Electronic Supplementary Information for

$Fe_3O_4/MnCO_3$ microbubbles for efficient elimination of bacterial biofilms by mechanical/sonodynamic effects under ultrasound irradiation and magnetic field targeting

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Characterization

The morphology of the nanomaterials was characterized by using transmission electron microscopy (TEM, HT-7700, 120 kV, Hitachi) and high-resolution transmission electron microscopy (HRTEM, Talos-F200X, FEI). The structure and composition of the nanomaterials were characterized through scanning transmission electron microscopy (STEM, Talos F200X, FEI), X-ray photoelectron spectroscopy (XPS, AXIS Supra, Al Kα 1486.6 eV, Kratos), and powder X-ray diffraction (XRD, D8 Advance A25, Bruker). The morphology characterization of the microbubbles was performed on an inverted fluorescence microscope (IX71, Olympus) and a scanning electron microscope (SEM, S-4800, Hitachi). Ultraviolet-visible near-infrared (UV-vis-NIR) absorption spectra were recorded on a spectrophotometer (UV-3600, Shimadzu). An electron paramagnetic resonance spectrometer (EPR, EMX-10/12, Bruker) was used to evaluate the reactive oxygen species (ROS) generation of the sonosensitizers. The fluorescence images of methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms were recorded on a confocal laser scanning microscope (CLSM, FV1000, Olympus).

Cytotoxicity of FMMB

The cytotoxicity of FMMB was evaluated by CCK-8 assay. Mouse embryonic fibroblasts (3T3) were seeded in 96-well plates at 10^4 cells/well and incubated for 24 h. After the medium was removed, FMMB in DMEM medium (serum-free) at different concentrations (Fe_3O_4: 0, 0.5, 1.0, 2.0 mg/mL) were added to each well. After 24 h incubation, CCK-8 reagent was added. The absorbance at 450 nm was measured after incubation for 3 h. Cell viability was calculated according to the following formula: Cell Viability = $(A_{sample} - A_{blank\ control}) / (A_{negative\ control} - A_{blank\ control}) \times 100\%$.

Hemolysis of FMMB

Mouse red blood cell (RBC) saline dispersions were mixed with FMMB dispersions (Fe_3O_4: 0, 0.05, 0.1, 1.0, 2.0 mg/mL) and oscillated in a shaker at 37°C (100 rpm, 3 h). Triton solution (0.2%) and saline were used as the positive control and negative control, respectively. Then, the supernatant was collected after centrifugation, and the absorbance at 540 nm was measured. The hemolysis rate was calculated according to the following formula: Hemolysis (%) = $(A_{sample} - A_{negative\ control}) / (A_{positive\ control} - A_{negative\ control}) \times 100\%$.

MRSA biofilm Culture

A single colony of MRSA was suspended in an LB medium and incubated for 12 h (37°C, 220 rpm). MRSA suspension was centrifuged and washed three times and resuspended in LB medium supplemented with 1% glucose. The UV-vis-NIR absorption spectrum of MRSA suspension was measured. When the absorbance at 600 nm was 0.1, the concentration of MRSA was 10^7 CFU/mL. MRSA biofilms were cultured in 96-well plates in a 37°C incubator for 48 h. MRSA biofilms were also cultured in laser confocal dishes with a similar procedure for fluorescence imaging.

Fluorescence imaging of MRSA biofilms

MRSA biofilms were grown in confocal dishes before treatment. MF is provided by an annular permanent magnet with an outer diameter of 80 mm and an inner diameter of 40 mm, 32 mT. The ultrasound treatment was set as 1.0 W/cm^2, 1 MHz, 50% duty cycle, and the action time was 5 min. After treatment, the confocal dish was incubated for 30 minutes. Calcein-AM solution (4 μM) was added to the treated confocal dish and incubated at 37°C for 30 min. After the incubation, the staining solution was removed and washed three times with phosphate-buffered saline (PBS). The fluorescence image was captured by CLSM.

Supplementary Figures

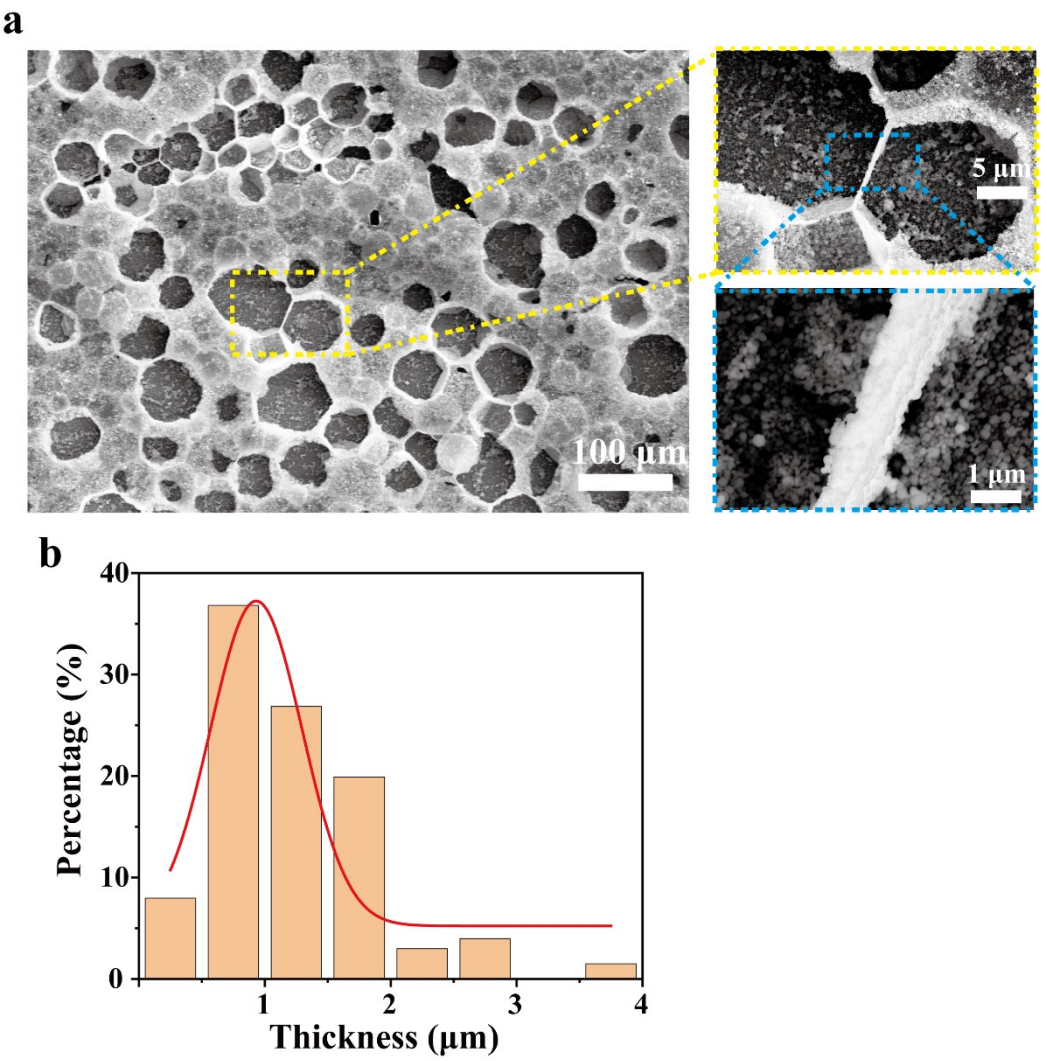

Figure S1 (a) SEM images of FMMB at different magnifications. (b) Statistical histogram of the shell thickness of FMMB.

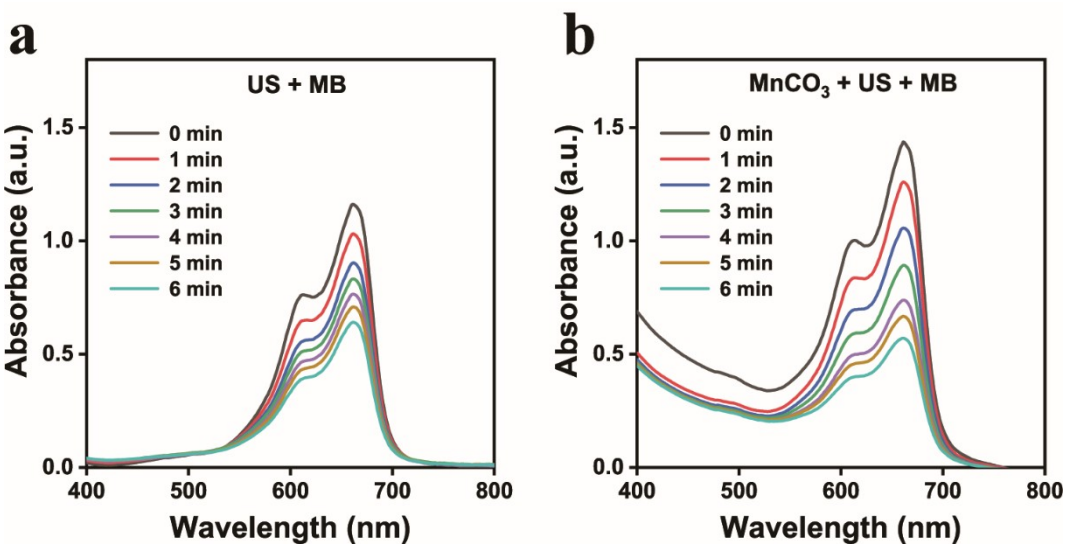

Figure S2 UV-vis-NIR absorption spectra of the MB solutions mixed with (a) H_2O and (b) $MnCO_3$ NPs at different time points of US treatment.

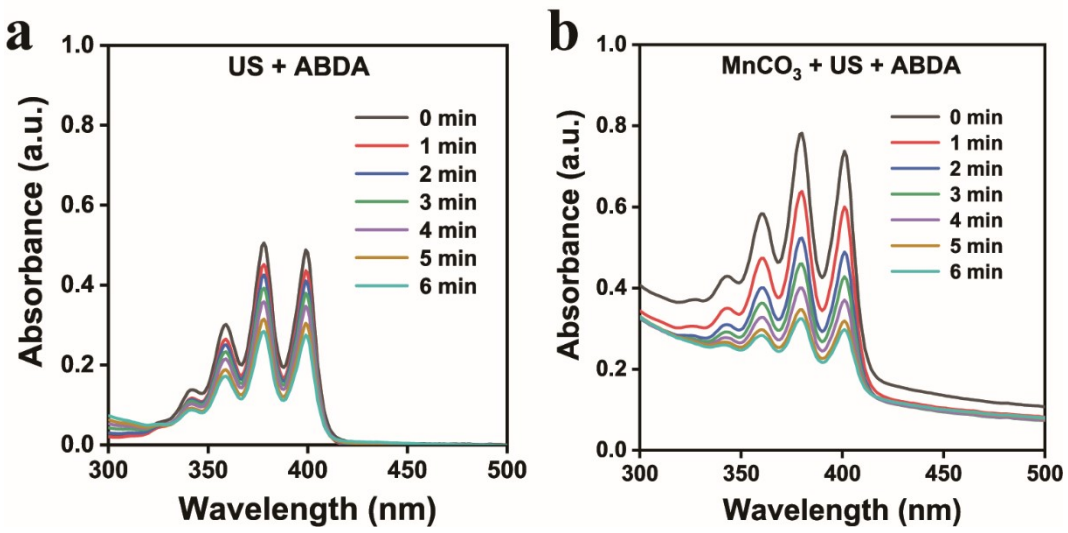

Figure S3 UV-vis-NIR absorption spectra of the ABDA solutions with (a) H_2O and (b) $MnCO_3$ NPs at different time points of US treatment.

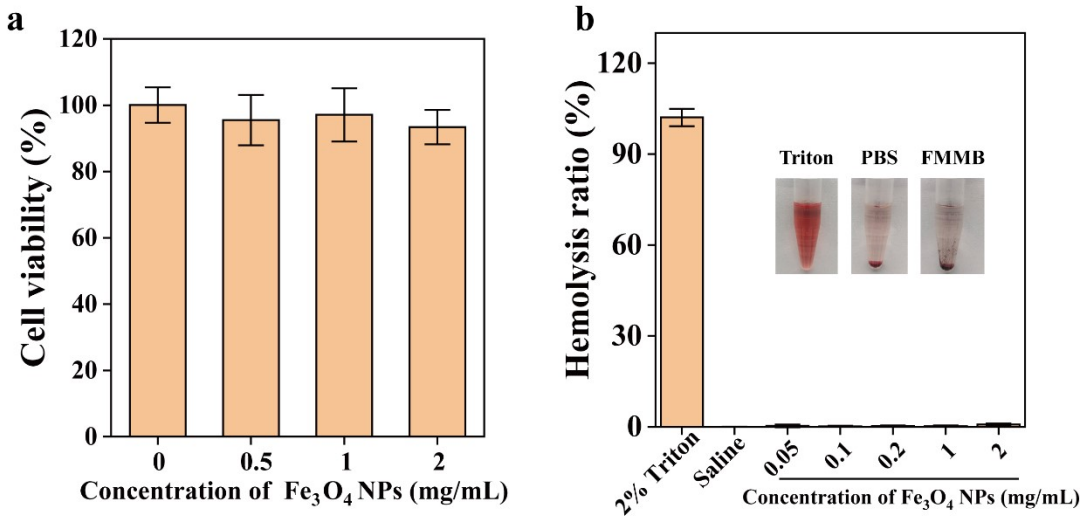

Figure S4 Cytotoxicity and hemolysis of FMMB. (a) Viability of 3T3 cells after co-incubation with different concentrations of FMMB. (b) Hemolysis of mouse RBC after incubation with FMMB. Inset: Photographs of RBC after incubation with Triton (0.2%), PBS, FMMB (Fe_3O_4: 2 mg/mL).

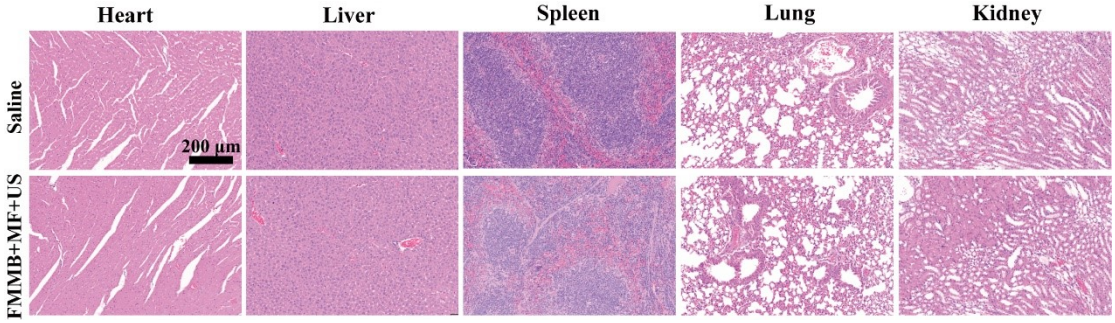

Figure S5 H&E staining images of the major organ tissue sections from the FMMB-administered mice and healthy mice. (Fe_3O_4: 1.00 mg/mL; $MnCO_3$: 0.13 mg/mL).