Supporting Information

Exosome-modified mesoporous polydopamine with saikosaponin D and ferric ion for immunotherapy of metastatic breast cancer

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Materials and methods

Materials

Pluronic F127, 1,3,5-trimethylbenzene (TMB, ≥98%), dopamine (≥99%), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, ≥98%), Saikosaponin D (SSd, ≥98%), Cell Counting Kit-8 (CCK-8) and Nile red (≥98%) were all purchased from Sigma-Aldrich (Shanghai, China). ExoQuick Exosome Precipitation Solution was acquired from System Biosciences (CA, USA). Live/Dead cell assay kit (L3224) was purchased from Invitrogen (USA). Anti-PCNA antibody (ab29) was obtained from Abcam (Cambridge, UK). All Enzyme-linked immunosorbent assay (ELISA) kits were purchased from ThermoFisher (Waltham, MA, USA).

Animals

The animal experiment was approved by the Animal Ethics Committee of Changchun University of Chinese Medicine (license No. 2023112). Female BALB/c mice, aged 4 to 6 weeks and weighing approximately 16 g, were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and were raised in a specific pathogen-free facility. The mice were allowed to acclimate to their environment for one week prior to the experiments and were housed under specific pathogen-free conditions with unrestricted access to food and water.

Photothermal performance study

To test the photothermal properties of MFSE, 100 μL of aqueous MFSE solution at concentrations of 0, 50, 100, 200 and 400 μg/mL was irradiated using an 808 nm continuous wavelength laser at a power of 1 W/cm^2. Another 200 μg/mL of MFSE solution was an irradiated with 808 nm continuous wavelength laser at 0, 0.5, 1.0 and 1.5 W/cm^2. The temperature changes before and after irradiation were finally recorded by infrared thermography. Photothermal conversion efficiency

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_0}{I(1 - 10^{-A_{808nm}})} \quad (1)$$

$$hS = \frac{m_d C_d}{t_s} \quad (2)$$

$$t_s = -\frac{t}{\ln \theta} \quad (3)$$

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (4)$$

T_{max}, T_{surr} and T are the maximum temperature of the sample in water, the ambient temperature and the cooling cycle stage respectively. h is the heat transfer coefficient, S is the laser irradiation area, Q_0 the energy absorbed by pure water under 808nm laser irradiation. I is the laser power density of the 808 nm laser output. A_{808nm} is the UV-visible absorption luminosity value at a wavelength of 808nm. m_d is the mass of the solution. C_d is the specific heat capacity of water. The final photothermal conversion efficiency (η) is derived from equation (1) (2) (3) and (4).

Cell culture

Breast cancer cell line 4T1 was purchased from American Tissue Culture Collection. 4T1 cells were cultured in 1640 culture medium containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, at 37°C, with 5% CO_2.

Cytotoxicity assay

The cytotoxicity assay was detected using CCK-8 reagent. Briefly, 4T1 and MCF-10A cells were inoculated into a 96-well plate at a density of 5000 cells per well and cultured for 12 h. The original medium was replaced with different concentrations of MFSE, incubated for 2 h, irradiated with an 808 nm laser at 1 W/cm^2 for 10 min and continued to incubate for 24 h. The original medium was replaced with fresh medium and 10 uL of CCK-8 was added to each well, further incubated at 37°C for 30 min. The absorbance of the samples was measured at 450 nm. All experiments were repeated in triplicate.

Live/dead cell experiment

The experiments were conducted with live/dead cell assay kit according to the manufacturer's instructions. 4T1 cells were inoculated into a 6-well plate at a density of 1.5×10^5 per well and cultured for 12 h. After that, the 4T1 cells were sequentially treated with PBS, PBS + NIR (1 W/cm^2, 5 min, 808 nm), SSd (0.2 μg/mL), SSd (0.2 μg/mL) + NIR (1 W/cm^2, 5 min, 808 nm), MFE (200 μg/mL), MFE (200/μg·mL) + NIR (1 W/cm^2, 5 min, 808 nm), MFSE (200 μg/mL), MFSE (200 μg/mL) + NIR (1 W/cm^2, 5 min, 808 nm), respectively. After incubation for the next 12 h, the cells in each well were stained

with Calcein-AM (1 μL) for 30 min and PI (2 μL) for 10 min in a blank medium and imaged by the fluorescence.

Wound-healing assays

4T1 cells were inoculated into a 6-well plate at a density of 1.5×10^5 per well and cultured for 12 h. The cells were scratched vertically at the bottom of the medium using a 1 mL gun tip, and then the 4T1 cells were sequentially treated with PBS, PBS + NIR (1 W/cm^2, 5 min, 808 nm), SSd (0.2 μg/mL), SSd (0.2 μg/mL) + NIR (1 W/cm^2, 5 min, 808 nm), MFE (200 μg/mL), MFE (200 μg/mL) + NIR (1 W/cm^2, 5 min, 808 nm), MFSE (200 μg/mL), MFSE (200 μg/mL) + NIR (1 W/cm^2, 5 min, 808 nm), and continued to incubate for 24 h. The cells were observed by fluorescence microscopy. Migration distances were processed with Image J. All experiments were repeated three times.

Cellular uptake assay

4T1 cells were inoculated into a 6-well plate at a density of 1.5×10^5 per well and cultured for 12 h, and then incubated with 200 μg/mL of MFSE and MFS labeled with Nile red for 6 h. Then, the treated cells were washed with PBS twice and fixed in 4% paraformaldehyde for 30 min. Then the fixed cells were stained with DAPI solution for 15 min and observed under LSM 710 confocal laser scanning microscope (CLSM, Carl Zeiss Microscopy LLC, Germany).

H&E staining

Briefly, collected tissues were fixed in 10% neutral buffered formalin and subjected to paraffin embedding procedures. Paraffin-embedded tissue specimens were cut into 5 μm slices using a microtome and stained with hematoxylin and eosin (H&E). The stained slices were observed and photographed using a confocal fluorescence microscope.

Immunofluorescence staining

The sections were baked at 60 °C for 10 minutes and then stained with xylene, graded ethanol (100%, 95%, 70%) and PBS for 5 min each. Sections were immersed in antigen retrieval solution for 10 min at a temperature of 80°C. Sections were then closed with 10% BSA in PBS for 30 min at room temperature and incubated with anti-PCNA overnight at 4°C. Sections were then incubated with FITC-labeled secondary antibody for 1 h blocked with DAPI. Finally, confocal fluorescence microscopy was used for observation and photography.

ELISA analysis

ATP, CRT, HMGB1, and IFN-γ of tumor tissues were detected using commercial ELISA kits. Briefly, the mice tumor tissue was washed with PBS, homogenized, and the supernatant was collected by centrifugation. The levels of ATP, CRT, HMGB1 and IFN-γ

116 in the supernatant were detected according to the manufacturer's recommended
117 procedure.

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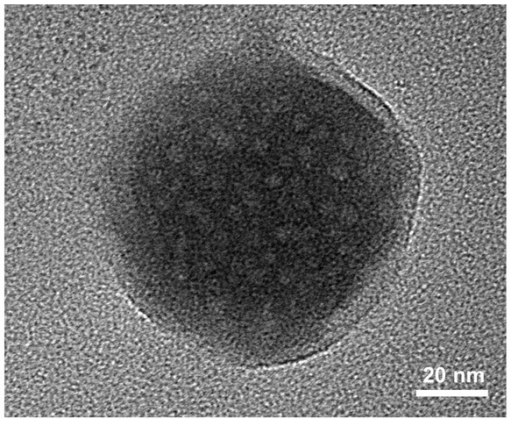
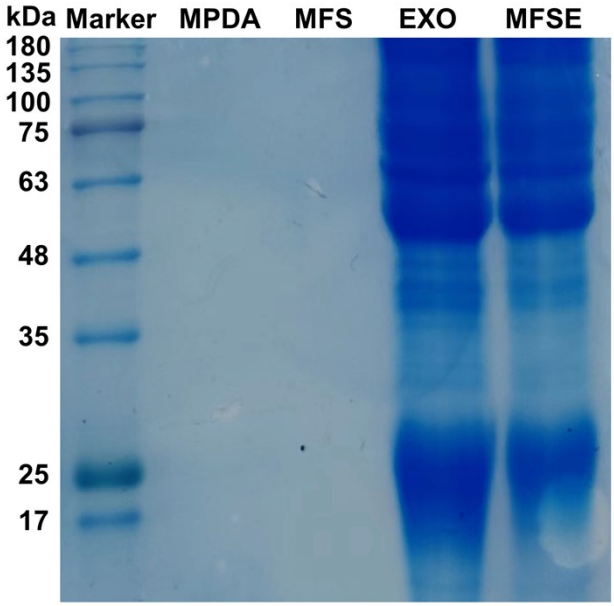


Figure S1. Representative TEM images of exosome.



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123 **Figure S2.** SDS-PAGE analysis of MPDA, MFS, EXO and MFSE.

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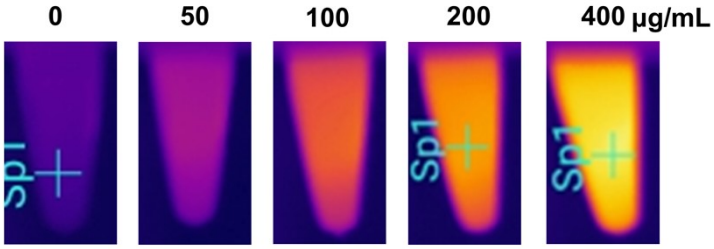
125 

126 **Figure S3.** Infrared thermal images of MFSE under irradiation at different concentrations

127 (1 W/cm^2, 5 min).

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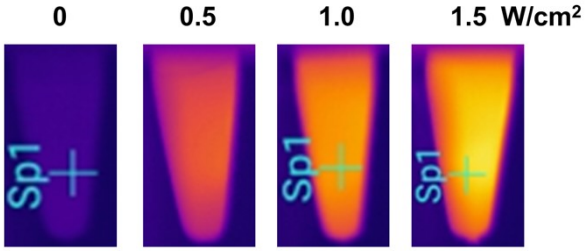

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130 **Figure S4.** Infrared thermal images of MFSE at different laser power densities under

131 irradiation (200 μg/mL, 5 min).

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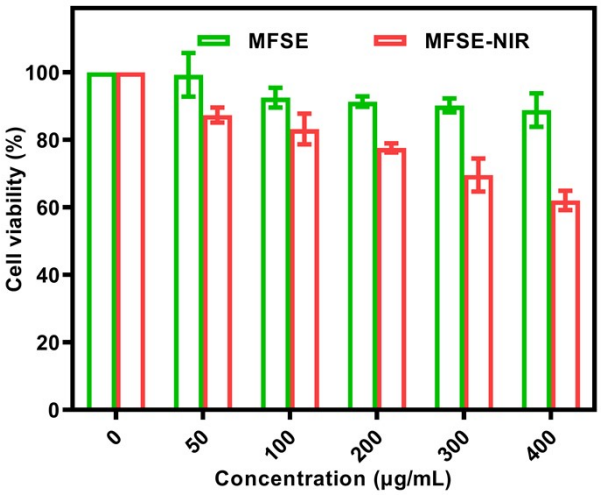
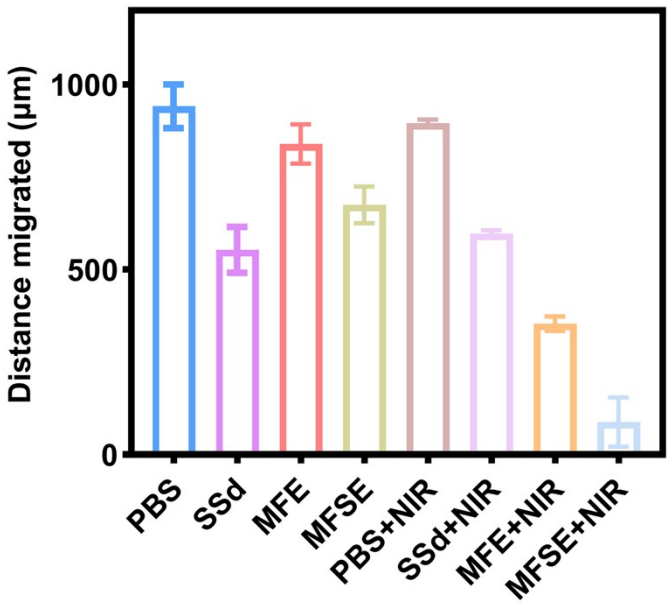


Figure S5. Cell viabilities of MCF-10A cells treated with varying concentrations of MFSE with or without 808 nm laser (n = 3).



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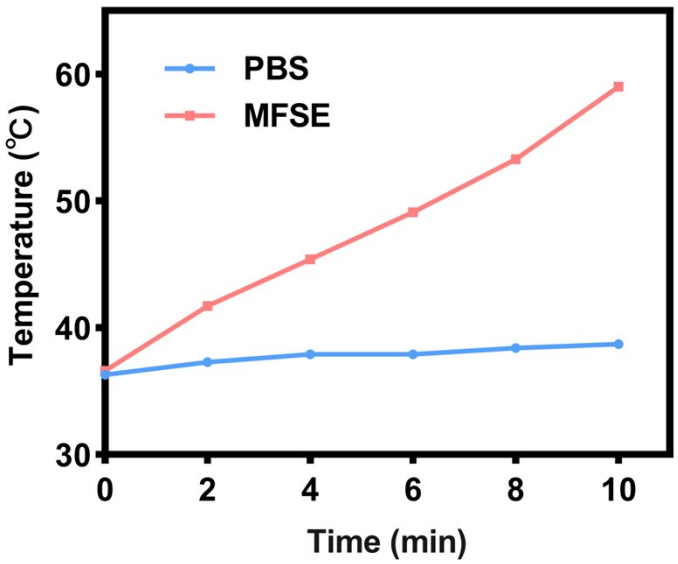
137 **Figure S6.** Migration distances of 4T1 cells following various treatments (mean ± SD, n

138 = 3).



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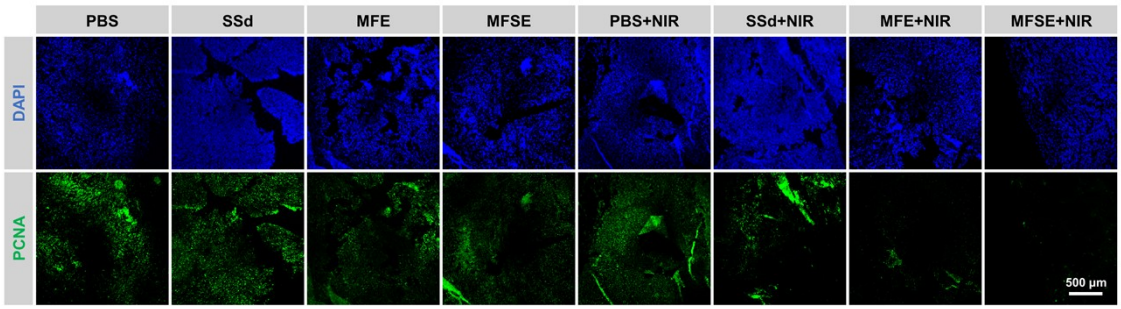
140 **Figure S7.** Temperature changes of PBS and MFSE during 10 min of irradiation at 1

141 W/cm² with a concentration of 200 μg/mL.

142 

143 **Figure S8.** PCNA staining of the tumor tissues after different treatments.