Sequential SPECT and NIR-II Imaging of Tumor and Sentinel Lymph Node Metastasis for Diagnosis and Image-Guided Surgery

Xiaolu Zhang[1, 2, ‡], Meng Zhao[2, ‡], Ling Wen[3, ‡], Manran Wu[2], Yi Yang[2], Yujuan Zhang[4], Yan Wu[2], Jian Zhong[1, *], Haibin Shi[2], Jianfeng Zeng[2], Guanglin Wang[2, *], Mingyuan Gao[2, *]

[1] Integrated Scientific Research Base on Comprehensive Utilization Technology for By-Products of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, National R&D Branch Center for Freshwater Aquatic Products Processing Technology (Shanghai), Shanghai Engineering Research Center of Aquatic-Product Processing and Preservation, College of Food Science & Technology, Shanghai Ocean University, Shanghai, 201306, China

[2] State Key Laboratory of Radiation Medicine and Protection, School of Radiation Medicine and Protection & School for Radiological and Interdisciplinary Sciences (RAD-X), Collaborative Innovation Center of Radiation Medicine of Jiangsu Higher Education Institutions, Soochow University, Suzhou 215123, PR China.

[3]Department of Radiology, the First Affiliated Hospital of Soochow University; Institute of Medical Imaging, Soochow University, Suzhou 215000, PR China.

[4] Experimental Center of Soochow University, Department of Medicine, Soochow University, Suzhou 215123, PR China.

*Corresponding author:

E-mail: jzhong@shou.edu.cn

E-mail: glwang@suda.edu.cn

E-mail: gaomy@iccas.ac.cn

Experimental

Chemicals

Chemicals: NaI (^{125}I) was bought from Shanghai GMS Pharmaceutical Co., Ltd.. All

the reagents were purchased commercially and used directly without further purification.

All animal experiments were conducted following a protocol approved by the Animal Care and Use Committee of Soochow University. Female BALB/c mice of specific pathogen-free grade were bought from Shanghai SLAC Laboratory Animal Co., Ltd.

Radiolabeling of MT

0.5 μL of NaI (^{125}I) (200 μCi) was added to 10 μL of chloramine T (10 mg/mL) and 10 μL of MT (10 mg/mL) in DMSO solution. After 20 min reaction, 5 μL of $Na_2S_2O_3$ was mixed with the solution to stop the reaction. Thin layer chromatography assay was applied to determine the radiolabeling yield and radiostability by using saline as a mobile phase. The R_f of ^{125}I-MT was ~0.1, and free ^{125}I was ~0.8.

Preparation, characterization of MT nanoparticles (MT NPs) and ^{125}I-MT nanoparticles (^{125}I-MT NPs).

To prepare MT NPs nanoparticles and ^{125}I-MT NPs, nanoprecipitation method was used. Briefly, 200 μL of PEO-PPO-PEO solution in THF (100 mg/mL) mixed with 10 μL of MT (10^{-2} mmol/mL) or ^{125}I-MT (10^{-2} mmol/mL, 100 μCi) in THF (200 μL). Then the mixed solution was dropwise added into 2 mL water under continuous sonication for 30 min with temperature below 30 ^{o}C. After sonication, THF was evaporated overnight in fume cupboard in dark. The concentration of nanoparticles was determined by UV-Vis absorption according to their absorption coefficients. The final concentration of MT NPs and ^{125}I-MT NPs was fixed at (5 x 10^{-2} μmol/mL).

The hydrodynamic size of MT NPs and ^{125}I-MT NPs was measured by dynamic light scattering (DLS). All experiments were performed on a Malven Zetasizer Nano ZS90 equipped with a solid-state He-Ne laser (λ=633 nm) in triplicate at 20 ^{o}C. A transmission electron microscope (TEM, FEI Tecnai G2) was used to visualize the size and morphology of MT NPs, manipulating at an accelerating voltage at 120 kV. MT NPs (10 μL, 0.5 mg/mL) was dropped onto a carbon-coated copper grid of 200 mesh,

and the redundant solution of MT NPs was removed by using a filter paper after 15 min. Then, aqueous solution of phosphotungstic acid (10 μL, 1 mg/mL) was dropped onto the copper grid to dye the MT NPs. Fluorescence spectra of MT and MT NPs were conducted on a FLS980 spectrometer (Edinburgh Instruments, UK). NIR-II images of MT NPs were acquired with the NIR-II imaging system (Serious II 900-1700 nm, Suzhou NIR-Optics Co., Ltd, China). For in vitro imaging, MT in DMSO and MT-NPs was excited with the 808 nm laser. The power density of laser was 45 mW/cm^2 and the exposure time was 50 ms.

To evaluate the colloid stability of MT NPs in PBS, the size of MT NPs was monitor up to 96 h by DLS. The radiostability of ^{125}I-MT NPs was examined by TLC in PBS and 10 % fetal bovine serum (10% FBS), incubated with varied time from 1 h to 96 h.

Cytotoxicity Assay

Methyl thiazolyl tetrazolium (MTT) assays were conducted to evaluate the cytotoxicity of MT, MT NPs and ^{125}I-MT NPs. In a 96-well plate, 4T1 cells were seeded and cultured in DMEM with 10 % FBS for 24 h at 37 ^{o}C in a 5% CO_2 atmosphere. The culture medium was replaced by MT and MT NPs with different concentrations (0, 1.64, 3.28, 6.56, 13.12, 26.25 and 52.50 μg/mL), and ^{125}I-MT NPs with different activities (0, 2, 4, 6, 8 and 10 μCi/mL at concentration 3.28 μg/mL) for 24 h at 37 ^{o}C. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (100 μL, 0.5 mg/mL) was added into 4T1 cell for 4 h following with dimethyl sulfoxide (100 μL). The absorption of each solution was detected by a microplate reader (Thermo, Varioskan Flash).

Immunofluorescence cell experiment

4T1 cells seeded on the cover slips were treated with MT, MT NPs, ^{125}I, ^{125}I-MT and ^{125}I-MT NPs for 4 h, fixed with paraformaldehyde (4 %) for 10 min and then washed by phosphate buffered saline (PBS) for three times. The cells were treated with Triton X-100 (2%) for 15 min to rupture the cell membrane, washed with PBS for three times, immersed in a blocking buffer (5% bovine serum albumin in tris-buffered saline

solution) for 1 h at 37 ºC, incubated with anti-histone γH2A.x mouse monoclonal antibodies in the dark overnight at 4 ºC, washed with PBS for three times, and incubated in rabbit anti-mouse secondary antibody for 1 h at 37 ºC in the dark. The cell nuclei were, stained with Hoechst (20 μL, 5 mg/mL) for 5 min. The images were taken by confocal laser scanning microscope (Olympus FV1200).

The 4T1 breast cancer cells were incubated with culture medium or ^{125}I-MT NPs (13.12μg/mL, 10μCi/mL) in culture medium for 24 h. Following, new medium replaced for incubated one week. The medium was absorbed and washed with PBS twice. After fixation of methol, the Giemsa was used to color the 4T1 cells. The formation of colony was counted under microscopy (more than 50 cells as one colony).

Pharmacokinetics and biodistribution

To evaluate the pharmacokinetic studies in vivo, blood samples were drawn from the retinal veins of BALB/c mice (n=3) after postinjection of ^{125}I-MT NPs (30 μCi, 2.22 x 10^{-4} mmol/mL) at 0.05, 0.25, 0.5，1，2，4，6，8，10，12 and 24 h, respectively. In contrast, ^{125}I-MT in 10% DMSO water solution was injected via the tail vein. The blood samples were weighed, and the radioactivity was counted by a γ counter (Multi Crystal LB2111γ counter). Further, major organs (heart, kidneys, liver, lungs, and spleen) were harvested, weighed and the radioactivity was counted at 24 h postinjection.

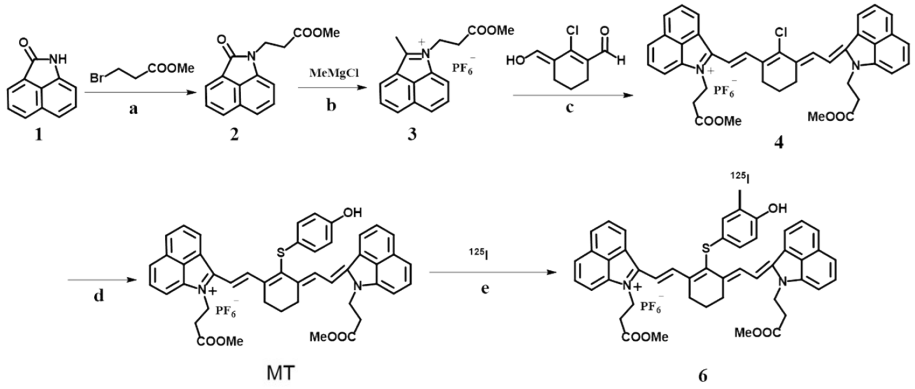
Establishment of tumor modal and lymphatic metastasis model

4T1 tumor bearing BALB/c mice modal were established by subcutaneous injection of $1x10^6$ 4T1 cells in 50 μL PBS into the right hindlimb. The mice were used for NIR-II imaging and SPECT dual modal imaging when the volume of tumor reached 100 mm^3. Lymphatic metastasis model was established by subcutaneous injection of $5x10^5$ 4T1 cells into foot pads of 6-8 weeks old BALB/c mice. The lymph node was sliced and stained to confirm the metastasis.

In Vivo SPECT/CT and NIR-II imaging

The imaging of MT NPs and ^{125}I-MT NPs in vivo was performed on NIR-II imaging

system (Serious II 900-1700 nm, Suzhou NIR-Optics Co., Ltd, China) and microSPECT/CT scanner (Milabs, Utrecht, the Netherlands), separately. In contrast, MT or ^{125}I-MT in 10% DMSO water solution was injected via the tail vein. The mice were imaged at 0, 4, 8, 10, 24, 48, 72, and 96 h post intravenous injection (0.5 μmol/mL, 200 μL) via tail vein under the power density of 808 nm laser at 45 mW/cm^2 with exposure time 200 ms. After 96 h imaging, the mice were sacrificed and major organ was imaged. The SPECT scan was fixed for 15 min/frame, and the CT scan was set as an accurate mode with full angle, three frames averaging, at 615 mA tube current and 55 kV tube voltage with the dose of 0.5 μmol/mL, 150 μCi, 200 μL. The constructed imaging was analyzed with Pmod software. After 96 h scan, the major organs were harvest, weighted and the activity was counted by γ counter. For mapping of lymphatic metastasis, the probes (2.22 x 10^{-4} mmol/mL, 30 μCi, 50 μL) were injected by intratumoral injection.



Scheme S1. Schematic representation of the synthesis procedure of MT and ^{125}I-MT. (a) $BrCH_2CH_2COOMe$, K_2CO_3, DMF, 100 °C, 18 hours; (b) MeMgCl, tetrahydrofuran, KPF_6, 70°C, 3 hours; (c) 2-chloro-3-(hydroxymethylene)cyclohex-1-enecarbaldehyde, CH_3COONa, $(CH_3CO)_2O$, 40 °C, 2 hours; (d) 4-mercaptophenol, DMF, 25 °C, 4 hours; (e) Chloramine-T, DMSO, 25 °C, 5 minutes.

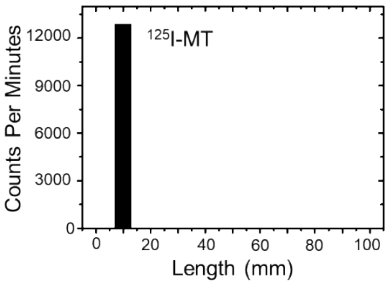


Figure S1. Thin layer chromatography analysis of ^{125}I-MT, the R_f of ^{125}I-MT was ~0.1, and free ^{125}I was ~0.8.

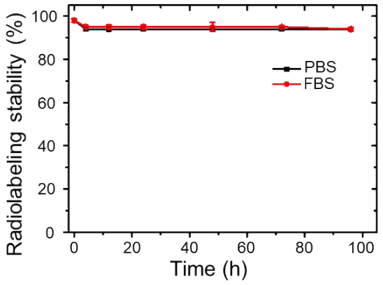


Figure S2. Radiostability of ^{125}I-MT in PBS and 10% FBS over time.

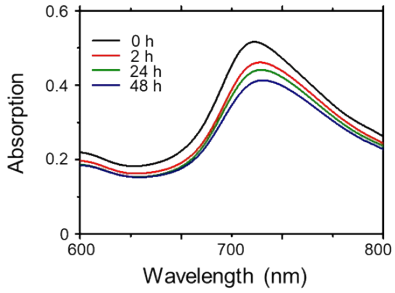


Figure S3. Optical stability of MT-NPs was measured over time.

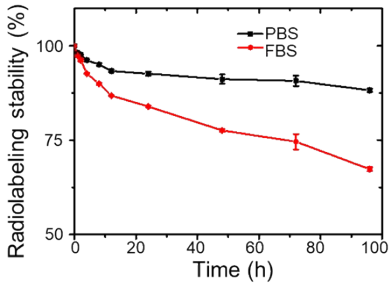


Figure S4. Radiostability of 10 times diluted ^{125}I-MT NPs in PBS and 10 % FBS.

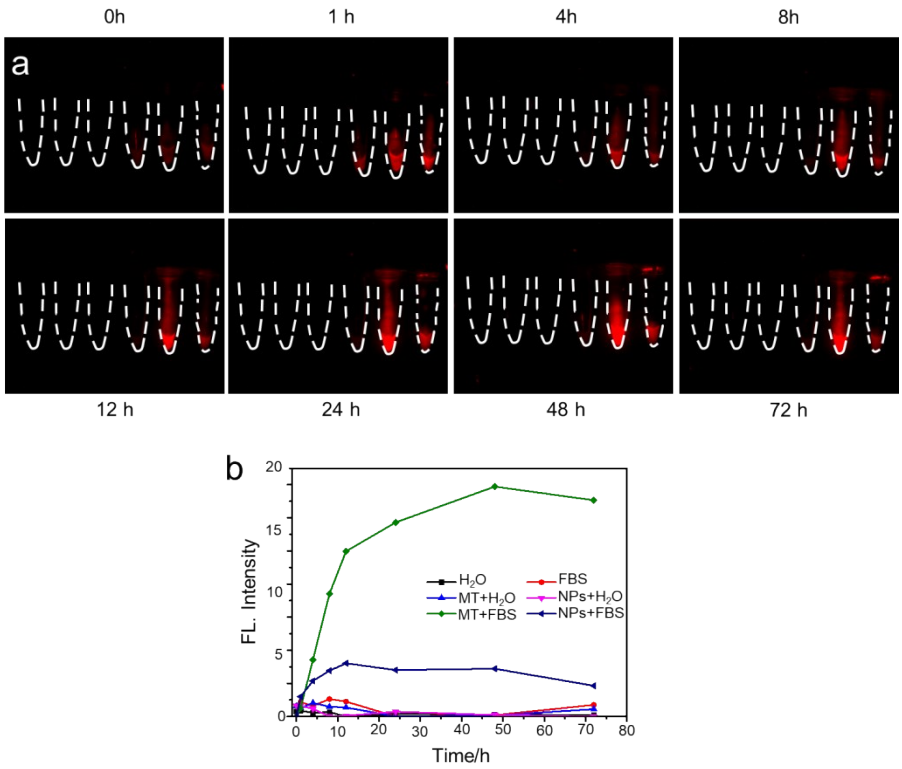


Figure S5. (a) NIR-II images of phantom of (1) H_2O, (2) 10 % FBS, (3) MT in H_2O, (4) MT NPs in H_2O, (5) MT in 10 % FBS, and (6) MT NPs in 10 % FBS (from left to right) overtime. (b) Quantification of NIR-II images of fluorescence intensity overtime.

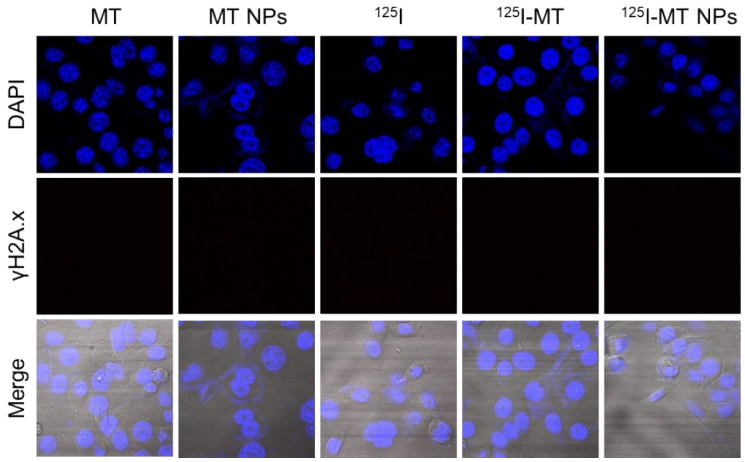


Figure S6. DNA damage analysis of 4T1 cells following treatment with MT, MT NPs, ^{125}I, ^{125}I-MT, ^{125}I-MT NPs, respectively.

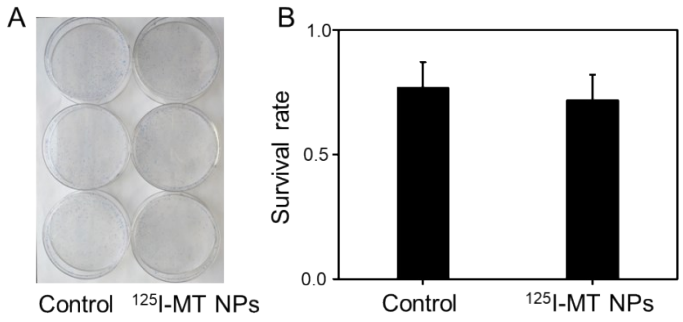


Figure S7. (A) Colony formation images and (B) the corresponding survival fraction of 4T1 which incubated with PBS and ^{125}I-MT NPs

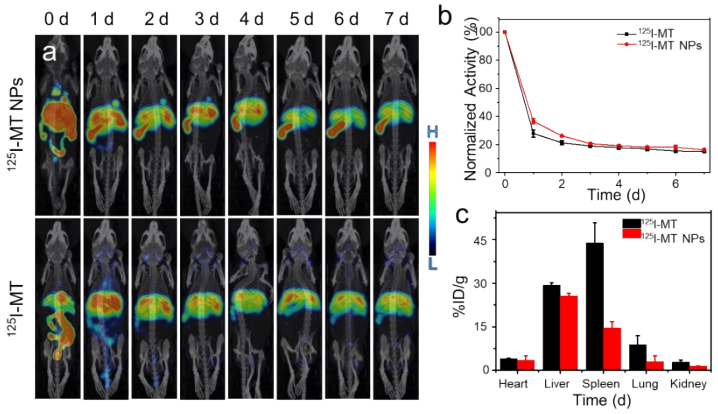


Figure S8. (a) 3D images of mice at 0, 1, 2, 3, 4, 5, 6 and 7th day postinjection of ^{125}I-MT and^{125}I-MT NPs. (b) Relative active of mice of ^{125}I-MT and ^{125}I-MT NPs at 0, 1, 2, 3, 4, 5, 6 and 7th day postinjection. (c) Biodistribution of ^{125}I-MT and ^{125}I-MT NPs at 7th day postinjection.

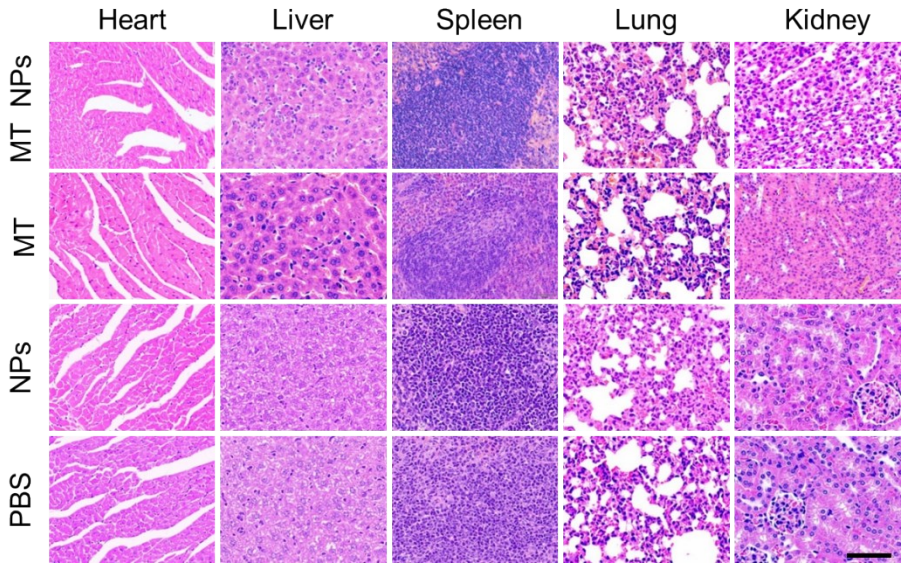


Figure S9. Macroscopic images of H&E stain of major organs excised on day 7. The scale bar corresponds to 100 μm.

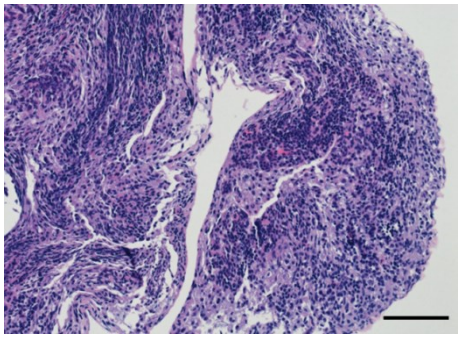


Figure S10. Macroscopic images of H&E stain of lymph node of 4T1 tumor metastasis. The scale bar corresponds to 100 μm.