Electronic Supplementary Information

Iron oxide nanoparticles as a drug carrier reduce host immunosuppression for enhanced chemotherapy

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Keywords: iron oxide nanoparticles, chemotherapy, immunosuppression, immune response, MR imaging

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Part of Experimental Details:

Materials

Amine functionalized IONPs, MTX, BSA were purchased from Sigma-Aldrich (St. Louis, MO). Dulbecco's Modified Eagle Medium (DMEM), RPMI-1640 medium, fetal bovine serum (FBS), 0.25% Trypsin-EDTA, penicillin, and streptomycin were obtained from Gibco (Grand Island, NY). Cell Counting Kit-8 (CCK-8) assay kit and double stain apoptosis detection kit (calcein-AM/PI) were purchased from Dojindo Laboratories (Shanghai, China). BCA assay kit was obtained from Thermo Scientific (Mass).

***In vitro* drug release**

The IONPs@BSA-MTX complexes (0.5 mg/mL MTX) was dispersed into 0.8 mL of PBS (pH 7.4) or acetate buffer (pH 5.5) and placed in a dialysis bag (MWCO = 14 000). The dialysis bag was then immersed in the corresponding buffer medium with a volume of 7.2 mL and kept in a vapor-bathing constant temperature vibrator at 37 ^{o}C with a vibration speed of 200 rpm. At each time point, 0.8 mL of outer phase medium was taken out and the same volume of fresh corresponding buffer medium was replenished. The MTX release was quantified by UV-vis spectroscopy.

Cell culture

4T1 cells, RAW264.7 macrophages, and DC2.4 cells were purchased from American Type Culture Collection (ATCC; Bethesda, MD). Bone-marrow-derived dendritic cells (BMDCs) were generated from C57BL/6 mice. 4T1 cells were cultured with RPMI-1640 medium containing 10% FBS and 1% penicillin and streptomycin. RAW264.7 macrophages, DC2.4 cells and BMDCs were cultured with DMEM containing 10% FBS, 1% penicillin and streptomycin.

Cytotoxicity assay

For in vitro cytotoxicity assay of IONPs@BSA-MTX complexes, 4T1 cells (5000 cells per well) were seeded into a 96-well plate. After overnight incubation, the cells were cocultured with IONPs@BSA-MTX, IONPs@BSA or MTX at different concentrations for 24 h. A standard CCK-8 assay was then performed to obtain the relative cell viability according to the manufacturer’s instructions. The morphology of the treated 4T1 cells was also investigated. Likewise, after 4T1 cells being treated with

those samples at different final concentrations for 24 h, the cells were also observed using Leica DM IL LED inverted phase contrast microscope (Wetzlar, Germany) with a magnification of 100 × for each sample.

In vivo MR imaging

Mice bearing orthotopic 4T1 breast tumors were first anesthetized using 1.5% isoflurane at 0.7 L/min oxygen and placed in an MR probe at a supine position before imaging using a Bruker Biospec 7 T horizontal-bore imaging spectrometer (Bruker BioSpin MRI GmbH, Ettlingen, Germany). T_2 weighted MR images were generated by a Bruker S116 gradient coil (2.0 mT/m/A), a 72 mm quadrature multirung radiofrequency coil for radiofrequency transmission and signal reception. For in vivo MR imaging, multiple MR slices were put in the transverse plane through a spin-echo multislice (repetition time, 0.8 s; echo time (TE), 63 ms; 256 ×256 pixels matrix; 4 steps per acquisition; FOV, 3.5 × 3.5 cm^2; and slice thickness, 1mm).

Table S1. The hydrodynamic size of IONPs, IONPs@BSA, and IONPs@BSA-MTX complexes.

Samples	Hydrodynamic size (nm)	PDI
IONPs	36.6 ± 1.6	0.169 ± 0.041
IONPs@BSA	383.2 ± 55.6	0.261 ± 0.221
IONPs@BSA-MTX	376.8 ± 31.1	0.415 ± 0.105

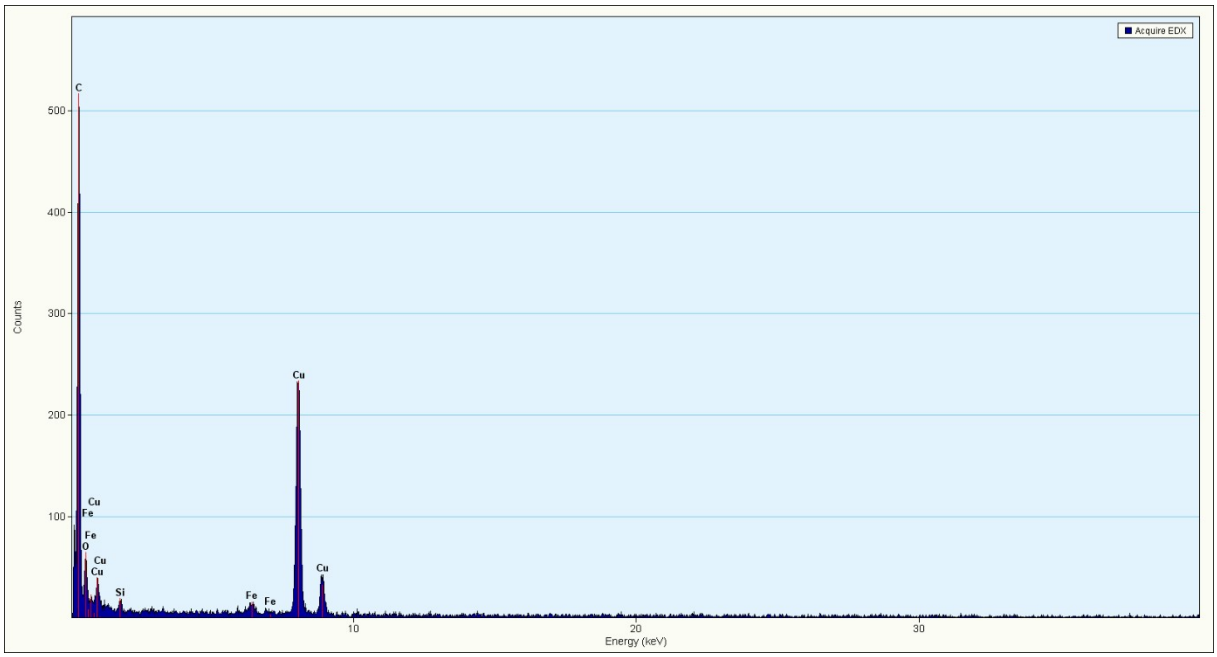

Fig. S1. The energy-dispersive X-ray spectroscopy (EDS) of the IONPs.

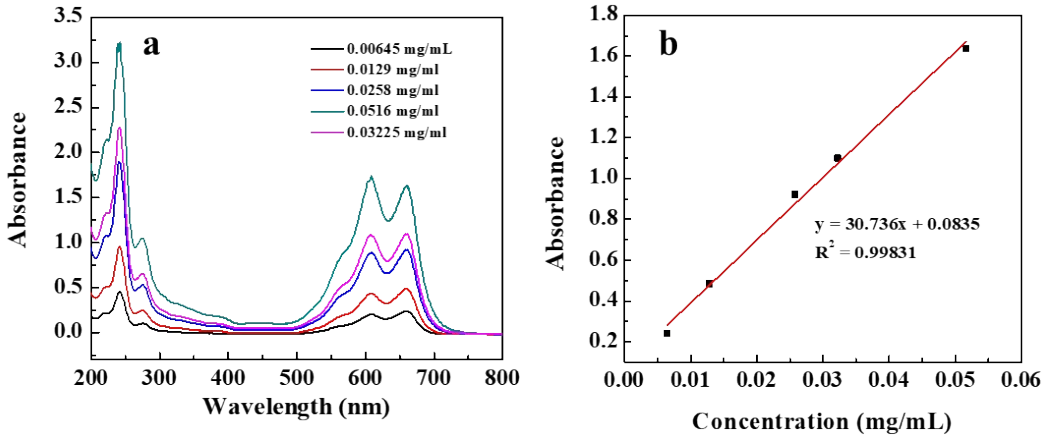

Fig. S2. (a) UV-vis spectra of MTX water solution at different concentrations. (b) The standard curve of calibration curve of MTX solution.

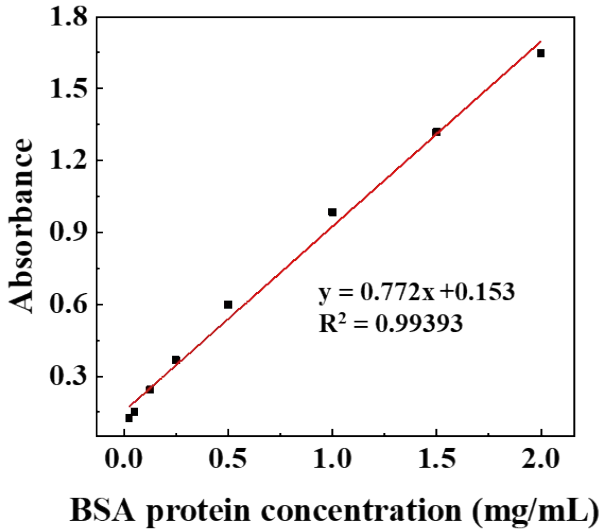

Fig. S3. The standard curve for BSA protein in the BCA protein assay.

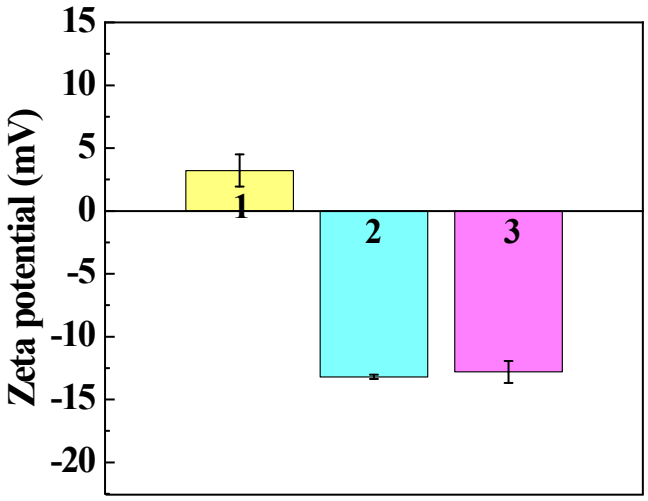

Fig. S4. Zeta potential of IONPs (1), IONPs@BSA (2), IONPs@BSA-MTX (3).

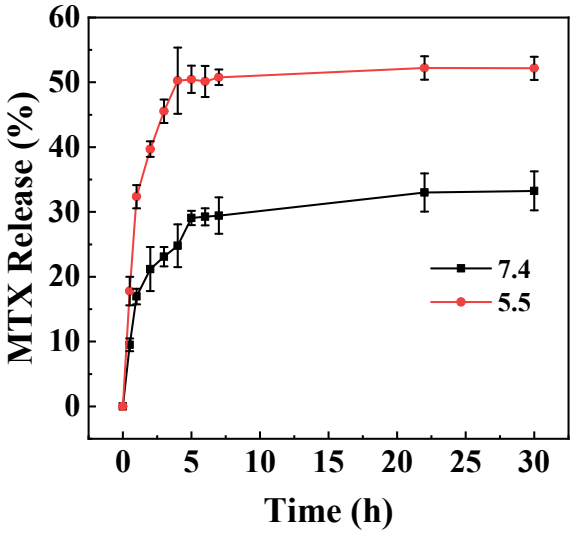

Fig. S5. Cumulative release of MTX from IONPs@BSA-MTX complexes in PBS (pH 7.4) and acetate buffer (pH 5.5) at 37 °C.

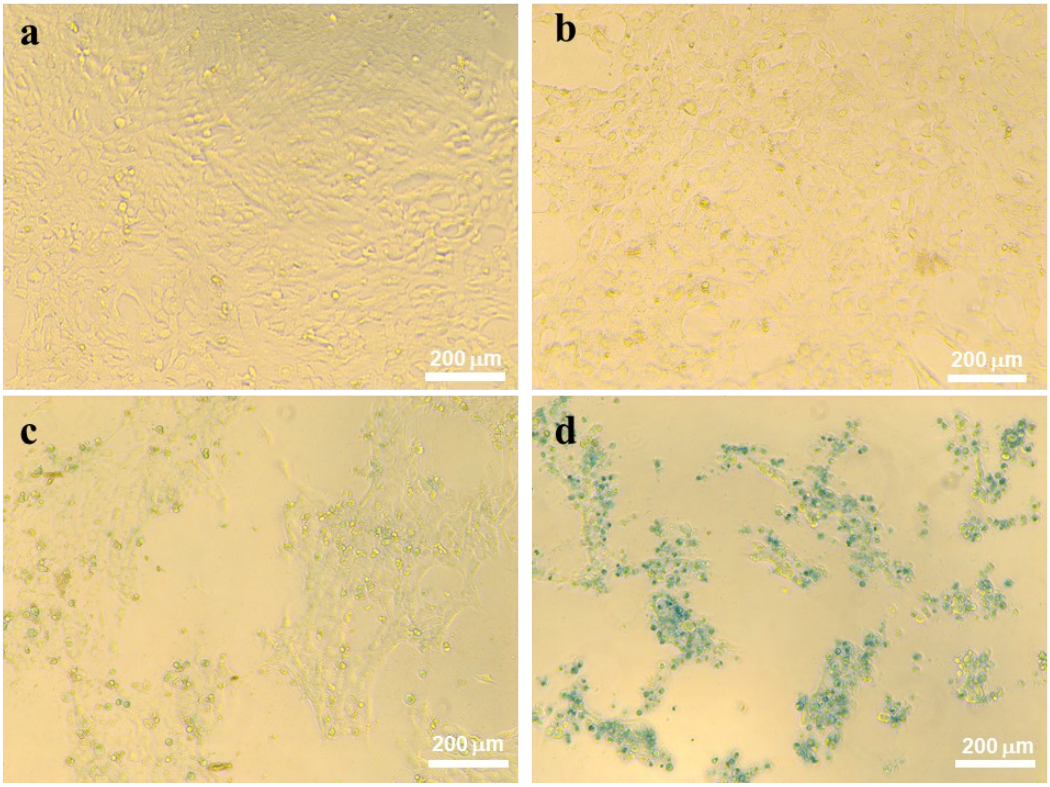

Fig. S6. The micrographs of 4T1 cells treated with PBS (a), IONPs@BSA (b) with same equivalent concentrations of IONPs@BSA-MTX complexes, IONPs@BSA-MTX complexes (c), MTX (d) at the 15 μg/mL of MTX concentration, respectively.

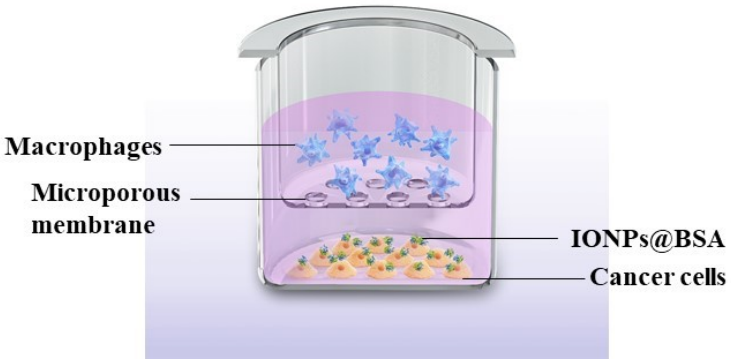

Fig. S7. Diagram shows macrophage-cancer cell co-culture set up in transwell plates.

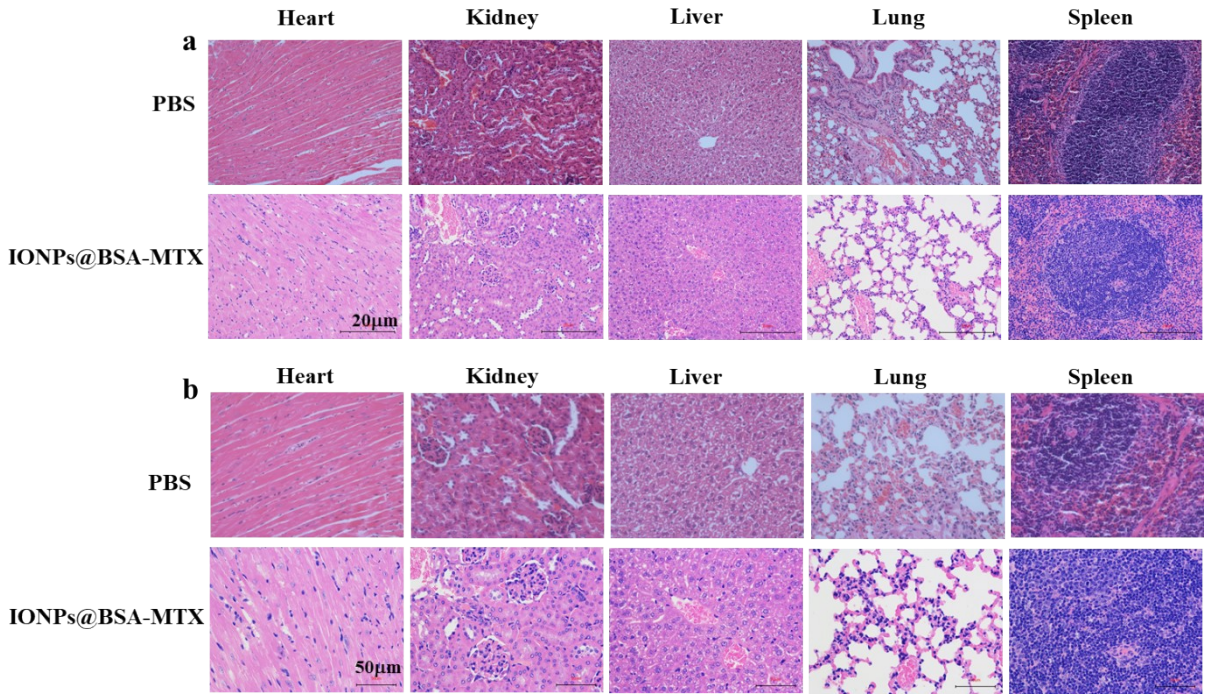

Fig. S8. Histological changes in the heart, kidney, liver, lung, and spleen of the healthy mouse at one month after intravenous injection of the IONPs@BSA-MTX complexes using hematoxylin-eosin (H&E) staining. The healthy mice treated by PBS as a control group. (a) and (b) were 200- and 400-times magnification photos, respectively.