Supporting Information

# Microfluidic Sonication to Assemble Exosome Membrane-Coated Nanoparticles for Immune Evasion-Mediated Targeting

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**Materials**

Cholesterol, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000] (DSPE-PEG-2000), polycarbonate membranes (pore sizes: 400 nm and 100 nm), and mini-extruder were purchased from Avanti Polar Lipids (USA). Hoechst 33342 (ex/em: 346/460 nm), DiO (ex/em:484/501), DiI (ex/em: 549/565 nm), DiD (ex/em: 644/663 nm), DiR (ex/em: 748/780 nm), and Fixable Aqua Dead Cell Stain Kit (ex/em: 367/526 nm) were purchased from ThermoFisher (USA). Coomassie blue, dimethylformamide (DMF), and trifluoroethanol (TFE) were purchased from Sigma (USA). Protease Inhibitor Cocktail (mini-Tablet, EDTA-free) was purchased from MCE (USA). Poly(lactide-*co*-glycolide) (PLGA, lactide:glycolide = 75:25) was purchased from SurModics (USA). BCA Kit, Cell Counting Kit-8 (CCK-8), RAPI Lysis Buffer, phenylmethanesulfonyl fluoride (PMSF), Mouse TNF-α ELISA Kit and Mouse IL-6 ELISA Kit were purchased from Beyotime (China). Dulbecco's modified Eagle's medium (DMEM), Leibovitz's L-15 medium, RPMI-1640 medium, fetal bovine serum (FBS), penicillin-streptomycin, and phosphate buffered saline (PBS) were purchased from Gibco (USA). Mouse BD Fc Block and anti-CD11b antibody were purchased from BD Bioscience (USA).

**Design and fabrication of the microfluidic chip**

The microfluidic chip consisted of three inlets with a width of 100 μm, a two-stage microchannel with a width of 300 μm, and an outlet for collecting the

resultant biomimetic nanoparticles. The first stage was a straight section with a length of 10 mm. The straight section ended with two branches that served as the two side inlets of the second stage, and the second stage also had a middle inlet with a width of 300 μm. The second stage was a 3-loop double spiral section with a width of 300 μm. The entire microchannel had a uniform height of 50 μm. The microfluidic chip mold was fabricated with SU-8 2050 photoresist (MicroChem Corp, USA) by standard soft lithography. The SU-8 2050 photoresist was spin-coated on the 4-inch silicon wafer at 500 rpm for 10 s and 3000 rpm for 30 s under a vacuumed atmosphere to obtain the desired thickness of 50 μm. The coated silicon wafer was baked at 95 °C for 60 min, then exposed to ultraviolet light through a photomask containing microfluidic channels pattern and baked at 95 °C for 25 min. The silicon wafer was soaked in SU-8 developer (MicroChem Corp, USA) to remove the unexposed photoresist and baked at 150 °C for 15 min. Degassed PDMS (PDMS base/curing agent = 10:1) was poured into the mold and peeled off after baking at 100 °C for 15 min. Then the PDMS was punched with a needle (ID = 0.75 mm, OD = 1 mm) and bounded with glass substrate (25 mm × 75 mm) through oxygen plasma treatment (220 W, 70 S). Five plastic tubes (ID = 0.5 mm, OD = 1 mm) were inserted into the channels and glued with sealant (3145 RTV, Dow Corning, USA).

**Numerical simulation.**

To evaluate fast mixing process within the microchannel, the flow field and species transportation for the entire microchannel at the experimental flow rate were

numerically simulated by solving the Navier-Stokes equations and species governing equation based on Fick's law[1]

$$\nabla \cdot \mathbf{u}_0 = 0$$
$$\frac{\partial \mathbf{u}_0}{\partial t} + (\mathbf{u}_0 \cdot \nabla)\mathbf{u}_0 = -\frac{1}{\rho_0}\nabla p_0 + \nu \nabla^2 \mathbf{u}_0 \quad (1)$$
$$\frac{\partial c_i}{\partial t} + \nabla \cdot (\mathbf{u}_0 c_i) = \nabla \cdot (D_i \nabla c_i)$$

where $\mathbf{u}_0$ was the flow velocity vector, $\rho_0$ was the fluid density, $p_0$ was the pressure, $\nu$ was the kinetic viscosity, $c_i$ was the species concentration, and $D_i$ was the mass diffusion coefficient of the species and set as $10^{-10}$ $m^2$ $s^{-1}$. Hexahedral grids inside the two-stage chip were generated by Gambit (Fluent 6.4, Ansys Inc.). All walls of the microfluidic channels were set as non-slip boundary conditions. Mass flow boundary condition was applied at the four inlets and outflow condition was applied at the outlet. The solving of the equations was performed using commercial CFD software Fluent 6.4 (Ansys Inc., USA).

To evaluate the pressure induce by acoustic pulses, the acoustic pressure for a section of microchannel was obtained by solving the governing equations of the propagation of acoustic waves

$$\frac{\partial \rho}{\partial t} + (\mathbf{u}_0 \cdot \nabla)\rho + (\mathbf{u}_0 \cdot \nabla)\rho_0 + \rho(\nabla \cdot \mathbf{u}_0) + \rho_0(\nabla \cdot \mathbf{u}) = 0$$
$$\frac{\partial \mathbf{u}}{\partial t} + (\mathbf{u}_0 \cdot \nabla)\mathbf{u} + (\mathbf{u} \cdot \nabla)\mathbf{u}_0 + \frac{1}{\rho_0}\nabla p - \frac{\rho}{\rho_0^2}\nabla p_0 = 0 \quad (2)$$
$$p = c_0^2 \rho$$

where $\mathbf{u}$, $\rho$, and $p$ were the oscillatory velocity, density, and pressure induced by the acoustic waves, and $c_0$ was the acoustic speed. The acoustic wave was exerted from the bottom wall of the microchannel. The frequency was set to be 80 kHz and

area density of the sonication power was $2.2\times10^3$ W/m$^2$. The solving of the equations was performed using commercial FEM package COMSOL 5.4 (COMSOL Inc., Sweden).

**Cell lines**

Human lung carcinoma cell line, A549, was cultured in Dulbecco's modified eagle medium (DMEM). Human breast cancer cell line, MDA-MB-231, was cultured in Leibovitz's L-15 medium. Murine macrophage-like cell, RAW 264.7, was cultured in RPMI-1640 medium. All the media were supplied with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. When culturing A549 cells for harvesting exosomes, exosome-depleted FBS (a 0.22 μm membrane filtration, overnight ultracentrifugation at 100000× g, and a second 0.22 μm membrane filtration) was used. All the cells were provided by American Type Culture Collection (ATCC) and cultured at 37 °C with 5% $CO_2$.

**Isolation of exosomes**

The exosomes used in the present study were isolated from cell culture medium using differential centrifugation at 4 °C. The collected supernatant (240 mL) was first centrifuged at 300× g for 10 min to remove cells and large cell debris. The supernatant was then centrifuged at 2000× g for 10 min to remove small cell debris. To remove larger extracellular vesicles, the supernatant was then centrifuged at 10000× g for 60 min. The supernatant was then processed by membrane filtration

(0.22 μm, Millipore, USA), followed by ultracentrifugation at 100000× g for 3 h. The exosomes were collected by resuspending the pellet in 5 mL of 1× PBS. Before ultracentrifugation, the cell culture medium was packaged into 8 ultracentrifuge tubes, and the weight difference between every pair of tubes was less than 50 mg.

**Preparation of cancer cell membrane (CCM)**

To derive CCM, A549 cells were first washed in 1× PBS for twice and then suspended in cold hypotonic buffer containing 10 mM Tris, 10 mM $MgCl_2$, and EDTA-free protease inhibitor for 1 h. The cells were then mechanically disrupted by grind for three times (2 min each), followed by a centrifugation at 10000× g for 10 min and a centrifugation at 100000× g for 1 h to obtain the membrane from the homogenate. The resulted membrane was extruded through 400 nm and 100 nm polycarbonate porous membranes for 8 passes immediately before the fabrication of CCM-PLGA NPs.

**Optimization of experimental parameters**

To investigate the effect of flow rates, we performed computational fluid dynamics (CFD) simulation to characterize the flow field and species transportation within the microfluidic chip at a high flow rate (80 mL/h for each side inlet, and 7 mL/h for middle inlet) at the first stage (Figure S2A). At the high flow rate, four vortices perpendicular to the flow direction at the intersection of the inlets were generated to achieve a chaotic mixing, allowing for efficient and rapid interfacial

deposition of small-sized and mono-disperse PLGA NPs. We thus used a total flow rate of 174 mL/h (80 mL/h for each side inlet, and 7 mL/h for each middle inlet) for fabrication of biomimetic NPs. Moreover, the application of sonication (80 kHz frequency and 100 W power) cannot affect the velocity field of the microfluidic channel (Figure S2B).

To optimize the concentrations of EM (or CCM), EM-PLGA NPs (or CCM-PLGA NPs) were fabricated at different EM concentrations of 0.3, 0.5, and 0.65 mg/mL under the optimal conditions of flow rates and sonication. EM-PLGA NPs (or CCM-PLGA NPs) fabricated at 0.5 mg/mL of EM (or 0.15 mg/mL of CCM) showed an average diameter of 177.4 nm (or 172 nm) with the lowest PDI of 0.193 (or 0.222) (Figure S2C-D).

**Synthesis of NPs.**

To prepare EM-PLGA NPs, a 5 mg/mL solution of PLGA in DMF and TFE (7 mL/h), two 0.5 mg/mL solutions of exosomes in PBS (80 mL/h each), and a PBS solution (7 mL/h) were injected into the microfluidic device from the middle and two side inlets at the first stage, and the inlet at the second stage. To prepare CCM-PLGA NPs, a 5 mg/mL solution of PLGA in DMF and TFE (7 mL/h), two 0.15 mg/mL solutions of CCM in PBS (80 mL/h each), and a PBS solution (7 mL/h) were injected into the microfluidic device from the middle and two side inlets at the first stage, and the inlet at the second stage. To prepare Lipid-PLGA NPs, a 10 mg/mL solution of PLGA in DMF and TFE (5 mL/h), two aliquot of DI water (40 mL/h each), and a

lipid solution (45.5 mg DPPC, 4.8 mg cholesterol, and 8.5 mg DSPE-PEG in 40 mL ethanol, 5 mL/h) were injected into the microfluidic device from the middle and two side inlets at the first stage, and the inlet at the second stage. During the preparation of EM-PLGA NPs and CCM-PLGA NPs, the microfluidic chip was subjected to sonication using a bath sonicator (KQ-200TDE, Kun Shan Ultrasonic Instrument Ltd, China) at a frequency of 80 kHz and a power of 100 W. The flow rates of all inlets were precisely adjusted using syringe pumps (PHD Ultra, Harvard Apparatus, USA). Prior to usage, the amount of fluorescent dye loaded by different types of biomimetic NPs were unified. After dialysis, 30 μL of fabricated NPs were added to 270 μL of acetonitrile. After sonication for 15 min to release the loaded dye, the fluorescence intensities of different NPs were measured by a plate reader, based on which the concentrations of different NPs were adjusted to ensure the equal loading of dye.

**Purification of NPs**

A dialysis step was employed to purify biomembrane-coated NPs by removing organic solutions and free dyes post synthesis. 1 mL of prepared NP solution was loaded into a dialysis bug with a MWCO of 7 kDa (SEP, USA). The NPs were dialyzed in 100 mL of PBS buffer at 4 °C. The PBS solution was changed at 2 h and 6 h during the dialysis process. The dialysis period was 12 h.

**Encapsulation efficiency**

The encapsulation efficiency of dyes into NPs was determined as a measure of

the purity of NPs. NP solutions after dialysis were collected, suspended in acetonitrile, sonicated (100 W, 15 min) to release dye, and subjected to dye quantification using a microplate reader (Synergy H1, BioTek, USA). The amount of dye encapsulated in the NPs ($Q_{np}$) was determined using a standard curve established by a series of acetonitrile solutions of dyes at different concentrations. The encapsulation efficiency of the biomimetic NPs was determined as $Q_{np}/Q_{total}$, in which $Q_{total}$ was the total amount of dyes that infused into the microfluidic chip during the synthesis of NPs. Using this method, the encapsulation efficiencies of DiO in EM-PLGA NPs, CCM-PLGA NPs, and lipid-PLGA NPs were 94 %, 93 %, and 88 %, respectively.

**Dynamic light scattering (DLS)**

The size, polydispersity index (PDI), and surface zeta potential of the replicate NP samples ($n$ = 3 for each type of NP) were obtained by DLS measurements using a Malvern 3000HS Zetasizer (Malvern Instruments Ltd, England). The stability of NPs (20 μg/mL of PLGA) was examined in 1× PBS for 5 days in succession.

**Transmission electron microscopy (TEM)**

NP samples (5 μL each) were directly absorbed on Formvar/carbon-coated cooper grids for 5 min, followed by drying with filter paper. The dried NPs were then negatively stained with 4 drops of 1% uranyl acetate. After air drying, the morphology of the NPs was characterized using an HT7700 TEM (Hitachi, Japan) at 80 kV. To evaluate the efficiency of encapsulation of PLGA cores into membranes, at

least 10 images, corresponding to at least 120 core-shell NPs, were taken for each type of NP.

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)**

SDS-PAGE was performed to analyze the protein content of cells, exosomes, and NPs. To obtain A549 cell protein and CCM protein, the A549 cells and CCM were lysed for 30 min by RAPI Lysis Buffer (Beyotime, China), and centrifuged at 16000× g for 10 min to remove cell debris. To obtain exosome protein, the A549 cell-derived exosomes obtained by ultracentrifugation were resuspended in 1× PBS and ultracentrifuged again to remove the non-exosome protein. The pellet was lysed for 30 min by RAPI Lysis Buffer and centrifuged at 16000× g for 10 min to remove debris. To obtain protein in EM-PLGA NPs and CCM-PLGA NPs, the NPs were centrifuged at 20000× g for 1 h; the pellet was lysed for 30 min by RAPI Lysis Buffer, and centrifuged at 16000× g for 30 min to remove debris and PLGA. All RAPI Lysis Buffer was spiked with 1 mM PMSF to inhibit protease and the protein concentration was measured by BCA assay. The protein solution was added into SDS-PAGE loading buffer and boiled for 10 min, then 30 μg protein were added into each well at gel (4 – 20 %) and run at 120 V. Before observation, the gel was stained by Coomassie blue solution for 1 h and washed overnight.

**Dot blotting**

The expression levels of CD9, CD47, CD63, and GAPDH on EM, EM-PLGA NPs, CCM, and CCM-PLGA NPs were detected by dot blotting. The concentrations

of EM-PLGA NPs, CCM-PLGA NPs, EM vesicles, and CCM vesicles were adjusted to exhibit equal total protein content. For each dot, 2 μL of sample was spotted on a polyvinylidene difluoride (PVDF) membrane. After spots dried, the membrane was blocked and incubated with anti-CD63 (5 μg/mL, Abcam, UK), anti-CD9 (5 μg/mL, Abcam, UK), anti-CD47 (5 μg/mL, Abcam, UK), and anti-GAPDH (5 μg/mL, Abcam, UK) primary antibodies and horseradish peroxidase (HRP)-conjugated secondary antibody (5 μg/mL, Santa Cruz, USA). Dots were detected with chemiluminescence method (ECL substrate, Pierce, USA).

**Aptamer labeling**

The orientation of membrane proteins after synthesis was assessed using Cy5-conjugated aptamers specific for the extracellular domain of CD63 and EpCAM. EM-PLGA NPs, EM vesicles, CCM-PLGA NPs, and CCM vesicles with equal total protein content (determined by BCA assay) were incubated with 0.1 μM of Cy5-conjugated aptamers targeting CD63 and EpCAM at room temperature for 2 h. Lipid-PLGA NPs and random sequence were used as negative control. The incubated samples were injected into a 10-μm microchamber, followed by fluorescence observation under a 100× objective on a fluorescent microscope (DMi8, Leica, Germany) equipped with a sCMOS camera (95B, Photometrics, Canada). Three replicates were performed for each type of aptamer/random sequence and each replicate consisted of 20 images (> 100 particles). ImageJ was used to recognize particles and calculate the total fluorescent intensity for all images.

**Mechanism of cellular uptake**

To reveal the enhanced cellular uptake of NPs with the support of rigid PLGA

cores, EM-PLGA NPs (40 μg/mL of PLGA) fabricated from A549 exosomes and A549 exosomes were labeled with DiO at membranes and incubated with A549 cells ($2\times10^5$ cells) at 37 °C for 4 h. Prior to the incubation, the cells were cultured in the wells for overnight to stabilized the cells. After the incubation, cell nuclei were stained with Hoechst 33342 and washed 2 times with 1× PBS. Cell imaging was performed on a LSM710 confocal laser scanning microscope (Zeiss, Germany) with a 63× objective.

### *In vitro* homotypic targeting study

A549 and MDA-MB-231 cells were used to examine the specific targeting of the biomimetic NPs. The two types of cells were seeded in confocal dishes (for fluorescent imaging) or 12 well plate (for flow cytometric analysis) at a destiny of $2\times10^5$ cells per dish/well. After overnight culture at 37 °C, the culture medium was discarded and cells were incubated in medium containing EM-PLGA NPs, CCM-PLGA NPs, and Lipid-PLGA NPs with DiO-labeled membranes (40 μg/mL of PLGA) for 4 h. The incubated cells were washed 3 times with 1× PBS and then stained with Hoechst 33342. Cell imaging was performed on a LSM710 confocal laser scanning microscope (Zeiss, Germany) with a 63× objective. For flow cytometric analysis, cells incubated with the 3 types of NPs were digested without staining of nucleus.

### Endocytic pathway

To determine the endocytosis pathways, A549 cells were treated with different endocytosis inhibitors for 1 h. Specifically, 20 μM of amiloride (AMI, inhibitor of

Na+/H+ pump related macropinocytosis), 5 μg/mL of dynasore (DYN, inhibitor of dynamin), and 30 μM of chlorpromazine (CHL, inhibitor of clathrin-mediated endocytosis) were used. A549 cells were then incubated with DiO-labeled biomimetic NPs for 4 h. After incubation, A549 cells were washed, detached, and analyzed by the flow cytometry (BD Accuri C6, USA) for quantification of cellular uptake.

### *In vitro* evaluation of cytotoxicity

Cell Counting Kit-8 (CCK-8) was used to test *in vitro* cytotoxicity of the core-shell NPs. A549 cells were seeded in a 96 well plate at a density of 5000 cells per well. After overnight culture at 37 °C, the cells were incubated with different concentrations (40 ng/mL – 40 μg/mL of PLGA) of EM-PLGA NPs, CCM-PLGA NPs, and Lipid-PLGA NPs. After incubation for 48 h, the medium was removed and the cells were washed once with 1× PBS. Fresh culture medium containing 10 % of CCK-8 solution was added to each well. After incubation for 1 h, the absorbance at 450 nm were measured by a microplate reader (Syneryy H1, BioTek, USA).

### *In vivo* homotypic targeting study

To establish a mouse model bearing two different kinds of tumors (A549 and MDA-MB-231), NOD SCID mice (female, 4-5 weeks old, Charles River, USA) were subcutaneously inoculated at right flank with $10^7$ A549 cells and at left flank with $2\times10^5$ MDA-MB-231 cells. The tumor size was monitored by vernier caliper and calculated as $V = \pi LW^2/6$, where the L and W are the length and width of tumor,

respectively. When tumor size reached 100 ± 10 $mm^3$, the mice randomly separated into four groups (1 mouse for each type of biomimetic NP and PBS). Equal volume (200 μL) of EM-PLGA NPs, CCM-PLGA NPs, lipid-PLGA NPs (loaded with 2 % of DiR), and 1× PBS were injected into the mice of respective groups *via* tail vein. The fluorescence distribution was monitored at 4 h, 8 h, 12 h, 24 h, and 48 h post administration by *in vivo* IVIS Spectrum (PerkinElmer, USA). The mice were euthanized at 48 h, the tumors and major organs were imaged. All data were analyzed by IVIS system (living imaging 4.5.5).

**Biodistribution calculation**

The mean fluorescence intensities of region of interest were measured using the *ex vivo* images. Standard curve was constructed using serum solution of DiR with different known concentrations, whose images were taken under the same imaging set ups. The concentration ($c$) of dye in the organs and tumors were determined by the standard curve. The accurate mass ($M$) and volume ($V$) of fresh organs and tumors of the administrated mice were measured by microbalance and graduated cylinder. The percent injected dose per gram was calculated as $\%ID/g = cV/MQ \times 100\%$, where $Q$ was the injected dose of DiR.

**Measurement of cytokine levels**

Equal volume (200 μL) of EM-PLGA NPs, CCM-PLGA NPs, Lipid-PLGA NPs, and 1×PBS were injected into mice (Kun Ming, female, 4-5 weeks old) via tail vein.

The blood was collected 24 h after the injection and added with 1 mg/mL of EDTA. The concentrations of TNF-α and IL-6 in plasma were detected by mouse TNF-α and IL-6 ELISA Kits (Beyotime, China).

**Evaluation of safety of biomimetic NPs**

Equal volume (200 μL) of EM-PLGA NPs, CCM-PLGA NPs, Lipid-PLGA NPs, and 1×PBS were injected into NOD SCID mice (female, 4 weeks old) and the mice were sacrificed 3 days after injection. Liver-function markers: ALT, ALP, and kidney-function markers: BUN, CREA in serum were quantified by biomedical analyzer (HCP-7100X, Hitachi, Japan). The major organs from the sacrificed mice were fixed in 4% paraformaldehyde solution, embedded in paraffin, and sectioned into slices with a thickness of 4 μm. The slices were stained with hematoxylin and eosin, and imaged by an optical microscope (BX51, Olympus, Japan).

***In vitro* evaluation of phagocytosis**

Phagocytosis was evaluated by characterizing the cellular uptake of NPs by murine macrophage cell line RAW264.7. RAW264.7 cells were seeded in confocal dishes (for fluorescent imaging) or 12 well plate (for flow cytometric analysis) at a destiny of $2\times10^5$ cells per dish/well. After overnight culture at 37 °C, the culture medium was discarded and cells were incubated in medium containing EM-PLGA, CCM-PLGA, and Lipid-PLGA NPs with DiO-labeled membranes (40 μg/mL of PLGA) for 4 h. The incubated cells were washed 3 times with 1× PBS and then

stained with Hoechst 33342. Cell imaging was performed on a LSM710 confocal laser scanning microscope (Zeiss, Germany) with a 63× objective. For quantitative analysis of the uptake by flow cytometry, cells incubated with the 3 types of NPs were digested without staining of nucleus.

***In vivo* evaluation of phagocytosis**

Equal volume (200 μL) of EM-PLGA NPs, CCM-PLGA NPs, and Lipid-PLGA NPs loaded with DiD (2 %) in the PLGA cores were injected into Balb/c mice (female, 4-5 weeks old) by tail vein and the blood was collected 3 h after the injection. Erythrocytes were lysed by ACK lysis buffer (Invitrogen, USA) and the remaining cells collected by a centrifugation at 800× g for 5 min and blocked by mouse BD Fc Block (1 μg per $1\times10^6$ cells) for 5 min. The cells were stained with anti-CD11b (1 μg/million cells, Percp-Cy5.5) and Fixable Aqua Dead Cell Stain Kit (1 μL per $1\times10^6$ cells) for 30 min. The stained cells were washed by centrifugation to remove extra antibody and processed by membrane filtration (pore size: 40 μm) and analyzed by a CytoFlex LX flow cytometry (Beckman Coulter, USA).

**Flow cytometric analysis**

To evaluate the uptake of biomimetic NPs by monocytes, total cells were first identified on the SSC-A/FSC-A gating. Live monocytes were then identified as cells that are positive for CD11b (Cy5.5, threshold of $10^4$) and negative for Live/Dead Aqua dye (DAPI, threshold of $10^5$). The internalization of DiD-labeled NPs by

monocytes was subsequently identified as the cells positive for DiD (threshold of $10^5$). All the thresholds were determined from the control samples without fluorescence labeling.

**References**

(1). Sun, J.; Li, Y.; Yan, F.; Liu, C.; Sang, Y.; Tian, F.; Feng, Q.; Duan, P.; Zhang, L.; Shi, X.; Ding, B.; Liu, M., Control over the Emerging Chirality in Supramolecular Gels and Solutions by Chiral Microvortices in Milliseconds. *Nat. Commun.* **2018,** *9*, 2599.

**Table S1. Summary of size and PDI of synthesized core-shell NPs**

| Nanoparticles | Payload | Size (nm) | PDI | Percentage of NPs showing a typical core-shell structure |
|---|---|---|---|---|
| EM-PLGA NPs | - | 177.4 | 0.193 | 90.5 % (*n* = 389 particles under TEM) |
| EM-PLGA NPs without sonication | - | 237.6 | 0.474 | 47.3 % (*n* = 224 particles under TEM) |
| EM-PLGA NPs | DiR | 179.2 | 0.221 | |
| EM-PLGA NPs | DiO | 177.2 | 0.200 | |
| EM-PLGA NPs | DiI/DiO | | | |
| CCM-PLGA NPs | - | 172.3 | 0.222 | 92.7 % (*n* = 192 particles under TEM) |
| CCM-PLGA NPs | DiR | 170.3 | 0.202 | |
| CCM-PLGA NPs | DiO | 181.1 | 0.191 | |
| CCM-PLGA NPs | DiI/DiO | | | |
| Lipid-PLGA NPs | - | 157.2 | 0.195 | 87.5 % (*n* = 125 particles under TEM) |
| Lipid-PLGA NPs | DiR | 185.6 | 0.214 | |
| Lipid-PLGA NPs | DiO | 178.9 | 0.231 | |
| Lipid-PLGA NPs | DiI/DiO | | | |

**Table S2. Summary of methods for assembling biomimetic core-shell NPs**

| Method | Synthesis mode | Synthesis time | Material for core-shell structures | Encapsulation efficiency | Size (nm) and PDI |
|---|---|---|---|---|---|
| Bulk method (Liu, 2018) | Batch mode | Overnight stirring for PLGA NPs, 1 h for PLGA NP collection, overnight for forming CCM-PLGA NPs | CCM; PLGA cores | N.A. | 123 – 160; 0.12 – 0.14 |
| Bulk method (Zhang, 2017) | Batch mode | 2.5 h for forming PLGA NPs, 2 min for forming CCM-PLGA NPs | CCM; PLGA cores | N.A. | ~ 130 nm; N.A. |
| Bulk method (Zhang, 2017) | Batch mode | 4 h for PLGA NPs, 2 min for membrane coating | RBC-platelet hybrid membrane; | N.A. | ~ 100 nm; N.A. |
| Microfluidic electroporation (Liu, 2017) | Continuous | ~ 1 s in the microfluidic device with patterned electrodes | RBC membrane; $Fe_3O_4$ magnetic cores | N.A. | ~ 100 nm; N.A. |
| Microfluidic sonication (Present study) | Continuous | 30 ms in the two-stage microfluidic device | EM, CCM, lipid; PLGA cores | Up to 91 % | ~ 200 nm; ~ 0.2 |

*EM, exosome membrane; CCM, cancer cell membrane.

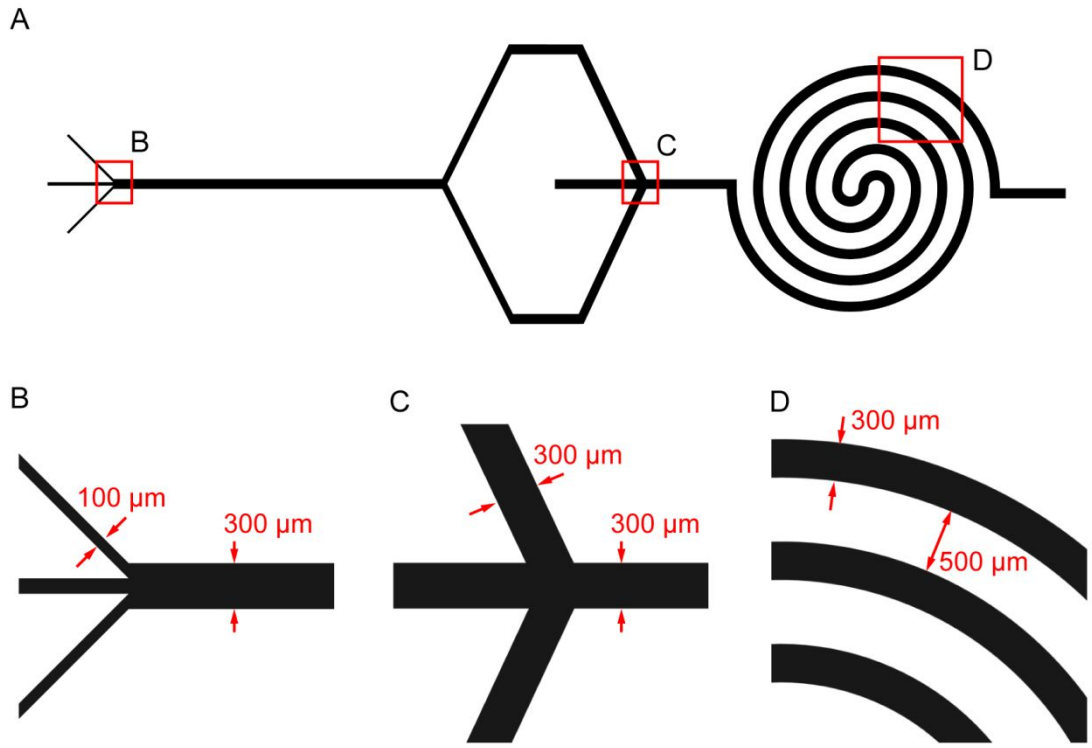


Figure S1. Microchannel design. The first stage of microfluidic device has three inlets and one straight microchannel, and the second stage has one inlet, one double-spiral microchannel, and one outlet. The entire microchannel has a uniform height of 50 μm.

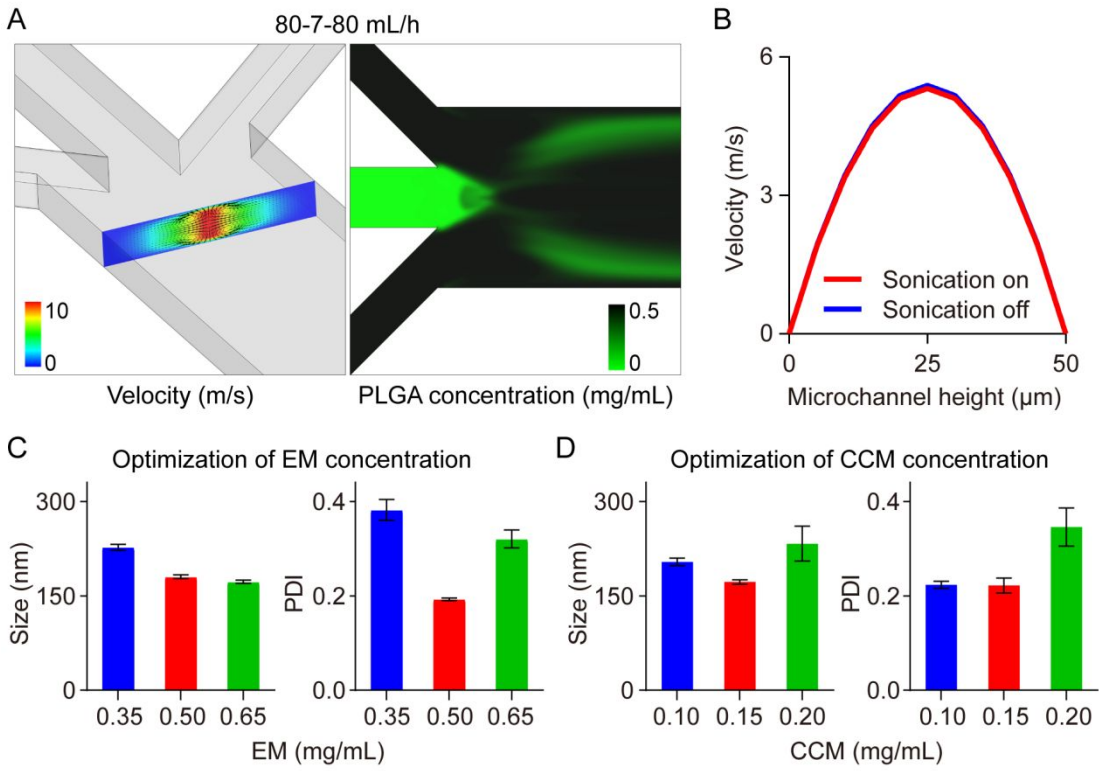


Figure S2. Optimization of the flow condition and biomembrane concentration. (A) Computational fluid dynamics (CFD) simulation of flow field and species transportation showing the effective hydrodynamic mixing in the microchannel under a total flow rate of 167 mL/h at the first stage. (B) Fully developed velocity profiles along the microchannel height for cases of sonication on and sonication off. (C) Average size and polydispersity index (PDI) of EM-PLGA NPs fabricated at different EM concentrations of 0.35, 0.5, and 0.65 mg/mL ($n$ = 3, mean ± s.d.). (D) Average size and PDI of CCM-PLGA NPs fabricated at different EM concentrations of 0.35, 0.5, and 0.65 mg/mL ($n$ = 3, mean ± s.d.). In (C,D), a 5 mg/mL solution of PLGA in DMF and TFE (7 mL/h), two solutions of EM/CCM in PBS (80 mL/h each), and a PBS solution (7 mL/h) were injected into the microfluidic device from the middle and two side inlets at the first stage, and the inlet at the second stage.

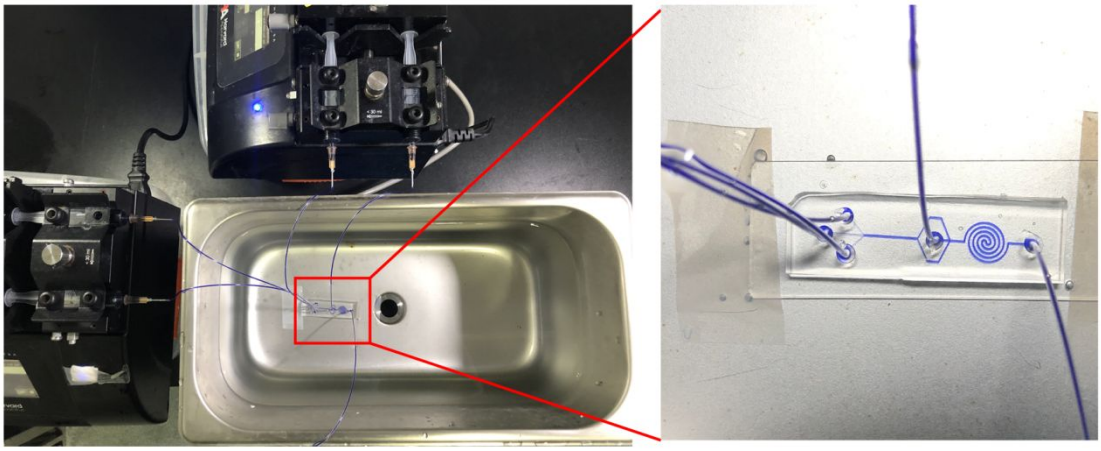

Figure S3. Experimental setup of the microfluidic sonication synthesis. The two-stage microfluidic device was immersed into an ultrasonic bath. The ultrasonic sources were under the bath bottom.

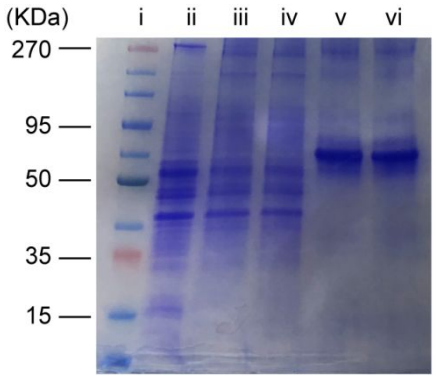


Figure S4. Protein contents of (i) marker, (ii) A549 cell, (iii) membrane derived from A549 cells, (iv) CCM-PLGA NPs, (v) exosomes derived from A549 cells, and (vi) EM-PLGA NPs characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at equivalent loading of protein.

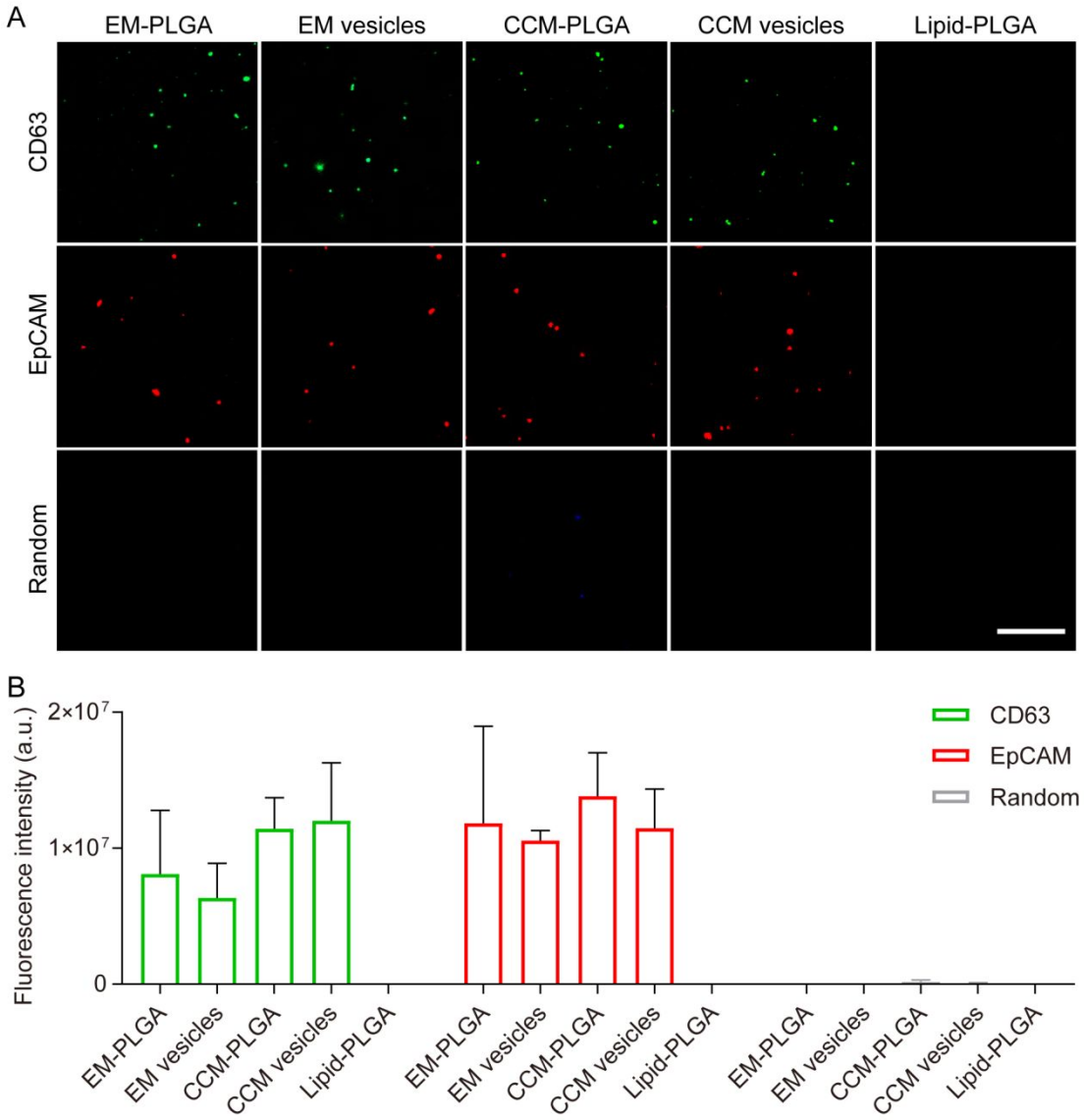


Figure S5. Examination of the orientation of surface proteins on EM-PLGA NPs and CCM-PLGA NPs after synthesis. (A) Immunolabeling analysis using Cy5-conjugated aptamers specific for the extracellular domain of CD63 and EpCAM. EM-PLGA NPs and CCM-PLGA NPs had similar protein profiles with EM vesicles and CCM vesicles. The incubation of Cy5-random sequence with all the biomimetic NPs and the incubation of CD63- and EpCAM-aptamers with lipid-PLGA NPs revealed the absence of unspecific binding. Scale bar, 20 μm. (B) Quantification of the fluorescence intensities ($n$ = 3, mean ± s.d.).

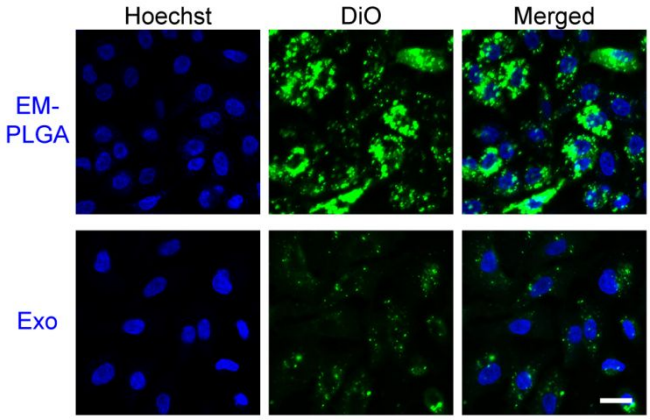


Figure S6. Enhanced cellular uptake of EM-PLGA NPs by A549 cells as compared with exosomes (Exo). Confocal fluorescence images were taken of A549 cells incubated with EM-PLGA NPs and Exo. The membranes of EM-PLGA NPs and Exo were labeled with DiO (green). The cell nuclei were stained with Hochest (blue). Scale bar, 40 μm.

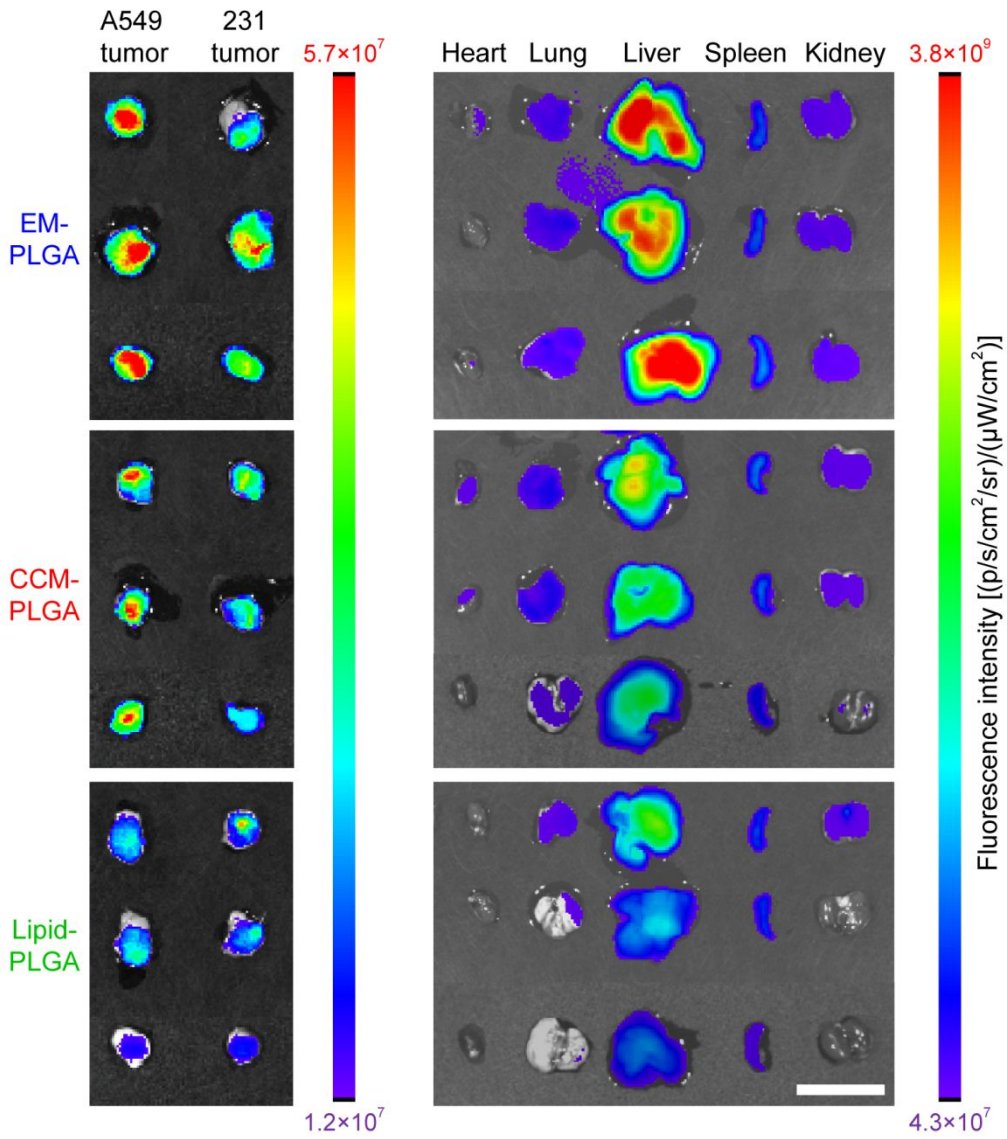


Figure S7. *Ex vivo* fluorescence imaging of tumors and major organs after euthanasia at 48 h post-injection. Scale bar, 2 cm.