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Brief Report

Light modulates plant-derived extracellular vesicle properties: a photosensitive-responsive nanodelivery system

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Abstract
Plant-derived extracellular vesicles (PDEVs) have emerged as innovative nanocarriers for drug delivery, offering advantages such as biocompatibility, stability, and cost-effectiveness. This study explores light-mediated strategies to optimize cargo encapsulation into PDEVs while preserving structural integrity. Leveraging the intrinsic photosensitizing properties of PDEVs, light irradiation (LED) triggered reactive oxygen species (ROS) generation, including superoxide anions and singlet oxygen, which transiently enhanced membrane permeability for controlled drug loading. Using FITC-dextran (70 kDa) as a model cargo, we optimized light-induced loading efficiency, achieving a peak (~ 80%) at 10 min of irradiation. Prolonged exposure (15 min) reduced efficiency (~ 50%), likely due to excessive ROS-induced membrane destabilization. The optimal PDEVs-to-cargo ratio (1:30) ensured maximal loading while maintaining stability over 30 days. Lipid peroxidation analysis further confirmed ROS-induced membrane modifications through malondialdehyde (MDA) accumulation. These findings demonstrate that PLDENs (Pueraria lobate-derived exosomes-like nanovesicles) function as light-responsive nanocarriers, balancing ROS-mediated permeability enhancement with structural integrity. This light-triggered strategy balances permeability modulation and structural integrity, advancing PDEVs as scalable, non-invasive platforms for precision drug delivery and photodynamic applications.

Keywords Plant-derived extracellular vesicles · Light · Reactive oxygen species (ROS) · Encapsulation · Photosensitizing properties · Lipid peroxidation

1 Introduction

Extracellular vesicles (EVs), including exosomes, have emerged as promising drug delivery vehicles due to their biocompatibility, targeting ability, and capacity to encapsulate therapeutic agents [1, 2]. Exosomes, nanoscale vesicles derived from endosomal compartments, mediate intercellular communication by transporting proteins, lipids, and nucleic acids [3]. Current drug-loading strategies include pre-loading, where donor cells are engineered to secrete

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cargo-loaded exosomes, and post-loading, which introduces therapeutics into isolated exosomes via electroporation, sonication, or chemical transfection [4]. While effective, these methods may compromise exosomal integrity [1–4].

Plant-derived extracellular vesicles (PDEVs) have emerged as a scalable, cost-effective, and non-immunogenic alternative. Readily extracted from edible plants, PDEVs exhibit stability under harsh conditions, making them particularly advantageous for oral drug delivery [5, 6]. Compared to synthetic drug delivery systems (DDS) like liposomes and micelles, PDEVs offer superior stability, reduced immunogenicity, and enhanced cellular uptake [7–10]. However, challenges such as scalability, variability in vesicle composition, and potential off-target effects remain to be addressed.

PDEVs also possess unique properties for light-enhanced cargo loading. Their membranes, enriched with sterols and flavonoids, exhibit high fluidity and stability, enabling responsiveness to light-induced permeability changes [11]. Photosensitive molecules such as chlorophyll and flavonoids generate photothermal effects or reactive oxygen species (ROS) under light, transiently enhancing drug loading without additives [6, 12]. Light exposure may also reorganize lipids, improving permeability while preserving vesicle integrity [13].

Conventional post-loading methods, including electroporation and sonication, can disrupt PDEV structure, reducing stability and activity [4, 12]. Light-based techniques, particularly red and near-infrared (NIR) wavelengths, offer a non-invasive approach to modulate membrane properties and improve drug encapsulation without compromising vesicle integrity [14]. While this method has been explored in mammalian exosomes, its application to PDEVs remains underexplored.

This study investigates the effects of light treatment on PDEV-based drug loading, aiming to establish a novel, scalable, and non-invasive strategy. By evaluating existing exosome-based techniques and highlighting PDEVs' advantages, this research seeks to advance next-generation drug delivery systems for clinical applications.

2 Results and discussion

2.1 PLDENs have photosensitizing properties

Our previous results demonstrated that isolated PLDENs (Pueraria lobate-derived exosomes-like nanovesicles) are membrane-structured, stable vesicles [15], characterized by an average particle size and a negative zeta potential [16]. To evaluate the potential of PLDENs as natural photosensitizing molecules, we conducted separate analyses of superoxide anion radicals ($\cdot O_2^-$), hydroxyl radicals (·OH), singlet oxygen ($^1O_2$), and total reactive oxygen species (ROS) generation (Fig. 1A–D). Upon irradiation with LED light, we observed a significant, time-dependent increase in their production from PLDENs (Fig. 1A–D), indicating the presence of photosensitizing molecules. The presence of photosensitizing molecules in PLDENs suggests that light irradiation can induce ROS generation, which may transiently alter the structural integrity of the PLDENs membrane. The oxidative effect of ROS could increase membrane permeability, facilitating the controlled diffusion or encapsulation of target molecules. Upon cessation of light exposure, the ROS production would be halted, allowing the membrane to restore its original structure. This reversible, light-triggered modulation of membrane permeability presents a promising strategy for the controlled loading of bioactive compounds into PLDENs, further underscoring their potential as stimuli-responsive delivery systems.

To evaluate the loading efficiency of PLDENs under light irradiation and determine the optimal exposure time, we utilized FITC-labeled dextran (70 kDa) as a trackable fluorescent cargo. Continuous monitoring of light treatment duration revealed that the highest loading efficiency (~ 80%) was achieved at 10 min of irradiation. However, when the exposure time was extended to 15 min, the loading efficiency decreased to approximately 50% (Fig. 1E).This observation suggests that while ROS-induced membrane permeability facilitates cargo loading, prolonged light exposure may lead to excessive oxidative stress, potentially compromising membrane integrity or inducing structural destabilization of PLDENs. The decline in loading efficiency beyond the optimal irradiation time could be attributed to increased ROS levels, which may cause irreversible membrane disruption or leakage of the encapsulated cargo. These findings highlight the importance of precise light modulation to balance ROS generation for controlled loading while maintaining the structural stability of PLDENs.

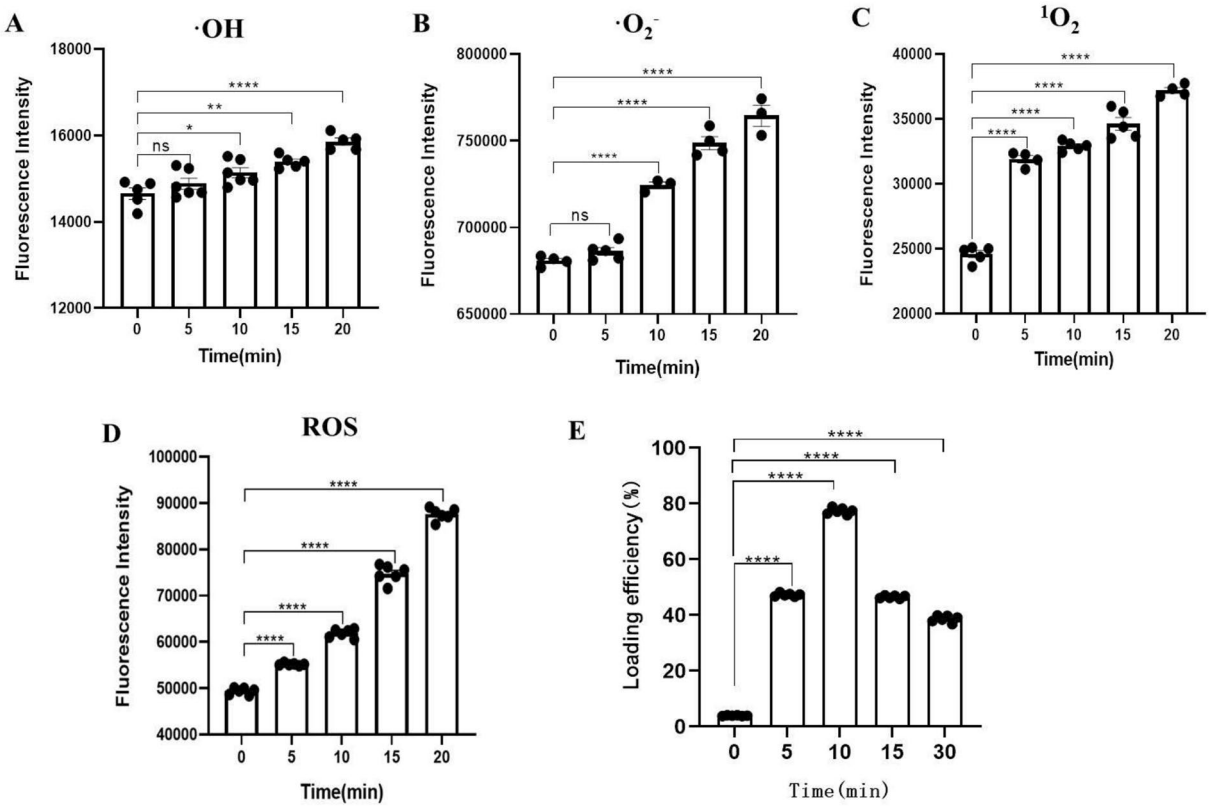

Fig. 1 HPDENs exhibit photosensitizer properties. **A**–**D** superoxide anions (O_2^-), hydroxyl radicals (·OH), singlet oxygen ($^1O_2$), and total ROS were measured in a time-dependent manner following LED light exposure for varying durations (0, 5, 10, 15, and 20 min). (**E**) FITC-labeled dextran (70 kDa) as a trackable fluorescent cargo. Loading efficiency was observed following LED light exposure for varying durations (0, 5, 10, 15, and 20 min). n = 3/group; *p < 0.05; **p < 0.01, ***p < 0.001; ****p < 0.0001

2.2 Optimization of PLDENs loading ratio with light treatment

To determine the optimal loading ratio, PLDENs were incubated with FITC-dextran at a fixed concentration of 25 µg, while PLDENs were tested at varying concentrations of 1, 2, 4, 8, 16, and 32 µg. The samples underwent a 10-min light treatment, followed by continuous monitoring of fluorescence intensity (Fig. 2A–C) and loading efficiency (Fig. 2D–F) over a 30-day period. During this time, the particle size and zeta potential of the loaded PLDENs were measured to assess their stability (Fig. 2G–J). The results indicated that a 1:30 ratio was optimal for encapsulation efficiency, with PLDENs maintaining excellent stability throughout the observation period.

Identifying the optimal loading ratio is crucial for achieving high encapsulation efficiency while preserving nanoparticle stability. The 1:30 ratio suggests that this proportion allows for maximal encapsulation without inducing excessive aggregation or destabilization. Higher PLDENs concentrations may lead to nanoparticle interactions that reduce encapsulation efficiency, while lower concentrations might result in insufficient loading. The stability assessment over 30 days demonstrated that PLDENs retained their physicochemical properties, including particle size and surface charge, which are critical for cellular uptake, biodistribution, and in vivo performance. The application of a 10 min light treatment introduces an additional variable that may influence nanoparticle behavior. The sustained stability observed suggests that light exposure did not cause significant structural degradation or aggregation, further supporting the robustness of this formulation.

2.3 ROS-Mediated lipid peroxidation

ROS-mediated lipid peroxidation involves the oxidation of polyunsaturated fatty acid side chains in biological membranes, producing malondialdehyde (MDA) and altering membrane fluidity and permeability. This disruption affects

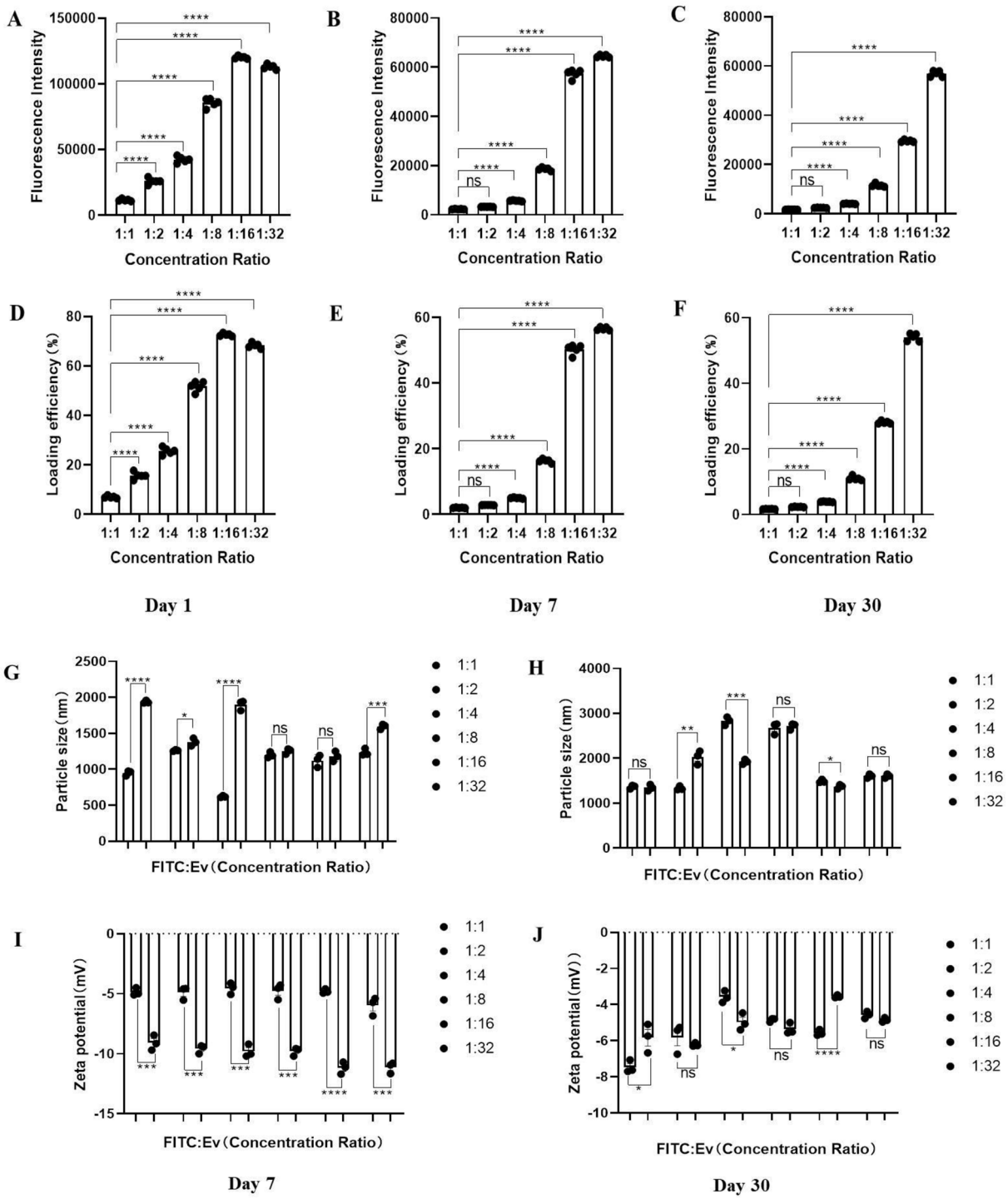

Fig. 2 Optimization of PLDENs loading ratio with light treatment. PLDENs underwent a 10-min light treatment, followed by continuous monitoring of fluorescence intensity (**A**–**C**) and loading efficiency (**D**–**F**) over a 30-day period. **G**–**J** FITC-dextran was added at a fixed concentration of 25 μg. The particle size and zeta potential were measured across a concentration gradient (1–32 μg)**.** FITC-dextran alone served as the negative control. n = 3/group; *p < 0.05; **p < 0.01, ***p < 0.001; ****p < 0.0001

cellular structure and function. In our study, light treatment increased MDA levels in a time-dependent manner (Fig. 3A), suggesting its impact on extracellular vesicle membrane properties.

To validate the cellular internalization of FITC-dextran-encapsulated PLDENs, we performed uptake experiments in human colonic epithelial cells (NCM460). Time-dependent fluorescence analysis revealed a progressive increase in intracellular signal intensity from 6 to 24 h post-incubation, reaching maximal accumulation at the 24-h time point (Fig. 3B). These findings confirm efficient internalization of FITC-dextran-loaded PLDENs following co-culture, demonstrating that light-trigged encapsulated PLDENs serve as effective nanocarriers for cargo delivery into human cells**.** Further investigations will elucidate the functional consequences of this light-dependent uptake mechanism, including intracellular trafficking and therapeutic potential.

Photothermal strategies show promise in antibacterial, drug delivery, and tissue regeneration applications [17–19]. Parallel advances in biomaterials include lipid nanoparticles for mRNA delivery [20] and non-viral gene carriers such as liposomes/extracellular vesicles [21]. PDEVs act as light-responsive nanocarriers, generating ROS under LED to transiently boost membrane permeability for controlled drug loading. Efficient cargo encapsulation was achieved under optimized irradiation, while excessive ROS disrupted membrane integrity. Stability and lipid peroxidation (via MDA) confirmed ROS-mediated membrane modulation (Fig. 3C). This strategy balances permeability and structural stability, advancing light-triggered drug delivery.

The natural origin and biocompatibility of PDEVs make them promising candidates for drug delivery. Our systematic investigation revealed that controlled LED irradiation induces moderate ROS generation, which transiently increases lipid peroxidation. This optimal ROS level creates temporary membrane pores that facilitate drug encapsulation, as demonstrated by: (1) dose-dependent loading efficiency peaking at 1:30 PLDEN:drug ratio, (2) MDA quantification showing controlled lipid peroxidation. These findings collectively established that light-mediated ROS generation enables reversible membrane reorganization for efficient loading while preserving vesicle stability, with excessive irradiation (> 15 min) causing irreversible membrane damage through overproduction of ROS. However, the behavior in physiological environments, such as biodistribution and clearance rates, requires further investigation. Smaller PDEVs may exhibit enhanced tissue penetration and longer circulation times, while surface modifications could reduce immune clearance. Future studies should focus on evaluating the behavior of light-responsive PDEVs in complex biological environments, including their stability under varying physiological conditions such as pH, enzymatic activity, and oxidative stress.

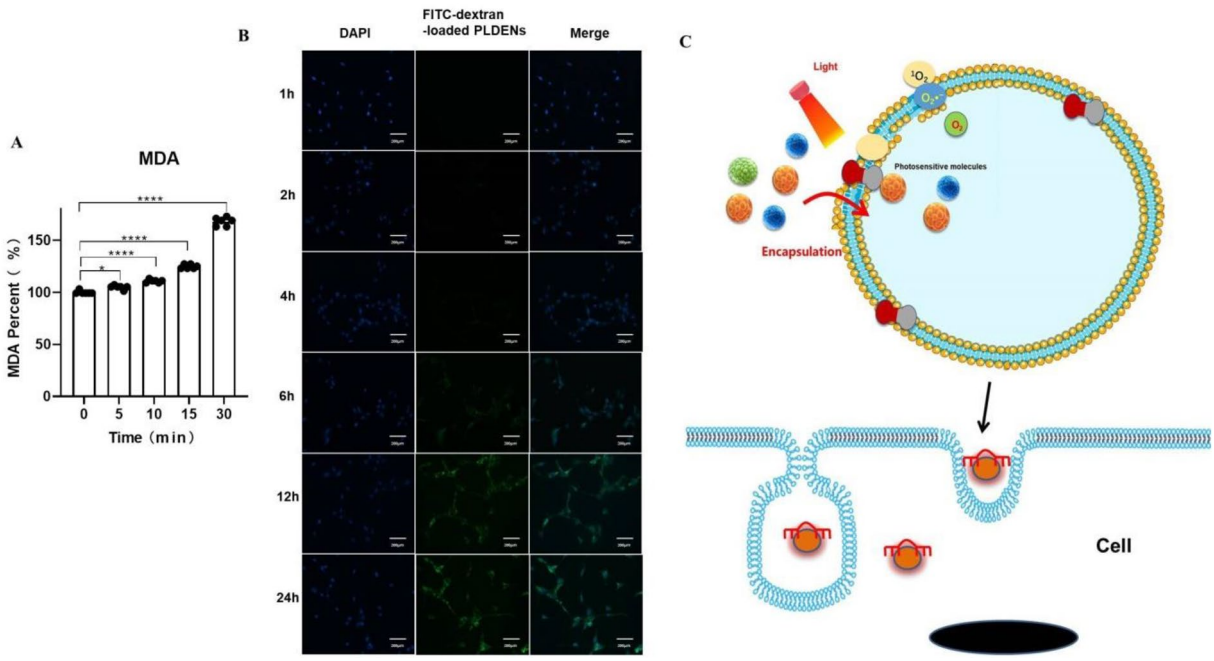

Fig. 3 Schematic diagram of light-triggered drug delivery in plant-derived extracellular vesicles. **A** MDA was assessed in PLDENs following LED light exposure for varying durations (0, 5, 10, 15, and 30 min). *p < 0.05; ****p < 0.0001. **B** Fluorescence microscopy images demonstrate the intracellular uptake of FITC-dextran-encapsulated PLDENs in NCM460 cells after a 24 h incubation period. The images reveal cytoplasmic localization of the FITC-dextran-encapsulated PLDENs, while nuclei were counterstained with DAPI (blue). Scale bar: 200 μm. **C** Schematic illustration of light altering plant-derived extracellular vesicle properties

Understanding how PDEVs interact with biological matrices (e.g., blood, extracellular fluids) and their performance in preclinical models will be essential for advancing their clinical translation. Additionally, exploring variations in light wavelength, surface modifications and targeting strategies could further enhance their specificity and efficacy in vivo.

2.4 Methods

2.4.1 Isolation of exosomes-like nanovesicles (PLDENs)

Fresh leaves and stems of *Pueraria lobata* (P. lobata) were collected from Luzhou, Sichuan, China. PLDENs were extracted by grinding the plant material, filtering the juice, and centrifuging at 10,000 × g for 10 min. Large debris was removed via 0.22 μm membrane filtration, followed by concentration using an Amicon Ultra-4 PL 100 KDa centrifugal filter at 5000 × g for 10 min at 4 °C. The PLDENs(Pueraria lobate-derived exosomes-like nanovesicles)suspension (10 μl) was diluted in calcium- and magnesium-free PBS to 1 ml for particle size and zeta potential analysis via NanoSight NS300. Protein content was quantified using the BCA assay.

2.5 Light treatment

PLDENs were resuspended and placed into the Light-loaded bioreactor and rotationally treated by light-emitting diode light. This device integrates monochromatic lights of three different wavelengths, including 580–595 nm (5–20 mW), 625–680 nm (10–100 mW) and/or 500–560 nm (10–60 mW) of monochromatic light.

2.5.1 Cell culture and uptake assay

Human colonic epithelial cells (NCM460) were purchased from Guangzhou Rongman Biotechnology Co., Ltd., and maintained in DMEM supplemented with 10% FBS (Gibco, USA), 1% L-glutamine, 10 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C, 5% CO_2. For the cellular uptake assay, FITC-dextran-loaded PLDENs were incubated with the cells, followed by DAPI staining for fluorescence microscopy observation.

2.6 Particle size analysis

PLDENs particle size was assessed using a NanoFCM instrument (N30E, NanoFCM, China). A diluted sample of 10 μL was analyzed for particle size distribution.

2.7 Zeta potential measurement

The Zeta potential of PLDENs was determined using a Malvern laser particle size analyzer (Malvern Company, UK).

2.8 General ROS detection

PLDENs were exposed to LED light for varying durations (0, 5, 10, 15, and 20 min) DCFH-DA used for ROS detection, and fluorescence was measured.

2.9 Superoxide radical detection

The concentration of superoxide radical in PLDENs was determined by superoxide detection assay kit (Abcom) following LED light exposure for varying durations (0, 5, 10, 15, and 20 min).

2.10 Hydroxyl radical detection

Hydroxyl radicals were similarly detected using dihydrorhodamine 123 (DHR123, MCE Co.) after LED light exposure for varying durations (0, 5, 10, 15, and 20 min).

2.11 Singlet oxygen detection

Singlet oxygen was detected by using SOSG (Thermo Fisher) in a mixture of PLDENs after LED light exposure for varying durations (0, 5, 10, 15, and 20 min).

2.12 Measurement of malondialdehyde (MDA)

PLDENs were exposed to LED light irradiation for different durations (0, 5, 10, 15, and 30 min). Subsequently, the samples were mixed with the MDA reagent (Nanjing Jiancheng Bioengineering Institute) and incubated in a boiling water bath for 15 min. After cooling, the absorbance at 532 nm was measured using a microplate reader (Molecular Devices, USA).

A standard curve was generated using known MDA concentrations, where the optical density (OD) of the pink chromogen exhibited a linear correlation with MDA levels. The MDA concentration in test samples was determined by interpolating their absorbance values against the standard curve. The final MDA concentration was adjusted for dilution factors and expressed as a percentage relative to the control group.

2.13 Light-based encapsulation into PLDENs

PLDENs were incubated with dextran-FITC (Sigma–Aldrich) in PBS at 37 °C for 30 min. The concentration of PLDENs was quantified using the bicinchoninic acid (BCA) protein assay. To determine the optimal yield of exogenous protein encapsulation, FITC-dextran was added at a fixed concentration of 25 μg, while PLDENs were tested at varying concentrations of 1, 2, 4, 8, 16, and 32 μg. FITC-dextran alone served as the negative control.

2.14 Statistical analysis

Statistical analyses were conducted using SPSS 24.0, with data presented as mean ± SEM. Significance between groups was determined using one-way or two-way ANOVA, with $P < 0.05$ considered significant.

Author contributions Y.L. W, Z. and J.W. designed and performed experiments, analyzed data; P.Z. Y.H. X.H. and Y.L. collection and/or assembly of data, data analysis and interpretation; J.W. designed and analyzed experiments and wrote the manuscript. All authors reviewed the manuscript.

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Data availability Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication All authors are aware of this submission.

Competing interests The authors declare no competing interests.

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