Supporting Information

Photo/thermo-responsive and size-switchable nanoparticles for

chemo-photothermal therapy against orthotopic breast cancer

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Materials and methods

Materials and Instruments

All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, US) and used without further purification unless otherwise noted. Dialysis membranes (3.5 kDa and 7 kDa MWCO) were purchased from Pierce Inc. Methoxy PEG acrylate (mPEG-acrylate) was purchased from JenKem Technology USA Inc. (Allen, TX, US). Carbon Coated Copper Grids were purchased from Canemco Inc. (Core, Quebec, Canada). 4T1 cell line was purchased from ATCC. 4T1-luc cell line was gifted from Caliper Life Sciences Inc. Dulbecco's Modified Eagle's Medium (DMEM, Gibco, America) was purchased from Gene Tech Co. (Shanghai, China).

The fluorescence intensity was recorded by a Varian Cary Eclipse fluorescence spectrophotometer. Hydrodynamic diameters (Dh) and size distributions were characterized by Malvern Zetasizer Nano-S dynamic light scattering (DLS) (Malvern Instruments Ltd. Worcestershire, UK). Measurements were conducted at room temperature. Photographs of the nanoparticles were taken by a HT7700 transmission electron microscope operating at 100 kV. Flow cytometry (FACS) experiments were performed by FlowSight® Imaging Flow Cytometer (Merck Millipore, Darmstadt, Germany).

Transcellular transport

4T1 cells were seeded in a culture dish containing coverslips on the bottom at a density of 2×10^4 cells/well. After 24 h, a predetermined amount of NPs-DOX was added into the culture dish and allowed to be incubated with 4T1 cells for 8 h at 37 ° C. Afterward, coverslip I was carefully washed with pre-warmed fresh media, and then co-incubated with coverslip II for 1 h. After NIR irradiation (1 W/cm², 2 min), coverslip I and II were co-incubated for another 12 h. Similarly, coverslip II was washed with pre-warmed fresh media and co-incubated with coverslip II. After NIR irradiation and co-incubation, the cells on the coverslips were fixed with

paraformaldehyde and stained with Hoechst 33342. The average fluorescent intensity of DOX was quantified by the software ImageJ.

Orthotopic Breast Cancer Model and Treatment

Female BALB/c mice, aging 5-6 weeks and weighing 18±2 g, were obtained from Slac Laboratory Animal Incorp. (Shanghai, China), and kept in SPF laboratory animal environment with standard rodent chow diet and water ad libitum, and a 12 h light/dark alternate cycle. The study was performed after the mice were allowed to acclimate for 1 week. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Wenzhou Medical University and approved by the Animal Ethics Committee of Wenzhou Medical University.

In the orthotopic breast cancer model, $1 \times 10^5 4T1$ or 4T1-Luc cells suspended in 50 µL PBS were injected into the breast fat pad. When the average tumor volume reached ~60 mm³, mice were randomized into six treatment groups: PBS, DOX, NP, NP-DOX, NP+NIR, NP-DOX+NIR. The treatment was conducted at a dose of 5 mg/kg (DOX/weight) once a week for two consecutive weeks via tail vein injection. After 15 min, tumors were irradiated with or without NIR light (808 nm, 1 W/cm², 10 min). The mice were observed daily for clinical symptoms. Tumor sizes were measured by a digital caliper every three days until 15 days after first therapy, and tumor volume was calculated by the formula (L × W²)/2, where L is length (longest dimension) and W is width (shortest dimension). After the treatments, all animals were sacrificed, and the tumors were dissected and weighed.

Immunohistological examination

After mice were euthanized, samples of tumors were collected, and fixed by immersion in paraformaldehyde (PFA, 4 wt%) for 48 h. Then the tissues were dehydrated, cleared and paraffin-embedded. Paraffin-embedded slides (6-7 μ m) were stained with hematoxylin and eosin (H&E) staining dyes, and examined by an optical microscope. Furthermore, the tumor cellular proliferation was evaluated by

immunohistochemical study with a primary antibody against Ki67 (Abcam, USA) according to the protocol of the manufacturer (ZSGB-BIO, Beijing, Chnia).

FACS analysis of immune effector cells in tumor-bearing mice

The percentages of CD11c⁺, CD80⁺CD86⁺, CD4⁺, CD8⁺, CD4⁺FoxP3⁺, CD11b⁺Gr-1⁺ cells were investigated by FACS. The tumor-draining lymph nodes (TDLNs) and tumor tissues were harvested from tumor-bearing mice and teased apart into single cell suspension by pressing with plunger of a syringe. After being washed by PBS for twice, cells were stained with PE anti-mouse CD8, APC anti-mouse CD4, FITC anti-mouse FoxP3, PE anti-mouse CD86, FITC anti-mouse CD80, APC anti-mouse CD11c, FITC anti-mouse CD11b and Percp/Cy5.5 anti-mouse Ly-6G/Ly-6c(Gr-1) antibody for 30 min at 4 °C according to the manufacturer instructions. After being washed by 2 % FBS and filtering by a nylon membrane, cell samples were analyzed by FACS.

Statistics

All data were expressed as the mean value \pm standard deviation unless otherwise indicated. One-way ANOVA with Tukey's post-hoc test was applied to determine the *P* values. *P* < 0.05 was accepted as a statistically significant difference.



Fig. S1 (A) The percentage of CD11c⁺ dendritic cells in TDLNs of tumor-bearing mice after the treatments. (B) The percentage of CD80⁺CD86⁺ cells in CD11c⁺ dendritic cells of tumor-bearing mice after the treatments. *, P < 0.05; **, P < 0.01.



Fig. S2 The percentages of CD8⁺ T cells (A) and CD4⁺ T helper cells (B) in tumor tissues of mice after the treatments. *, P < 0.05.



Fig. S3 The percentage of CD4⁺FoxP3⁺ cells in TDLNs of tumor-bearing mice after the treatments.



Fig. S4 The percentage of CD11b⁺Gr-1⁺ myeloid-derived suppressor cells in tumor tissues of mice after the treatments. **, P < 0.01.