significant local adverse effects.

4 Discussion

Plant-derived exosome-like nanoparticles have recently attracted considerable attention owing to their inherent biocompatibility and bioactivity [47]. Notably, exosome-like nanoparticles derived from garlic [48], celery [49], *Allium tuberosum* [50], and potato [51] have

demonstrated significant anti-inflammatory properties. *Portulaca oleracea* L. has been reported to possess anti-inflammatory, antioxidant, and immunomodulatory activities, suggesting therapeutic potential for inflammatory skin diseases, including AD [30, 31]. Following isolation and purification via ultracentrifugation combined with size-exclusion chromatography, we confirmed that PELN retained anti-inflammatory activity consistent with previous reports [52].

Despite their therapeutic promise, PELNs have several

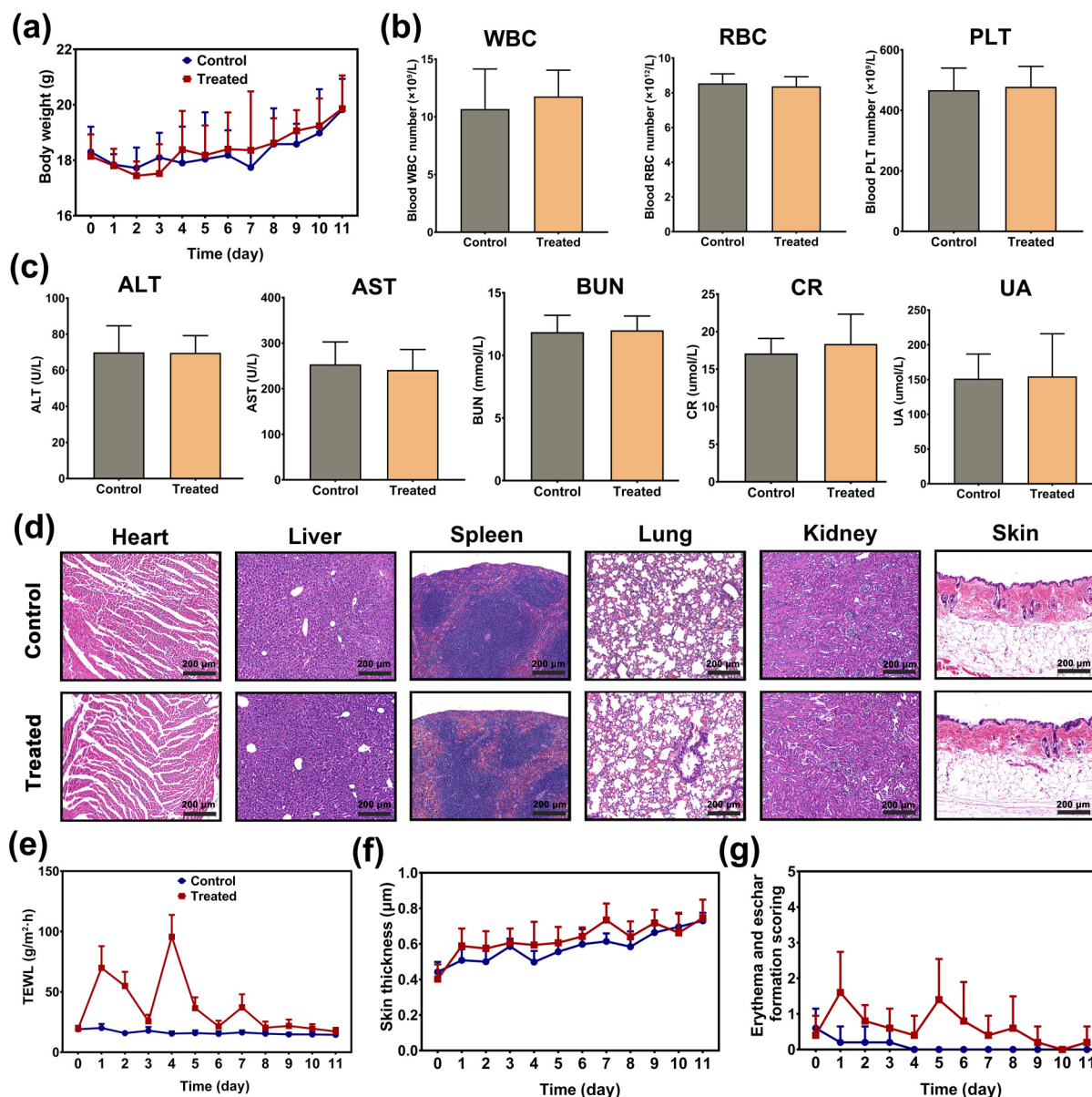


Figure 5 *In vivo* biocompatibility assessment of HA-TEMPO@PELN-MN. (a) Body weight changes of mice with or without HA-TEMPO@PELN-MN administration ($n = 5$). (b) Blood WBC, RBC, and PLT concentrations in mice with or without HA-TEMPO@PELN-MN administration on day 11 ($n = 5$). (c) Blood ALT, AST, BUN, CR, and UA concentrations in mice with or without HA-TEMPO@PELN-MN administration on day 11 ($n = 5$). (d) H&E staining of major organs (heart, liver, spleen, lungs, and kidneys) and skin tissues from mice with or without HA-TEMPO@PELN-MN administration. Scale bars: 200 μm . (e) TEWL of mice during safety evaluation ($n = 5$). (f) Skin thickness in 11 days ($n = 5$). (g) Erythema and eschar formation scoring of mice during safety evaluation ($n = 5$). Data are presented with mean $\pm$ s.e.m.

limitations. Mechanistic studies indicate that ROS accumulation in AD disrupts epidermal homeostasis and drives overexpression of Th2 cytokines, thereby amplifying Th2 polarization and exacerbating disease progression [5, 53]. Importantly, PELNs lack the intrinsic antioxidant capacity of their source plant, and their cellular uptake is significantly impaired under high ROS conditions, potentially compromising therapeutic efficacy. Therefore, incorporation of ROS-scavenging components into PELN-based therapeutic platforms is essential to maximize their effectiveness.

In AD-affected skin, the hyperkeratotic stratum corneum poses a substantial barrier to drug permeation, particularly for large therapeutics such as exosomes, thereby limiting treatment efficacy [18]. Significant progress has been made in the therapeutic application of transdermal delivery microneedle preparations for

skin diseases [54–56]. To overcome the transdermal delivery limitations of PELN, we employed dissolving MNs as a minimally invasive administration platform. Previous studies have demonstrated that MNs can effectively deliver exosomes while maintaining their bioactivity, including those derived from mesenchymal stem cells, regulatory T cells, and milk [57–59]. In addition, recent clinical trials in AD patients have reported favorable safety profiles for MN interventions, with no serious adverse events and only minor transient side effects [60, 61].

To address the dual limitations of PELNs, restricted antioxidant capacity and poor transdermal delivery, we developed a multifunctional HA-TEMPO composite that simultaneously enables PELN encapsulation and cutaneous ROS scavenging. This composite was further fabricated into dissolvable MNs to achieve

effective skin penetration. HA, a natural component of the extracellular matrix, was selected as the carrier due to its biocompatibility, high permeability, and porous architecture [62]. Although the high permeability of HA hydrogels facilitates drug delivery, it can compromise retention of functional agents [63]. Thus, HA was chemically modified via hydroxyl activation to conjugate with 4-amino-TEMPO, yielding HA-TEMPO. As previously demonstrated, HA-TEMPO preserved the ROS-scavenging activity of TEMPO and promoted cutaneous revascularization [24]. Furthermore, the use of low-molecular-weight HA (10 kDa) enabled complete dissolution of HA-TEMPO within 5 min of application, enabling rapid therapeutic release.

Our experimental results confirmed the successful incorporation of PELNs within HA-TEMPO@PELN-MN, which exhibited marked therapeutic efficacy against AD-like lesions. Compared to PELN, HA-TEMPO-MN, or HA@PELN-MN alone, HA-TEMPO@PELN-MN more effectively reduced scab areas in AD mice, indicating a synergistic enhancement of therapeutic outcomes through combinatorial action. This improved efficacy may be attributed to TEMPO-mediated ROS scavenging, which potentially facilitates PELN cellular uptake. Furthermore, only HA-TEMPO@PELN-MN, but not HA-TEMPO-MN, significantly restored the Th1/Th2 cell balance, indicating that the immunomodulatory effects are predominantly mediated by PELNs within the AD microenvironment.

The pathogenesis of allergic diseases is closely linked to Th1/Th2 immune imbalance, with Th2-dominant polarization representing a central driver of AD [64]. Acute AD is characterized by Th2-skewed responses, whereas chronic lesions shift toward Th1-biased immunity. Th2-derived cytokines, such as IL-4 and IL-13, exacerbate disease progression through the following mechanisms [65]: (i) IL-4 induces IgE class switching in B cells, with IgE binding to FcεRI on mast cells triggering degranulation and promoting mast cell infiltration [3]; (ii) IL-4/IL-13 binding to the type II IL-4R complex (IL-13Rα1/IL-4Rα) activates the JAK1/TYK2-STAT6 signaling axis, driving Th2 cell expansion; and (iii) IL-13 binding to the decoy receptor IL-13Rα2 promotes collagen deposition and fibrotic remodeling, resulting in increased epidermal thickness [66]. Consistent with these mechanisms, both PELN and HA-TEMPO@PELN-MN treatments significantly attenuated epidermal hyperplasia, reduced mast cell density, and restored Th1/Th2 homeostasis, thereby confirming the immunomodulatory role of PELNs in AD therapy.

Emerging evidence highlights the pivotal role of the JAK/STAT signaling pathway in cutaneous pathophysiology [67]. STAT6, a key effector of this pathway, drives AD progression by mediating B cell differentiation and IgE class switching [68]. Western blot analysis indicated that PELNs may modulate Th cell response by interfering with STAT6 activation.

Plant-derived exosome-like nanoparticles have been reported to mediate cross-kingdom gene regulation and exert diverse therapeutic bioactivities, such as anti-inflammatory, immunomodulatory, and tissue-repair functions [17, 48, 69, 70]. These effects may originate from their cargo of bioactive molecules, including miRNAs, lipid metabolites, and functional proteins. Consistently, our sequencing analysis confirmed that PELNs are enriched in miRNAs, lipids, and proteins, although the precise constituents mediating their therapeutic effects remain to be elucidated.

5 Conclusions

In this study, we developed a dissolving MN patch capable of effectively penetrating the stratum corneum and rapidly delivering PELNs. The therapeutic efficacy of this system in AD can be attributed to its combined ability to modulate inflammatory responses, scavenge ROS, and restore immune homeostasis, highlighting its potential as a promising therapeutic strategy for AD. Additionally, HA-TEMPO@PELN-MN showed no apparent toxicity. This biocompatible and readily fabricated drug-loaded MN system represents a promising candidate for the management of AD and related dermatological disorders.

Electronic Supplementary Material: Supplementary material (sequencing data, additional TEM images, additional SEM images, additional immunofluorescence images) is available in the online version of this article at <https://doi.org/10.26599/NR.2025.94908151>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon request.

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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.

Author contribution statement

B. Z.: Data curation, project administration, validation, writing manuscript, experimental design. X. C. J.: Project administration, validation, experimental design. W. S. L.: Data curation, project administration. L. Z.: Validation. Y. H. X.: Project administration. W. T. H.: Project administration. T. Y. Z.: Validation, experimental design. N. G. C.: Funding acquisition. J. Q. G.: Project administration, experimental design. H. J. L.: Project administration, funding acquisition. All the authors have approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Zhejiang

Chinese Medical University and approved by the Animal Ethics Committee of Zhejiang Chinese Medical University (IACUC-20221121-01).

Use of AI statement

None.

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