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Supramolecular engineering of cell membrane vesicles for cancer immunotherapy

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ABSTRACT

The clinical translation of nanomedicines has been strongly hampered by the limitations of delivery vehicles, promoting scientists to search for novel nanocarriers. Although cell membrane-based delivery systems have attracted extensive attention, further functionalizations are urgently desired to augment their theranostic functions. We propose a cell-friendly supramolecular strategy to engineer cell membranes utilizing cyclodextrin-based host–guest molecular recognitions to fix the defects arising from chemical and genetic modifications. In this study, the supramolecular cell membrane vesicles (SCMVs) specifically accumulate in tumors, benefiting from tumor-homing capability and the enhanced permeability and retention effect. SCMVs co-delivering indocyanine green and an indoleamine 2,3-dioxygenase inhibitor effectively ablate tumors combining photodynamic therapy and immunotherapy. Driven by host–guest inclusion complexation, SCMVs successfully encapsulate resiquimod to repolarize tumor-associated macrophages into M1 phenotype, synergizing with immune checkpoint blockade therapy. This supramolecular engineering methodology based on noncovalent interactions presents a generalizable and cell-friendly tactic to develop living cell–originated nanomaterials for precise cancer therapy.

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1. Introduction

The overall rate of successful clinical translations is extremely low despite the approval granted for the use of several anticancer nanomedicines such as Abraxane (albumin-bound paclitaxel), Doxil (liposomal doxorubicin), and Onivyde (liposomal irinotecan) [1–4]. Current drug delivery systems are mainly fabricated from organic compounds, inorganic nanostructures, synthetic polymers, and biomacromolecules. However, these exogenous invaders easily stimulate the immune clearance mechanism by the reticuloendothelial systems, significantly reducing the therapeutic efficacy and causing undesirable side effects [5–10]. Synthetic polymers with good biodegradability have been widely used to prepare nanomedicines, but it takes several weeks to degrade the polymeric carriers into oligomers that inevitably interact with immune cells, resulting in severe adverse reactions and immunotoxicity [11–13]. PEGylated liposomal formulations have achieved several breakthroughs, but these medicinal benefits are at a price. The generation of anti-PEG antibodies after repeat injection of PEGylated nanomedicines always attenuates the therapeutic benefits and causes strong hypersensitivity reactions [14,15].

Cell membrane-derived delivery systems with desirable characteristics different from artificial delivery vectors have been extensively investigated [16–20]. A wide spectrum of biological membranes has been employed to fabricate nanomedicines that effectively prolong their circulation time, improve their targeting capability, and modulate the immune responses, thus optimizing the final therapeutic outcomes of these nanoformulations [21– 26]. However, it remains challenging to rationally engineer the cell membrane for better theranostic applications. Chemical modification has emerged as a formidable tool for cell membrane engineering to resurface the molecular landscape of cells; however, the

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perturbation of inherent cellular physiology based on chemical strategies always interferes with the cellular processes governed by the biomacromolecules on cell membrane. Genetic and metabolic engineering strategies harbor different ligands on cell membrane nanomaterials via natural biosynthetic procedures [27–29]. These approaches need rational designs and costly reagents, and also suffer from genetic contamination, low incorporation efficiency, and limited scalability. The complexity of the preparative processes remarkably hampers their clinical applications, prompting scientists to develop a facile, cell-friendly, and general approach to engineer the cell membrane to develop sophisticated nanomedicines.

Herein, we report a supramolecular approach to engineer cell membrane by taking advantage of noncovalent interactions without disrupting their intrinsic biological functions (Fig. 1). Supramolecular cell membrane vesicles (SCMVs) successfully loaded adamantane (Ad)-modified indocyanine green (Ad-ICG) through the host-guest complexation between β -cyclodextrin (β -CD) and Ad, allowing near-infrared-II (NIR-II) tumor imaging and photodynamic therapy (PDT). SCMVs co-delivering Ad-ICG in the membrane and an indoleamine 2,3-dioxygenase (IDO) inhibitor in the inner cavity highly accumulated in the tumor ascribing to tumor-homing capability and the enhanced permeability and retention (EPR) effect. The combination of PDT, PDT-triggered immunogenetic cell death (ICD), and IDO blockade resulted in the synergistic anticancer efficacy that ablated the tumors without relapse. Intriguingly, a Toll-like receptor 7 and 8 (TLR7/8) agonist, resiguimod (R848), was encapsulated in SCMVs through the hostguest inclusion complexation with β -CD, showing the ability to repolarize tumor-associated macrophages from M2 phenotype to M1 phenotype. The nanoformulation SCMVs@R848 effectively rewired tumor microenvironment and suppressed the tumor growth, and the antitumor performance was further boosted in synergy with immune checkpoint blockade therapy.

2. Experimental

2.1. Materials

Indocyanine green (ICG), p-toluenesulfonyl chloride (TsCl), β-cyclodextrin (β-CD), ICG-NH₂, fluorescein isothiocyanate (FITC) diaminohexane. 1-adamantanemethylamine, isomer. N-hydroxysuccinimide, rhodamine isothiocyanate, cholesteryl hemisuccinate (Chol-COOH), 1-adamantanecarbonyl chloride, N, *N*-dimethylformamide (DMF), 4-(dimethylamino)pyridine (DMAP), and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) were purchased from TCI (Tokyo, Japan) and Sigma-Aldrich (MA, USA). The MTT cytotoxicity assay kits for the detection of cell viability were bought from ThermoFisher Scientific (MA, USA). Anti-CD3-PerCP-Cy5.5, anti-CD4-FITC, anti-CD80-PE, anti-CD11c-FITC, anti-CD86-APC, anti-FoxP3-PE, anti-IFN-γ-APC, and anti-CD25-APC, antibodies were obtained from BioLegend, Inc. (CA, USA). Antibodies for calreticulin (CRT), high-mobility group box 1 (HMGB1), CD3, and CD8 were purchased from Abcam (Cambridge, UK). The IFN- γ enzyme-linked immunosorbent assay (ELISA) kit was purchased from Neobioscience Technology (Shenzhen, China). Solvents were purchased from commercial suppliers or dried according to the reported procedures described in the literature. Water was purified with the Milli-Q purification system. UV-vis absorption spectra were measured by a Hitachi U-3010 spectrophotometer (Tokyo). Fluorescence spectra were collected using a Hitachi F-7000 fluorescence spectrophotometer (Tokyo). Transmission electron microscopy (TEM) was performed on an HT-7700 instrument (Tokyo). Confocal laser scanning microscopy (CLSM) images were captured using a LSM710META (Zeiss) microscope (Oberkochen, Germany). The high-performance liquid chromatography (HPLC) analysis was conducted on an Agilent1200 machine (Agilent Technologies, CA, USA). The particle sizes were measured by the dynamic light scattering (DLS) on analyzer Zetasizer Nano ZS90 (Malvern Panalytical, Malvern, UK) with a detection angle of 90° at 25 °C using an incident He-Ne laser ($\lambda = 633$ nm).

2.2. Preparation of SCMVs

The 4T1 cells or U87MG cells were seeded to 6-well plates at a density of 2×10^5 per well and placed in a 37 °C humidified incubator with 5% CO₂/95% air supply. After 24 h culture, the cells were incubated with Chol-CD (100 µmol/L) for 8 h. The culture medium was removed, and the cells were washed with phosphate-buffered saline (PBS) thrice. Fresh medium was added in the presence of Ad-ICG (20 μ mol/L), and the cells were further cultured for 45 min. The culture medium was discarded, and the cells were washed with PBS thrice. The harvested cells were suspended in a hypotonic lysing buffer, and disrupted using a Dounce Homogenizer (CA, USA). Following DNase and RNase treatment (Invitrogen), the solution was centrifuged at $3200 \times g$ for 5 min. The supernatant was collected and centrifuged at 20,000 \times g for 30 min, and then at $80,000 \times g$ for 1.5 h. The pellets were collected and washed with protease inhibitor tablet-mixed PBS thrice, sonicated for 5 min, and finally extruded through 400-, 200-, and 100-nm polycarbonate porous membranes on a Mini-Extruder (Avanti Polar Lipids, AL, USA). The obtained SCMVs@Ad-ICG were stored at 4 °C for further in vitro and in vivo experiments. The contents of the supramolecular complex in the membrane could be adjusted by changing the amount of Chol-CD and Ad-ICG during incubation; the loading content could be calculated by detecting the amount of Ad-ICG through fluorescence measurement using a standard curve. 1-MT was dissolved in PBS and added into the membrane suspension to prepare SCMVs@Ad-ICG/1-MT. The mixture was shaken for 1 h and then extruded through 400-, 200-, and 100-nm polycarbonate porous membranes using a Mini-Extruder. After dialysis against PBS for 4 h to remove unloaded 1-MT, SCMVs@Ad-ICG/1-MT was obtained and stored at 4 °C for further in vitro and in vivo experiments. HPLC was used to measure the loading content using the equation: Loading content (%) = $(M_{loaded}/M) \times 100$, where M_{loaded} is the mass of 1-MT loaded in SCMVs@Ad-ICG/1-MT, and M is the mass of SCMVs@Ad-ICG/1-MT.

2.3. Evaluation of anticancer efficacy in vitro

The cytotoxicity of the different formulations against the 4T1 cells was determined using the MTT assays in a 96-well cell culture plate. Filters of 0.22 µm were used to sterilize all solutions before tests. The 4T1 cells were seeded at a density of 1.0×10^4 cells per well in a 96-well plate and incubated for 24 h for attachment. The cells were then incubated with free Chol-CD, Ad-ICG, SCMVs@Ad-ICG, or SCMVs@Ad-ICG/1-MT at different concentrations for 48 h. For SCMVs@Ad-ICG + L or SCMVs@Ad-ICG/1-MT + L, the cells were irradiated with a laser at 808 nm (50 mW/cm²) for 5 min. The cells were further cultured for another 48 h. After washing the cells with PBS buffer, 20 µL of an MTT solution (5 mg/mL) and 180 µL of media were added to each well. The MTT solution was removed after 4 h of incubation at 37 °C, and a volume of 100 µL dimethylsulfoxide (DMSO) was added to solubilise insoluble formazan crystals. The absorbance was measured at 570 nm using a spectrophotometer. Untreated cells in media were used as a control. All experiments were carried out with five replicates.



Fig. 1. (Color online) Fabrication of SCMVs. (a) Chemical structures and sketches of Chol-CD, Ad-ICG, Ad-Na, 1-MT, and R848. (b) Illustration of the procedures involving the preparation of SCMVs@Ad-ICG. (c) ¹H NMR spectra (400 MHz, D₂O, room temperature) of I, Ad-Na (3.00 mmol/L); II, β -CD (3.00 mmol/L) and Ad-Na (3.00 mmol/L); III, β -CD (1.00 mmol/L) and Ad-Na (3.00 mmol/L); III, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); IV, β -CD (3.00 mmol/L); III, β -CD (3.00 mmol/L); IIII for addition of Rho-CD. (f) TEM image of SCMVs@Ad-ICG. (g) CLSM images of the 4T1 cells after incubation with Ad-ICS hypertations of the 4T1 cells incubated with SCMVs@Ad-ICG. Scale bar: 20 µm. (I) Fluorescence intensity of the 4T1 and U87MG cells cultured with SCMVs@Ad-ICG from I, 4T1 cells and II, U87MG cells.

2.4. Induction of immunogenic cell death

Flow cytometric measurement and immunofluorescence were used for the surface detection of CRT. For flow cytometry, the 4T1 cells were seeded in the 24-well plate at a density of 4×10^4 cells per well. After 12 h of preincubation, the cells were incubated with PBS, 1-MT, SCMVs@Ad-ICG, and SCMVs@Ad-ICG/1-MT at an identical 1-MT concentration of 2.00 µmol/L for 4 h. For SCMVs@Ad-ICG + L and SCMVs@Ad-ICG/1-MT + L, the cells were irradiated with a laser at 808 nm (50 mW/cm²) for 5 min. The cells were further cultured for another 8 h. Next, the cells were washed twice with cold PBS and fixed with 0.25% paraformaldehyde for 5 min. The cells were then incubated with primary antibodies after washing twice in cold PBS. The cells were washed again 30 min later and incubated with the Alexa Fluor Plus 488conjugated monoclonal secondary antibody for 30 min. Finally, the cells were analyzed using CLSM and flow cytometry (BD Biosciences, UK).

The extracellular secretion of adenosine triphosphate (ATP) was tested using a commercially available ATP assay kit. Briefly, the 4T1 cells were seeded in the 24-well plate at a density of 4×10^4 cells per well. After 12 h of preincubation, the cells were incubated with PBS, 1-MT, SCMVs@Ad-ICG, and SCMVs@Ad-ICG/1-MT at an identical 1-MT concentration of 2.00 μ mol/L for 4 h. For SCMVs@Ad-ICG + L and SCMVs@Ad-ICG/1-MT + L, the cells were irradiated with a laser at 808 nm (50 mW/cm²) for 5 min. The cells were further cultured for another 24 h. Then, the cell culture supernatant was collected, and the ATP content was tested using an ATP assay kit following the manufacturer's instructions.

The intracellular HMGB1 release was visualized using immunofluorescence analysis. Briefly, a cell density of 4×10^4 cells per dish was used for the propagation of 4T1 cells in live-cell imaging glass bottom dishs overnight and then treated with PBS, 1-MT, SCMVs@Ad-ICG, and SCMVs@Ad-ICG/1-MT at an identical 1-MT concentration of 2.00 µmol/L for 4 h. For SCMVs@Ad-ICG + L and SCMVs@Ad-ICG/1-MT + L, the cells were irradiated with a laser at 808 nm (50 mW/cm^2) for 5 min. The cells were further cultured for another 24 h. Following that, intracellular HMGB1 was stained with antibodies. In brief, cells were fixed for 15 min in 4% paraformaldehyde and permeabilized for 20 min in 0.1% Triton X-100. Nonspecific binding sites were blocked through preincubation with 1% fetal bovine serum in PBS for 45 min, followed by incubation with primary antibodies for 1 h, and then incubated with an Alexa Fluor Plus 488-conjugated monoclonal secondary antibody for 45 min after washed with PBS thrice. Finally, the cells were stained with DAPI and examined using CLSM.

2.5. DC maturation in vitro

Bone marrow–derived DCs (BMDCs) were generated from the bone marrow of 8-week-old BALB/c mice to examine DC maturation *in vitro*. The 4T1 cells were pretreated with PBS, 1-MT, SCMVs@Ad-ICG, and SCMVs@Ad-ICG/1-MT at an identical 1-MT concentration of 2.00 μ mol/L for 8 h. For SCMVs@Ad-ICG + L and SCMVs@Ad-ICG/1-MT + L, the cells were irradiated with a laser at 808 nm (50 mW/cm²) for 5 min. The cells were further cultured for another 24 h. Afterward, 1 × 10⁶ immature DC cells were co-cultured with 1 × 10⁵ pretreated 4T1 cells. After staining with anti-CD11c-FITC, anti-CD80-PE, and anti-CD86-APC antibodies, the maturation of the DC cells was examined using flow cytometry.

2.6. Animal studies

Under protocols approved by the Experimental Animal Committee at Tsinghua University (protocol number: 21-YGC1), all animal studies were conducted based on the principles and procedures outlined in the institutional guide for the care and use of animals. BALB/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co., ltd. (China). It was provided with bedding, nesting material, food, and water. An ambient temperature of 20–22 °C was maintained throughout the day with 12-h of light and 12-h of darkness. About 25 μ L (300 μ mol/L) of imaging agents were intradermally injected into the mouse footpad to visualize sentinel LN.

2.7. NIR-II tumor imaging

Prior to injecting imaging agents into mice, they were shaved using Nair hair removal cream and anesthetized with isoflurane. The injected dose of each formulation was 2.00 mg ICG per kg for high contrast imaging or real-time ultrafast imaging. The excitation laser was an 808-nm CW laser at a power density of ~0.15 W/cm² (50 ms exposure, 900 + 1000 LP). With excitation light close to the absorption peak of the fluorophore, similar images were obtained at lower power density and/or shorter exposure time.

2.8. In vivo antitumor efficacy evaluation

The cell suspension containing 5×10^6 4 T1 cells was injected subcutaneously in the left flank region of mice with 200 µL on day 14 and in the right flank region with 200 µL on day 7. Before experiments, a total of 100 mm³ of volume was allowed for primary tumors. The volume of tumors was calculated as follows: (tumor length) \times (tumor width)²/2. The mice were injected intravenously with PBS, 1-MT, SCMVs@Ad-ICG, SCMVs@Ad-ICG/1-MT, SCMVs@Ad-ICG + L, and SCMVs@Ad-ICG/1-MT + L every 3 d thrice. The injected dose of 1-MT was 2.00 mg/kg. For the mice treated with SCMVs@Ad-ICG + L and SCMVs@Ad-ICG/1-MT + L, laser irradiation (808 nm, 50 mW/cm², 15 min) was conducted at 24 h post injection. The tumor volume and body weight were measured every 3 d. The primary and distant tumors were harvested at the end of antitumor studies. Histological analyses tissues were fixed with paraformaldehvde (4%) for 24 h. A 5-mm-thick section of each specimen was cut after dehydration in graded ethanol, embedding in paraffin. The standard protocol was followed in deparaffinization and hydrating of the sections, and hematoxylin and eosin (H&E) were stained for microscopic observations.

2.9. Intratumoral infiltration of T lymphocytes

To examine the intratumoral infiltration of T lymphocytes, the tumors were harvested on day 3, cut into small pieces, and immersed in the solution of 1 mg/mL collagenase IV and 0.2 mg/mL DNase I for 45 min at 37 °C. The small tumor pieces were pressed gently to obtain a single-cell suspension solution. Finally, the single cells were stained with fluorescent-labeled antibody following the manufacturer's protocols. To analyze the Tregs (CD3⁺-CD4⁺CD25⁺ Foxp3⁺), the lymphocytes were stained with anti-CD3-PerCP-Cy5.5, anti-CD4-FITC, anti-CD25-APC, anti-ForxP3-PE according to the manufacturer's protocols. For the analysis of the CD8⁺ T cells (CD3⁺CD4⁻CD8⁺) and CD4⁺ T cells (CD3⁺CD4⁺CD8⁻), the lymphocytes were stained with anti-CD3-PerCP-Cy5.5, anti-CD4-FITC, anti-CD3-PerCP-Cy5.5, anti-CD4-FITC, anti-CD3-PerCP-Cy5.5, anti-CD4-FITC, anti-CD3-PerCP-Cy5.5, anti-CD4-FITC, anti-CD8-PE, and anti-IFN- γ -APC antibodies following the manufacturer's protocols. The cells were analyzed using flow cytometry.

2.10. BMDM polarized activation and verification

For the M2 activation, the bone marrow-derived macrophages (BMDMs) or RAW264.7 were incubated with DMEM containing IL-4 (10 ng/mL). The M2 BMDMs or RAW264.7 were re-educated

to M1 by incubating in a complete medium (DMEM with 10% FBS and 1% P/S) containing LPS (100 ng/mL), R848 (200 nmol/L), or SCMVs@R848 (the concentration of R848 was 200 nmol/L). The polarization was verified by cell morphology and biomarker expression. After the fixation and permeabilization, BMDMs were stained with FITC-conjugated phalloidin (KeyGEN BioTECH, Nanjing, China) and Hoechst (Beyotime Biotechnology, Shanghai, China) to observe the morphology using CLSM. The expression of nitric oxide synthase (iNOS) and IL-6 (M1 activation) or Arg-1 and MRC1 (M2 activation) was detected using qRT-PCR. The relative levels of each value were normalized with GAPDH. After polarization, the medium supernatants of the cells were harvested for ELISA analysis to verify the release of IL-12. All procedures followed the manufacturer's protocol.

2.11. Macrophage phagocytosis behavior

The BMDM cells were stained with DIL (5 µmol/L) after being polarized into M1 by SCMVs@R848. The 4T1 cells were labeled with DIO (5 µmol/L). The cells were incubated with the culture medium containing the dyes for 20 min. The stained cells were subsequently washed thrice with PBS. Then, the M1 macrophages (5×10^3) and 4T1 cells (5×10^3) were seeded into the glassbottom cell culture dishes. After 8 h of co-incubation, the cells were washed five times with PBS and fixed with paraformaldehyde. The cells were treated with a sealing solution containing DAPI and an antifluorescent quenching agent, and the images were taken using a CLSM.

2.12. In vivo antitumor treatments

Mice were randomly divided into six groups (n = 5) and treated with PBS, \alpha PD-L1, R848, SCMVs@R848, R848 + \alpha PD-L1, and SCMVs@R848 + α PD-L1, respectively. The injection doses of R848 and α PD-L1 were 2.00 mg/kg and 100 µg per mouse, respectively. For in vivo antitumor therapy, R848 and SCMVs@R848 were intratumorally injected, while α PD-L1 was injected intravenously. The injections were repeated every 3 d thrice. One mouse from each group was randomly selected and sacrificed on the third day after the third injection to donate tumors for H&E staining and immunofluorescence staining; the other mice were all euthanized on day 22. The body weight and tumor volume of mice were measured every 3 d from the first injection. For H&E staining and immunofluorescence staining, the tissues were dehydrated in graded ethanol after fixed in 4% paraformaldehyde for 24 h. The 5-mm thick section was cut from the paraffin embedding samples. For microscopic observation, fixed sections were deparaffinized and hydrated according to a standard protocol and stained with H&E or different immunofluorescent antibodies.

2.13. Statistical analysis

Data are presented as the mean \pm standard deviation (SD). Statistical analysis of data was performed with one-way analysis of variance.**P* < 0.05, ***P* < 0.01, ****P* < 0.001.

3. Results and discussion

3.1. Preparation of SCMVs and SCMVs@Ad-ICG

The building blocks were successfully synthesized and fully characterized (Figs. S1–S10 online). The host–guest molecular recognition in aqueous solution was verified by ¹H NMR spectra (Fig. 1c and Fig. S11 online); upfield shifts occurred for the protons on 1-adamantane carboxylate sodium (Ad-Na) upon formation of

an inclusion complex. Strong nuclear Overhauser effect correlations were detected in 2D NOESY NMR spectrum between the proton signals on β -CD and Ad-Na (Fig. 1d), indicating that the hydrophobic Ad group penetrated deep into the cavity of β -CD. The host-guest complexation was also studied by exploiting fluorescence resonance energy transfer. β -CD and Ad were modified using rhodamine (Rho-CD) and fluorescein (FITC-Ad), which acted as fluorescence acceptor and donor, respectively (Figs. S12 and S13 online). The emission at 525 nm diminished, while the peak at 585 nm was enhanced by the gradual addition of Rho-CD into the solution containing FITC-Ad because of the fluorescence resonance energy transfer, further confirming the formation of the inclusion complex (Fig. 1e). In situ molecular recognition between Chol-CD and Ad-ICG was directly monitored using CLSM. The red signal was monitored on the cell membrane of the 4T1 cells incubated with Chol-CD, followed by the incubation with Ad-ICG. In sharp comparison, the red signal was mainly located in the cytoplasm for the cells cultured with Ad-ICG without pretreatment of Chol-CD (Fig. 1g). TEM indicated that Ad-ICG itself selfassembled into nanoparticles in an aqueous solution (Fig. 1f), which were directly internalized through endocytosis. These results demonstrated that the membrane was modified through a supramolecular strategy using Chol-CD and host-guest molecular recognition could take place.

The membrane embedding the host-guest complex was harvested after removing the intracellular content through combination treatments, including hypotonic lysis, mechanical disruption, and gradient centrifugation. SCMVs@Ad-ICG were obtained after sonication and extrusion of the membrane through nanopores on a mini extruder. The anchoring amount of the supramolecular complex was calculated by measuring the absorption of Ad-ICG according to the standard curve, which was roughly determined to be 3.54% (Fig. S14 online). Due to the existence of Ad-ICG, the morphology of SCMVs@Ad-ICG could be visualized using CLSM, in which small spherical nanostructures with strong red fluorescence were observed (Fig. 1h). As shown in the TEM image, hollow vesicles with the size ranging from 60 to 100 nm were observed (Fig. 1i). The average diameter of SCMVs@Ad-ICG was measured as 110 ± 12 nm using DLS, which was in agreement with the result obtained from TEM (Fig. 1j).

The zeta potential of SCMVs@Ad-ICG was determined to be -42.6 mV due to the electronegative nature of the cell membrane (Fig. S15 online). The stability of SCMVs@Ad-ICG was evaluated in a physiological environment; a negligible amount of Ad-ICG leaked out from SCMVs@Ad-ICG in PBS containing 10% of fetal bovine serum (Fig. S16 online). DLS also demonstrated outstanding colloidal stability, as evidenced by the little changes in average diameter within 48 h of incubation (Fig. S17 online). The biocompatibility of Chol-CD, Ad-ICG, SCMVs, and SCMVs@Ad-ICG was confirmed by а 3-(4',5'-dimethylthiazol-2'-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Figs. S18-S21 online). Benefiting from the natural property, ignorable hemolysis (<5%) was monitored by culturing red blood cells with SCMVs@Ad-ICG at different times in its test concentration range (Fig. S22 online), which was a convincing indicator of its safety for in vivo studies.

The surface engineering of nanomaterials using natural cell membranes exhibited unique advantages by intact inheritance of protein diversity on the source cell membrane. Benefiting from the inherent homologous adhesion properties, the nanoformulations decorated with cell membrane were expected to possess the active homing ability. Two different SCMVs@Ad-ICG were prepared using the membranes harvested from U87MG and 4T1 cells to verify their membrane-mediated homologous targeting effect [30,31]. As shown in Fig. 1k and l, the cancer cells selectively internalized the SCMVs@Ad-ICG derived from homologous cell membrane: SCMVs@Ad-ICG from 4T1 cells showed strong affinity to

homologous 4T1 cells, leading to much higher cellular uptake compared with SCMVs@Ad-ICG from the U87MG cells.

3.2. NIR-II imaging of tumors and safety evaluations

It has been demonstrated that ICG emits NIR-II fluorescence in 1000–1700 nm window, allowing to clarify deep structures with extremely high contrast-to-noise ratio [32,33]. Since the size of SCMVs@Ad-ICG was located within the optimal range for the EPR effect, they offered potential for tumor imaging and diagnosis. *In vivo* NIR-II imaging was performed at different times after injection to evaluate the tumor retention of SCMVs@Ad-ICG. SCMVs@Ad-ICG highly accumulated in the tumor at 6 h post injection, and the fluorescence further increased at 8, 16, and 24 h post injection (Fig. 2a and b). At 48 h post injection, the intratumoral fluorescence intensity still remained high, the tumor could be distinguished clearly from the surrounding tissue. The metastatic nodes with extremely small size in the lung were also clearly detected (Fig. 2c and d), possibly benefiting from the tumor-homing effect, which was extremely important for cancer theranostics.

For further biomedical applications, the safety of SCMVs@Ad-ICG was carefully assessed by monitoring the body weight changes and tissue pathology of the treated mice. No significant differences in body weights were observed between the SCMVs@Ad-ICG-treated group and the PBS-treated control group (Fig. S23 online). The pathological analyses using H&E staining demonstrated negligible morphological changes in the tested main organs, such as heart, liver, spleen, kidneys, and lungs (Fig. 2e). The hematology test and serum biochemistry analyses also confirmed that the hematological parameters were in the normal range for the mice formulated with SCMVs@Ad-ICG (Figs. S23 and S24 online).

3.3. In vivo antitumor therapy combining the PDT and IDO inhibitor

ICG is an excellent photosensitizer for PDT with the ability to ablate tumors by generating singlet oxygen $({}^{1}O_{2})$ [34–36]. Besides, the PDT can also trigger an ICD by releasing damage-associated molecular patterns (DAMPs) to promote tumor sensitivity and activate immune responses for synergistic therapy (Fig. 3a) [37,38]. An IDO inhibitor (1-methyl-tryptophan, 1-MT) was encapsulated into the hollow cavity of SCMVs@Ad-ICG with a loading content of 4.2% to strengthen durable immune responses and reverse "cold" immunosuppressive microenvironment, which could block the catalysis of tryptophan (Trp) into kynurenine (Kyn) [39–42]. TEM, DLS, and zeta potential investigations demonstrated no obvious changes in size and morphology after encapsulation of 1-MT (Figs. S25–S28 online). The generation of ${}^{1}O_{2}$ was validated using singlet oxygen sensor green (SOSG) as a probe whose characteristic peak at 530 nm increased rapidly upon laser irradiation in the presence of SCMVs@Ad-ICG/1-MT (Fig. 3b). The intracellular ¹O₂ generation was detected using 2,7-dichlorodihydrofluorescein diacetate (DCF-DA); bright green fluorescence was monitored for the cells cultured with SCMVs@Ad-ICG or SCMVs@Ad-ICG/1-MT followed by laser irradiation (Fig. 3c and Fig. S29 online). A comparison of the fluorescence changes of DCF before and after laser irradiation revealed that the encapsulation of 1-MT did not alter the ${}^{1}O_{2}$ production.

The MTT assay confirmed low cytotoxicity of SCMVs@Ad-ICG and SCMVs@Ad-ICG/1-MT; the cells were highly viable in the absence of laser irradiation (Fig. 3d). On the contrary, the anticancer efficacy was activated after laser exposure, indicating the distinguished ability of ROS production by Ad-ICG anchoring on SCMVs. A robust anticancer efficacy of SCMVs@Ad-ICG/1-MT was further confirmed using Annexin-V FITC/propidium iodide dualstaining; more than 40% of the 4T1 cells were located in early and late apoptotic states upon laser irradiation (Figs. S30–S33 online). A concentration-dependent inhibition was monitored using SCMVs@Ad-ICG/1-MT by measuring the ratio of Trp to Kyn in the 4 T1 cells, which was comparable to that of 1-MT (Fig. 3e), benefiting from the efficient internalization of the nanoformulation.

The PDT-mediated ICD was characterized by the release of DAMPs, including CRT, HMGB1, and ATP. CRT exposed on the cell surface after PDT was evidenced by CLSM, and the green fluorescence was observed in the cells treated with SCMVs@Ad-ICG or SCMVs@Ad-ICG/1-MT followed by laser irradiation (Fig. 3f). The percentages of the CRT-positive 4T1 cells were 10.4% ± 1.3%, $13.2\% \pm 1.4\%$, $9.82\% \pm 1.1\%$, $11.6\% \pm 1.4\%$, $37.6\% \pm 4.1\%$, and 40.1% ± 4.9% after the treatment with PBS, 1-MT, SCMVs@Ad-ICG, SCMVs@Ad-ICG/1-MT, SCMVs@Ad-ICG + L, and SCMVs@Ad-IC G/1-MT + L, respectively (Fig. 3g). Besides, PDT induced significant extracellular release of HMGB1, as immunofluorescence analysis showed that the cells pretreated with SCMVs@Ad-ICG or SCMVs@Ad-ICG/1-MT followed by laser irradiation had tremendously reduced HMGB1 signal in the nuclei (Fig. 3h and i). An ATP assay was also employed to measure ATP secretion quantitatively. The laser exposure resulted in 2.5- and 3.0-fold higher ATP efflux for the 4T1 cells treated with SCMVs@Ad-ICG or SCMVs@Ad-ICG/1-MT compared with those in the absence of irradiation (Fig. 3j). DAMPs, including HMGB1 and ATP produced by dying cancer cells, possessed high immunogenicity that promoted the maturation of DCs to stimulate adaptive immune responses. The frequency of matured DCs was significantly elicited by culturing with the 4T1 cells pretreated with SCMVs@Ad-ICG + L and SCMVs@Ad-ICG/1-MT + L (Fig. 3k).

A bilateral tumor model was established to evaluate the therapeutic efficacy of PDT-synergized immunotherapy by subcutaneously inoculating the tumors in the left (primary tumor) and right (distant tumor) flanks (Fig. 4a). The formulation of 1-MT, SCMVs@Ad-ICG, or SCMVs@Ad-ICG/1-MT when administered to mice showed limited therapeutic benefits compared with PBS, as the tumors grew rapidly in them (Fig. 4b). PDT alone hardly ablated the infiltrating cancer cells: the tumor growth was inhibited in the initial days in the mice treated with SCMVs@Ad-ICG + L, resulting in tumor relapse. Intriguingly, the combination of PDT and IDO blockade exhibited the highest antitumor performance, which completely eradicated the primary tumors (Figs. S34-S40 online). The administration of SCMVs@Ad-ICG/1-MT + L regressed the growth of distant tumors effectively along with providing superior efficacy against the primary tumors (Fig. 4c and Figs. S41-S47 online) because the ICD-activated immune responses and 1-MT-mediated IDO inhibition elicited consistent abscopal effect. Consistent results were also obtained by measuring the tumor weight at the end of the treatment after various administrations (Figs. S48 and S49 online). The H&E staining further provided insights into the antitumor outcomes; the administration of SCMVs@Ad-ICG/1-MT + L resulted in the maximum growth of apoptotic and necrotic cancer cells at the tumor sites (Fig. S50 online).

The excellent antitumor outcomes of SCMVs@Ad-ICG/1-MT + L were ascribed to the synergy of PDT and immunotherapy, which were favorable for the intratumoral infiltration of cytotoxic T lymphocytes (CTLs). The CLSM images revealed that primary tumors presented more infiltration of CD4⁺ (Fig. 4h and Fig. S51 online) and CD8⁺ T cells (Fig. 4h and Fig. S52 online) in the group treated with SCMVs@Ad-ICG/1-MT + L. The proportions of CD4⁺ (Fig. 4d) and CD8⁺ T cells (Fig. 4e) were 22.6% and 16.4%, respectively, in the primary tumors of the mice formulated with SCMVs@Ad-IC G/1-MT + L, which was much higher than those in the other groups. Moreover, a significant enhancement was observed in the number of CD4⁺ and CD8⁺ T cells infiltrating into distant tumors in the



Fig. 2. (Color online) NIR-II imaging of tumor. (a) NIR-II fluorescence image of the 4T1 tumor-bearing mouse from lateral and prone positions at different times post *i.v.* injection of SCMVs@Ad-ICG. (b) Time-dependent tumor accumulation of SCMVs@Ad-ICG. (c) NIR-II fluorescence image of the lung tissue harvested from the mice bearing the 4T1 tumor. The metastatic tumor nodes were pointed out by red arrows. Scale bars: 200 µm. (d) H&E staining of the lung tissue; the metastatic tumor was circled by red curve. Scale bars: 25 µm. (e) H&E staining of the main organs of the mice administered with PBS and SCMVs@Ad-ICG. Scale bars: 50 µm.

SCMVs@Ad-ICG/1-MT + L group, which was responsible for the abscopal effect (Figs. S53 and S54 online).

The combination therapy induced $54.6\% \pm 4.8\%$ of the CRTpositive cells; the 6.31-, 5.92-, 6.11-, 5.15-, and 1.13-fold of the groups were treated with PBS, 1-MT, SCMVs@Ad-ICG, SCMVs@Ad-ICG/1-MT, and SCMVs@Ad-ICG + L, respectively (Fig. S55 online). SCMVs@Ad-ICG/1-MT + L markedly accelerated the DC maturation rate to $40.7\% \pm 5.6\%$, which was 1.6- and 1.2fold more efficient than SCMVs@Ad-ICG/1-MT and SCMVs@AdICG + L, implying that the inhibition of the IDO pathway was also responsible for the maturation of DCs and their functions (Fig. S56 online). The intratumoral ratio of Kyn to Trp was greatly reduced for the mice formulated with SCMVs@Ad-ICG/1-MT or SCMVs@Ad-ICG/1-MT + L (Fig. 4f), which was much lower than that of the free 1-MT because of the high-level tumor accumulation of the nanoformulations. The arrestment of the IDO activity facilitated to suppress the proliferation of regulatory T cells (Tregs) to reverse the immunosuppressive tumor microenvironment. The



Fig. 3. (Color online) *In vitro* photodynamic therapy and immunotherapy. (a) Schematic illustration of the PDT and immunotherapy mediated by SCMVs@Ad-ICG/1-MT. (b) Fluorescent spectra of SOSG solution upon the laser irradiation (808 nm, 50 MW/cm²) at different times in the presence of SCMVs@Ad-ICG/1-MT. (c) CLSM images of the 4T1 cells after the indicated treatments. The green fluorescence from DCF indicates the generation of ROS. Scale bar: 25 µm. (d) Cytotoxicity evaluation of SCMVs@Ad-ICG and SCMVs@Ad-ICG/1-MT with/without laser irradiation. (e) Inhibitory effect of 1-MT and SCMVs@Ad-ICG/1-MT on the IDO enzyme activity. (f) CLSM images of the 4T1 cells after different treatments. The CRT was stained with green fluorescence using Alexa Fluor Plus 488 CRT antibody. Scale bar: 25 µm. (g) Quantitative analysis of CRT-positive cells after different treatments. (h) Quantitative detection of HMGB1 in the nuclei of 4T1 cells after different treatments. (k) DC maturation cultured with the 4T1 cells pretreated with different formulations. I, PBS; II, 1-MT; III, SCMVs@Ad-ICG; IV, SCMVs@Ad-ICG/1-MT; V, SCMVs@Ad-ICG + L; VI, SCMVs@Ad-ICG/1-MT + L.

formulation of SCMVs@Ad-ICG/1-MT + L significantly decreased the frequency of intratumoral Tregs to 13.4% ± 1.9%, which was extremely important to elicit the immune responses (Fig. 4g). Correspondingly, the combination therapy resulted in the highest ratio of CD8⁺ T cells to Tregs cells (Fig. S57 online). Moreover, the administration of SCMVs@Ad-ICG/1-MT + L was the most efficient in inducing the intratumoral infiltration of IFN- γ -positive CTLs (Fig. S58 online).

The activation of antitumor immune responses *in vivo* was also verified by testing the excretion of the inflammatory cytokines using ELISA. Fig. 4i showed that localized PDT remarkably accelerated the secretion of interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α), and the IDO blockade further elicited the immune responses. Notably, the level of IFN- γ , TNF- α , interleukin-6 (IL-6), and IL-12 in the SCMVs@Ad-ICG/1-MT + L group was much higher than that in other groups at 24 h post treatments (Figs. S59–S63 online). Besides, an apparent reduction in the transforming growth factor- β (TGF- β) expression was observed in the SCMVs@Ad-ICG/1-MT + L group because of its ability to attenuate immunosuppression by combining the PDT and IDO inhibitor.

3.4. In vivo antitumor performance by modulating the tumor microenvironment

The therapeutic performance of cancer immunotherapy typically offers unsatisfactory clinical benefits because of nonimmunogenic and immunosuppressive tumor microenvironments. Tumorassociated macrophages (TAMs), a dominant proportion of the immune cells infiltrating tumor tissue, act as the main driver of the immunosuppressive tumor microenvironment, suppressing the T cell activation and promoting tumor metastasis [43–45]. R848, a Toll-like receptor 7 and 8 (TLR7/8) agonist with potent antitumor and immunostimulatory activity, has been demonstrated as a potent driver of macrophage reprogramming to polarize TAMs from tumor-supportive M2 phenotype to opposing tumoricidal M1 phenotype [46–48]. However, the systemic delivery of R848 is poorly tolerated due to its low solubility, nonspecific immune activation, and inadequate antitumor immunity [49–51].

The SCMVs were a suitable platform to encapsulate R848 through host–guest complexation using the anchored β -CD as the supramolecular container (Fig. 5a) [49]. The host–guest complexation between R848 and β -CD was studied using ¹H NMR and 2D



Fig. 4. (Color online) *In vivo* combination therapy. (a) Therapeutic schedule of SCMVs@Ad-ICG/1-MT-mediated combination therapy. Growth curves of (b) primary and (c) distant tumors for the mice treated with different formulations. ^{***}*P* < 0.001. The frequency of the intratumoral infiltration in (d) the CD3⁺CD4⁺ T cells and (e) CD3⁺CD8⁺ T cells after various treatments. (f) Ratio of Kyn to Trp and (g) proportions of the Tregs cells in the primary tumors after different treatments. (h) CLSM images of the intratumor infiltrating CD4⁺ T and CD8⁺ T cells after different treatments. Scale bars: 100 μm. (i) Fold changes in cytokines at 24 h post treatment in the mice after different administrations. I, PBS; II, 1-MT; III, SCMVs@Ad-ICG/1-MT; V, SCMVs@Ad-ICG/1-MT; V, SCMVs@Ad-ICG + L; VI, SCMVs@Ad-ICG/1-MT + L.

NOESY spectroscopy. The signals related to R848 shifted downfield upon the addition of β -CD, confirming the host–guest recognitions (Fig. S64 online). The strong nuclear Overhauser effect (NOE) correlations also suggested that the drug deeply penetrated into the cavity of B-CD (Fig. S65 online). Moreover, the size and stability of SCMVs@R848 were studied, which confirmed the successful preparation of the supramolecular nanomedicine (Figs. S66 and S67 online). The existence of Chol-CD promoted the loading efficiency and stability of SCMVs@R848; the loading content was 6.31% for SCMVs, which was around 2-fold of the cellular membrane vesicles without supramolecular engineering. The cell morphology was identified as a direct indicator to distinguish M0, M1, and M2 macrophages. Mature M0 BMDMs treated with macrophage colony-stimulating factor (M-CSF) adopted round morphology without antennae (Fig. 5b), while IL-4 incubation polarized BMDMs to M2 phenotype with elongated projections. Treatment with R848 or SCMVs@R848 successfully resulted in the reeducation of M2 BMDMs into M1 phenotype. The cell morphology changed from elongated to round and flattened, which was similar to the cells treated with lipopolysaccharide (LPS)—a bacterial component possessing powerful M1 activation ability.

The quantitative real-time polymerase chain reaction (qRT-PCR) was employed to investigate the macrophage repolarization after the treatment with SCMVs@R848. IL-4 exposure induced the polarization of BMDMs into M2 phenotype, as indicated by high mRNA levels of characteristic M2-like signature, including mannose receptor-1 (MRC1) and arginase-1 (Arg-1). These biomarkers were significantly downregulated by further incubation with R848 or SCMVs@R848 (Fig. 5e and f). In contrast, antitumor immunity genes, such as iNOS and IL-6, were upregulated for M2 macrophages treated with SCMVs@R848 (Figs. S68 and S69 online). The ELISA monitored the increased production of IL-12 in the supernatant of SCMVs@R848-treated macrophages (Fig. 5g). Moreover, the flow cytometric analysis indicated that the expression of

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Fig. 5. (Color online) *In vivo* antitumor performance by modulating the tumor microenvironment. (a) Schematic illustration of repolarization of TAMs by SCMVs@R848 and synergistic immunotherapy with α PD-L1. (b) CLSM images of BMDMs in different phenotypes after the indicated treatments. Scale bars: 20 μ m. (c) Flow cytometry analysis of CD86 expression on the surface of macrophages after the indicated treatments. (d) Representative CLSM images show the effect on phagocytosis of 4T1 by SCMVs@R848-treated M1-like macrophages, in which the 4T1 cells were labeled with DI0 and M1-like macrophages were labeled with DIL. Scale bars: 20 μ m. The normalized expression of (e) MRC1 and (f) Arg-1 in macrophages after the indicated treatments. (g) Secretion levels of IL-12 in the culture medium by macrophages after the indicated treatments. (h) Tumor growth curves of the mice administered with different formulations. (i) Representative immunofluorescence results to evaluate the presence of F4/80⁺, CD206⁺, and CD86⁺ macrophages after threatments. Scale bars: 200 μ m. I, PBS; II, α PD-L1; III, R848; IV, SCMVs@R848; V, R848 + α PD-L1; VI, SCMVs@R848 + α PD-L1. **P* < 0.05, ***P* < 0.001.

CD86 was prominently boosted by SCMVs@R848 administration (Fig. 5c), demonstrating that the macrophages were effectively activated. These studies demonstrated that the macrophages could be successfully repolarized from the M2 phenotype to the M1 phenotype using this supramolecular nanomedicine. The 4T1 cells and BMDMs were labeled with different fluorescence dyes to verify whether SCMVs@R848 promoted the phagocytic capacity of macrophages (Fig. 5d). The 4T1 cells were surrounded by BMDMs pretreated with SCMVs@R848, indicating that SCMVs@R848 reeducated M2 macrophages to polarize them to M1 phenotype with the ability to devour the cancer cells.

SCMVs@R848 possessed the ability to synergize checkpoint blockade considering the productive diversion of TAMs from the immunosuppressive phenotype to immune-supportive phenotype and the demonstrated involvement of adaptive *T*-cell immunity (Figs. S70–S75 online). Fig. 5h indicated that αPD-L1 alone exhibited limited therapeutic benefits in this tri-negative breast tumor model. The administration of R848 or SCMVs@R848 moderately

delayed the tumor growth mainly arising from the reprogramming of TAMs. Notably, the supramolecular encapsulation of R848 by SCMVs increased the cell response, which was indicative of improved drug bioavailability. The combination of SCMVs@R848 and α PD-L1 resulted in a superior tumor inhibition rate of 83.8%, which was higher than that for SCMVs@R848 (57.0%) and R848 + α PD-L1 (72.6%). The H&E staining also revealed the highest apoptosis level of tumor tissue in the mice after the combination therapy (Fig. S76 online). The side effect of R848 was greatly attenuated after the administration of supramolecular nanoformulation, as indicated by changes in the body weight (Fig. S77 online). The immunofluorescence staining further confirmed the repolarization of TAMs in vivo. The administration of SCMVs@R848 remarkably enhanced the number of M1 macrophages (CD86⁺ and F4/80⁺) and reduced the number of M2 macrophages (CD206⁺) in tumor sites (Fig. 5i). The adaptive immunity was also manifested by utilizing its capability in activating TLR-7/8 pathway, which facilitated the maturation and differentiation of DCs and thus the

expansion of antitumor T lymphocytes [50]. The administration of SCMVs@R848 significantly increased the percentage of CTLs that could directly kill the targeted cancer cells in tumor tissues (Fig. S78 online).

4. Conclusion

In conclusion, a general and cell-friendly supramolecular strategy was developed to engineer cell membrane vesicles by fully exploring the noncovalent interactions, avoiding potential damage to the cell membrane functions. A theranostic guest Ad-ICG was loaded in SCMVs through the host-guest recognition, allowing the NIR-II imaging and imaging-guided PDT. The inner cavity of SCMVs@Ad-ICG was further utilized to encapsulate an IDO inhibitor for the PDT-synergized immunotherapy. The precise therapy of PDT simultaneously triggered tumor cell apoptosis and evoked an ICD response, which promoted the maturation of DCs and antigen presentation. The 1-MT loaded in SCMVs@Ad-ICG/1-MT further inhibited the IDO activity and suppressed the infiltration of intratumoral Tregs to heat up the cold tumor microenvironment and light the flames of the antitumor war. SCMVs also served as delivery vehicles to load R848 using its host-guest complexation with β-CD. The nanoformulation modulated the tumor microenvironment by repolarizing TAMs from the tumor-supportive M2 phenotype to the tumor-suppressive M1 phenotype, significantly potentiating the therapeutic efficacy of immune checkpoint blockade therapy. The supramolecular engineering methodology might provide us with new opportunities for research in cell surface engineering based on noncovalent interactions, offering promising and enabling tools for biochemically remodeling cell membrane-based nanomaterials with immense potential in biotechnological and biomedical applications.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Author contributions

Shaolong Qi, Shoujun Zhu, Lang Rao, Feihe Huang, and Guocan Yu conceived and designed the research. Shaolong Qi, Qian-Fang Meng, Kai Yang, Bing Bai, and Lang Rao prepared and characterized the nanoformulations. Yajun Wang, Haiyan Zhang, Rui Tian, and Shoujun Zhu conducted the NIR-II imaging studies. Haiyan Zhang, Xueyan Zhang, and Xinyang Yu performed the *in vitro* and *in vivo* studies. Shaolong Qi, Lang Rao, Feihe Huang, and Guocan Yu wrote and revised the manuscript.

Appendix A. Supplementary materials

Supplementary materials to this article can be found online at https://doi.org/10.1016/j.scib.2022.08.030.

References

- Torchilin VP. Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery. Nat Rev Drug Discov 2014;13:813–27.
- [2] LaVan DA, McGuire T, Langer R. Small-scale systems for in vivo drug delivery. Nat Biotechnol 2003;21:1184–91.
- [3] Chen H, Zhang W, Zhu G, et al. Rethinking cancer nanotheranostics. Nat Rev Mater 2017;2:17024.
- [4] Elsabahy M, Heo GS, Lim SM, et al. Polymeric nanostructures for imaging and therapy. Chem Rev 2015;115:10967–1011.
- [5] Lang T, Dong X, Zheng Z. Tumor microenvironment-responsive docetaxelloaded micelle combats metastatic breast cancer. Sci Bull 2019;64:91–100.
- [6] Miao ZH, Hu DH, Gao DY, et al. Tiny 2D silicon quantum sheets: a brain photonic nanoagent for orthotopic glioma theranostics. Sci Bull 2021;66:147–57.
- [7] Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. Nat Rev Clin Oncol 2010;7:653–64.
- [8] Barua S, Mitragotri S. Challenges associated with penetration of nanoparticles across cell and tissue barriers: a review of current status and future prospects. Nano Today 2014;9:223–43.
- [9] Ouyang J, Xie A, Zhou J, et al. Minimally invasive nanomedicine: nanotechnology in photo-/ultrasound-/radiation-/magnetism-mediated therapy and imaging. Chem Soc Rev 2022;51:4996–5041.
- [10] Ji X, Ge L, Liu C, et al. Capturing functional two-dimensional nanosheets from sandwich-structure vermiculite for cancer theranostics. Nat Commun 2021;12:1124.
- [11] Elsabahy M, Wooley KL. Data mining as a guide for the construction of crosslinked nanoparticles with low immunotoxicity via control of polymer chemistry and supramolecular assembly. Acc Chem Res 2015;48:1620–30.
- [12] Dobrovoľskaia MA, Germolec DR, Weaver JL. Evaluation of nanoparticle immunotoxicity. Nat Nanotechnol 2009;4:411–4.
- [13] Yu G, Zhao X, Zhou J, et al. Supramolecular polymer-based nanomedicine: high therapeutic performance and negligible long-term immunotoxicity. J Am Chem Soc 2018;140:8005–19.
- [14] Zhang P, Sun F, Liu S, et al. Anti-PEG antibodies in the clinic: current issues and beyond PEGylation. J Control Release 2016;244:184–93.
- [15] Kozma GT, Shimizu T, Ishida T, et al. Anti-PEG antibodies: properties, formation, testing and role in adverse immune reactions to PEGylated nanobiopharmaceuticals. Adv Drug Deliv Rev 2020;154:163–75.
- [16] Liu Y, Luo J, Chen X, et al. Cell membrane coating technology: a promising strategy for biomedical applications. Nano Micro Lett 2019;11:100.
- [17] Thanuja MY, Anupama C, Ranganath SH. Bioengineered cellular and cell membrane-derived vehicles for actively targeted drug delivery: so near and yet so far. Adv Drug Deliv Rev 2018;132:57–80.
- [18] Zhang X, Angsantikul P, Ying M, et al. Remote loading of small-molecule therapeutics into cholesterol-enriched cell-membrane-derived vesicles. Angew Chem Int Ed 2017;56:14075–9.
- [19] Cheng Q, Xu M, Sun C, et al. Enhanced antibacterial function of a supramolecular artificial receptor-modified macrophage (SAR-Macrophage). Mater Horiz 2022;9:934–41.
- [20] Gao C, Cheng Q, Wei J, et al. Bioorthogonal supramolecular cell conjugation for targeted hitchhiking drug delivery. Mater Today 2020;40:9–17.
- [21] Fang RH, Kroll AV, Gao W, et al. Cell membrane coating nanotechnology. Adv Mater 2018;30:e1706759.
- [22] Hu CM, Zhang L, Aryal S, et al. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. Proc Natl Acad Sci USA 2011;108:10980–5.
- [23] Chen Z, Hu Q, Gu Z. Leveraging engineering of cells for drug delivery. Acc Chem Res 2018;51:668–77.
- [24] Parodi A, Quattrocchi N, van de Ven AL, et al. Synthetic nanoparticles functionalized with biomimetic leukocyte membranes possess cell-like functions. Nat Nanotechnol 2013;8:61–8.
- [25] Hu CM, Fang RH, Wang KC, et al. Nanoparticle biointerfacing by platelet membrane cloaking. Nature 2015;526:118–21.
- [26] Gao C, Cheng Q, Li J, et al. Supramolecular macrophage-liposome marriage for cell-hitchhiking delivery and immunotherapy of acute pneumonia and melanoma. Adv Funct Mater 2021;31:2102440.
- [27] Xue J, Zhao Z, Zhang L, et al. Neutrophil-mediated anticancer drug delivery for suppression of postoperative malignant glioma recurrence. Nat Nanotechnol 2017;12:692–700.
- [28] Ren E, Liu C, Lv P, et al. Genetically engineered cellular membrane vesicles as tailorable shells for therapeutics. Adv Sci 2021;8:e2100460.
- [29] Wang C, Sun W, Ye Y, et al. In situ activation of platelets with checkpoint inhibitors for post-surgical cancer immunotherapy. Nat Biomed Eng 2017;1:0011.
- [30] Chen Z, Zhao P, Luo Z, et al. Cancer cell membrane-biomimetic nanoparticles for homologous-targeting dual-modal imaging and photothermal therapy. ACS Nano 2016;10:10049–57.
- [31] Lei Z, Wang J, Lv P, et al. Biomimetic synthesis of nanovesicles for targeted drug delivery. Sci Bull 2018;63:663–5.
- [32] Xu C, Pu K. Second near-infrared photothermal materials for combinational nanotheranostics. Chem Soc Rev 2021;50:1111–37.
- [33] Zhu S, Hu Z, Tian R, et al. Repurposing cyanine NIR-I Dyes accelerates clinical translation of near-infrared-II (NIR-II) bioimaging. Adv Mater 2018;30:1802546.

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- [34] Wang D, Wang T, Yu H, et al. Engineering nanoparticles to locally activate T cells in the tumor microenvironment. Sci Immunol 2019;4:eaau6584.
- [35] Gowsalya K, Yasothamani V, Vivek R. Emerging indocyanine green-integrated nanocarriers for multimodal cancer therapy: a review. Nanoscale Adv 2021:3:3332-52.
- [36] Zhao H, Xu J, Feng C, et al. Tailoring aggregation extent of photosensitizers to boost phototherapy potency for eliciting systemic antitumor immunity. Adv Mater 2022:34:2106390.
- [37] Irvine DJ, Dane EL. Enhancing cancer immunotherapy with nanomedicine. Nat Rev Immunol 2020;20:321-34.
- [38] Zhang C, Zeng Z, Cui D, et al. Semiconducting polymer nano-PROTACs for activatable photo-immunometabolic cancer therapy. Nat Commun 2021:12:2934
- [39] Prendergast GC, Malachowski WP, DuHadaway JB, et al. Discovery of IDO1 inhibitors: from bench to bedside. Cancer Res 2017;77:6795-811.
- [40] Munn DH, Mellor AL. IDO in the tumor microenvironment: inflammation, counter-regulation, and tolerance. Trends Immunol 2016;37:193–207.
- [41] Li X, Wenes M, Romero P, et al. Navigating metabolic pathways to enhance antitumour immunity and immunotherapy. Nat Rev Clin Oncol 2019;16:425-41.
- [42] Huck BR, Kotzner L, Urbahns K. Small molecules drive big improvements in immuno-oncology therapies. Angew Chem Int Ed 2018;57:4412-28.
- [43] Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med 2013;19:1423-37.
- [44] Xia Y, Rao L, Yao H, et al. Engineering macrophages for cancer immunotherapy and drug delivery. Adv Mater 2020;32:2002054.
- [45] Yi Y, Yu M, Feng C, et al. Transforming "cold" tumors into "hot" ones via tumor-microenvironment-responsive siRNA micelleplexes for enhanced immunotherapy. Matter 2022;5:2285-305.
- [46] Yang G, Ni JS, Li Y, et al. Acceptor engineering for optimized ROS generation facilitates reprogramming macrophages to M1 phenotype in photodynamic immunotherapy. Angew Chem Int Ed 2021;60:5386-93.
- [47] Shi C, Liu T, Guo Z, et al. Reprogramming tumor-associated macrophages by nanoparticle-based reactive oxygen species photogeneration. Nano Lett 2018;18:7330-42.
- [48] Tang Y, Fan W, Chen G, et al. Recombinant cancer nanovaccine for targeting tumor-associated macrophage and remodeling tumor microenvironment. Nano Today 2021;40:101244.
- [49] Rodell CB, Arlauckas SP, Cuccarese MF, et al. TLR7/8-agonist-loaded nanoparticles promote the polarization of tumour-associated macrophages to enhance cancer immunotherapy. Nat Biomed Eng 2018;2:578-88.
- [50] Li T, Chen G, Xiao Z, et al. Surgical tumor-derived photothermal nanovaccine
- for personalized cancer therapy and prevention. Nano Lett 2022;22:3095–103. [51] Ovais M, Guo M, Chen C. Tailoring nanomaterials for targeting tumor-associated macrophages. Adv Mater 2019;31:e1808303.



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