Electronic Supplementary Information

Amylase degradation enhanced NIR photothermal therapy and fluorescence imaging of bacterial biofilm infections

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Materials

Manganese chloride ($MnCl_2·4H_2O$), H_2O_2 (30%), anhydrous glucose, sodium chloride, tetramethylammonium hydroxide, and crystal violet were purchased from Sinopharm Chemical Reagent Co., Ltd. BeyoPure™ LB broth, BeyoPure™ LB broth with agar, and calcein acetoxymethyl ester (Calcein-AM) were provided by Beyotime Co., Ltd. Bovine serum albumin (BSA), indocyanine green (ICG), and α-amylase were bought from Sigma-Aldrich. Tissue fixation solution was provided by Wuhan Google Biotechnology Co., Ltd. Sulfhydryl modified polyethylene glycol (PEG) was ordered from Xi'an Ruixi Biotechnology Co., Ltd. Completed DMEM medium, HeLa cells, and PBS solutions were purchased from Nanjing KeyGEN Biological Co., Ltd.

Characterization

The morphology and thickness of MnO_2 NSs, MA NSs, MAP NSs, and MAPI NSs were characterized by transmission electron microscopy (TEM, HT7700, Hitachi, Japan) and atomic force microscopy (AFM, Dimension Icon, Bruker, USA). The material composition and structure of nanomaterials were investigated by X-ray photoelectron spectroscopy (XPS, Axis Supra, Shimadzu, Japan) and Fourier transform infrared spectrometer (FTIR, Spectrum Two, PerkinElmer, USA). The optical properties and physical properties of materials were characterized by using UV-vis-NIR spectroscopy (UV3600, Shimadzu, Japan) and dynamic light scattering (DLS, Zeta-PALS, Brookhaven, USA). The concentration of MnO_2 was analyzed by inductively coupled plasma mass spectrometer (ICP-MS, NexION 2000, PerkinElmer). Fluorescence images of MRSA biofilms were recorded on a confocal laser scanning microscope (FV1000, OLYMPUS, Japan). Fluorescent imaging of mice were performed on a small animal live imaging system (IVIS LUMINAK, PerkinElmer, USA). The photothermal performance of NIR was evaluated with an NIR imaging camera (Fortic225, IRS, China) under 785 nm laser (BWT Beijing, China) irradiation at 0.8 W/cm^2. The morphology of bacteria was observed by scanning electron microscopy (SEM, S4800, Hitachi, Japan).

Preparation of MnO_2 nanosheets

Briefly, 0.6 g of $MnCl_2·4H_2O$ (3 mM) was added to 10 mL of H_2O and mixed

thoroughly in a flask. Then, 2 mL H_2O_2 (30%) was added to 12 mL of tetramethylammonium hydroxide solution (12 mM) and diluted to 20 mL with water. The above two solutions were mixed and stirred for 12 h, the solution was centrifuged at 3000 rpm for 10 min to remove the supernatant. The precipitate was washed with ethanol and water for three times. After that, the precipitate was resuspended in 30 mL of ultrapure water. Then, 10 mL of the resuspended solution was treated with probe ultrasonication for 10 h. After centrifugation at 12000 rpm for 45 min, the supernatant was further centrifuged at 18000 rpm for 1.5 h to harvest MnO_2 NSs. Finally, the MnO_2 NSs were dispersed in water and stored at 4°C before use.

Cytotoxicity assay

HeLa cells (100 μL, 10^5 cells/mL) were seeded into 96-well plates. After cell adhesion, the culture medium was discarded and 100 μL of fresh DMEM medium containing different concentrations of MPA NSs and MPAI NSs was infused into each well. After incubation for 24 h, 20 μL of MTT solution (5 mg/mL) was added into each well and incubated for 4 h under darkness. Then, the supernatant was removed, and 150 μL of dimethyl sulfoxide was added to dissolve the formazan product. The absorbance of each well at 490 nm was measured by using a microplate reader. The viability of HeLa cells was calculated by $C/C_0 \times 100\%$, where C and C_0 was the absorbance value of test sample and blank control, respectively.

Biosafety evaluation

Nine MRSA-infected mice were randomly divided into 3 groups, and injected with 200 μL of saline, MAPI NSs solution, and MBPI NSs solution, respectively. After eight days post-treatment, the mice were sacrificed to harvest the major organs (heart, liver, spleen, lungs, kidneys) for pathological analysis.

Supporting Figures

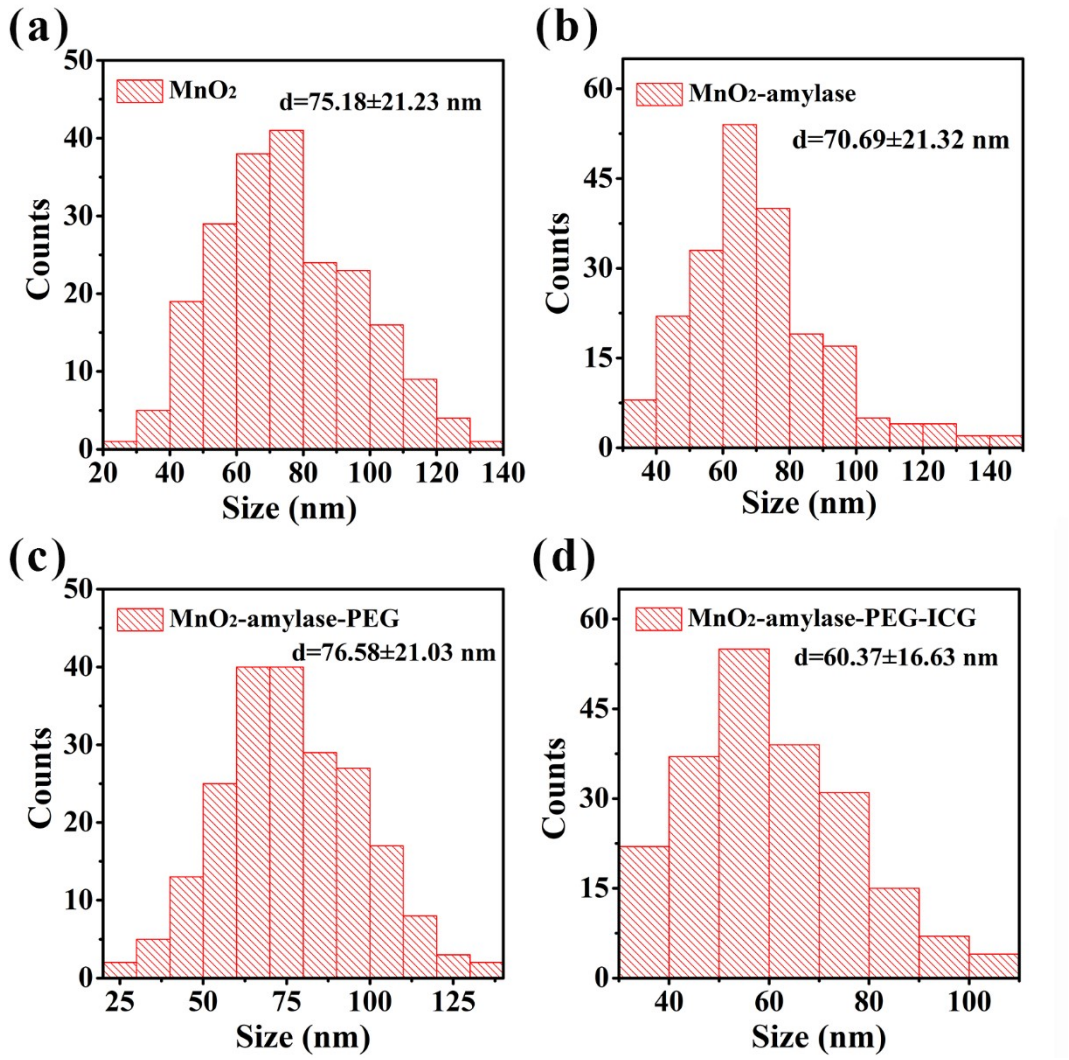

Figure S1. Size histograms of (a) MnO_2 NSs, (b) MA NSs, (c) MAP NSs, and (d) MAPI NSs based on more than 100 nanosheets in TEM images.

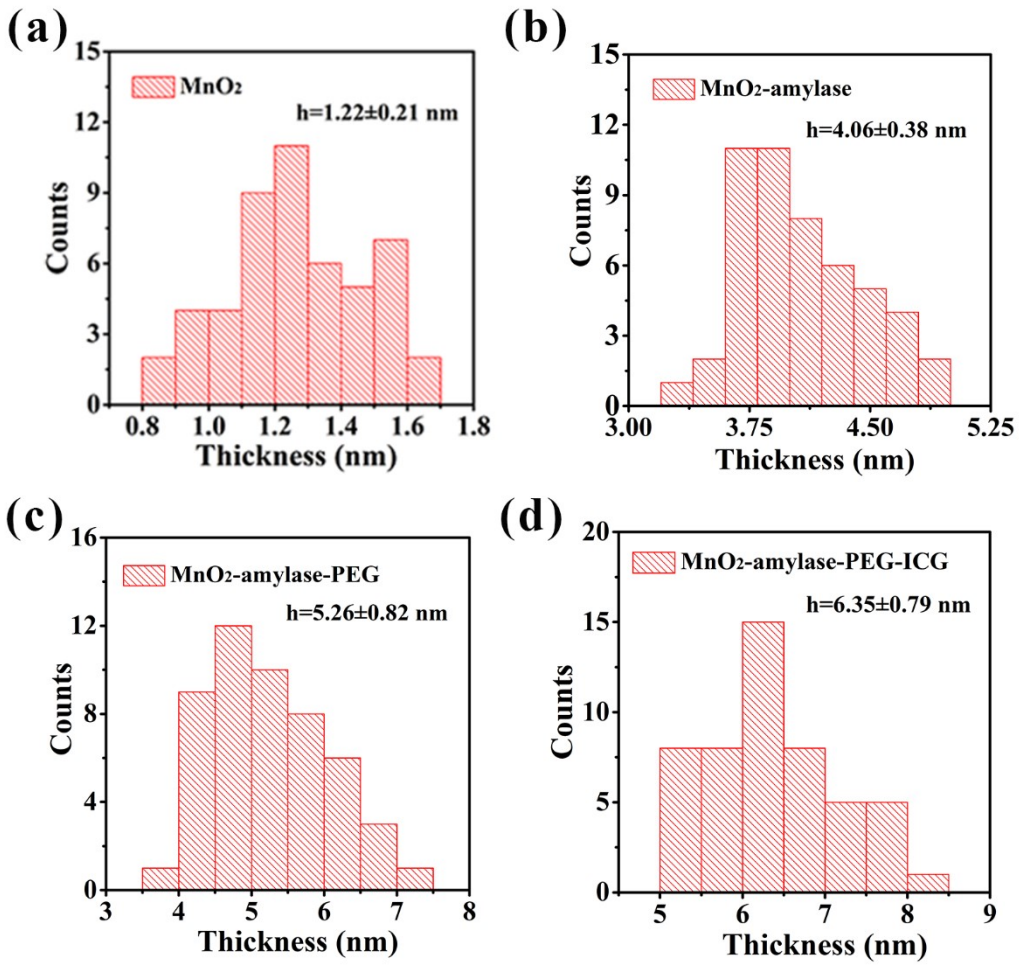

Figure S2. Thickness histograms of (a) MnO_2 NSs, (b) MA NSs, (c) MAP NSs, and (d) MAPI NSs based on more than 50 nanosheets in AFM images.

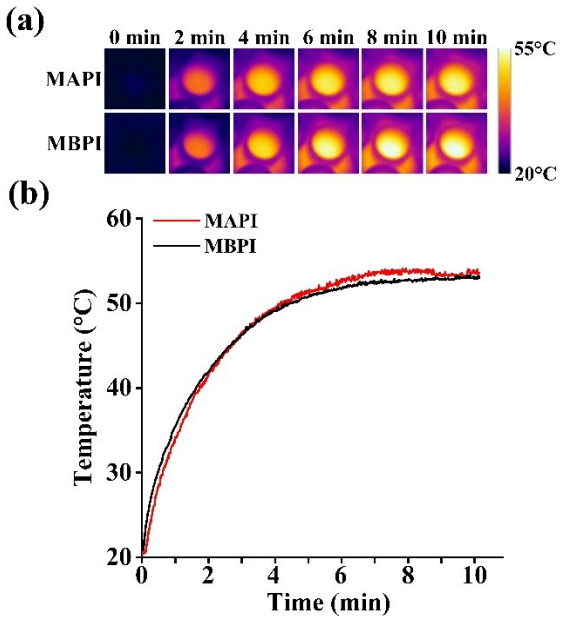

Figure S3. (a) Infrared thermal images of MRSA biofilms treated by MAPI NSs and MBPI NSs (ICG: 25 μg/mL) under 785 nm laser irradiation (0.8 W/cm^2). (b) Photothermal heating curves of MRSA biofilms treated by MAPI NSs and MBPI NSs during laser irradiation.

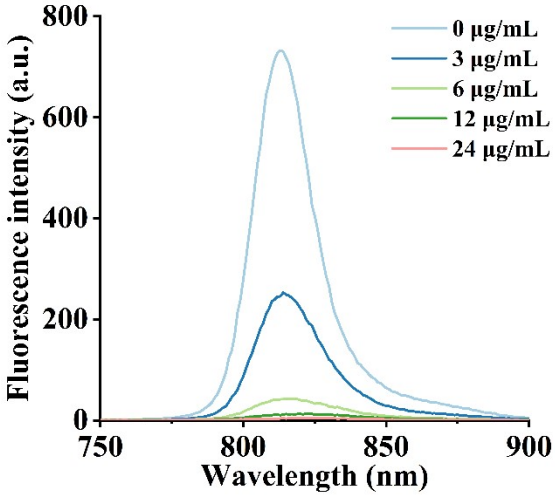

Figure S4. The fluorescence spectra of ICG aqueous solutions (10 μg/mL) after added MAP NSs (MnO_2: 0, 3, 6, 12, 24 μg/mL).

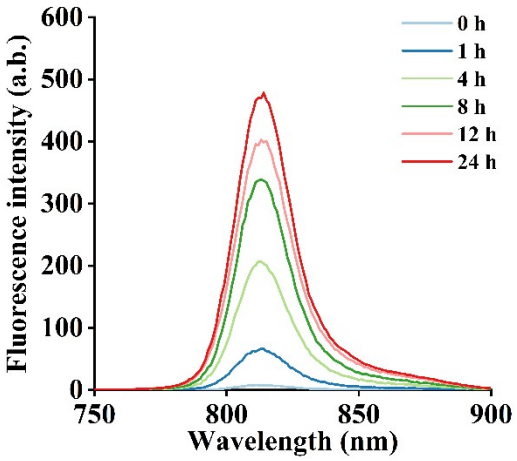

Figure S5. The fluorescence spectra of MAPI dispersion (ICG: 10 μg/mL) incubated with PBS (pH 5.5) for different times.

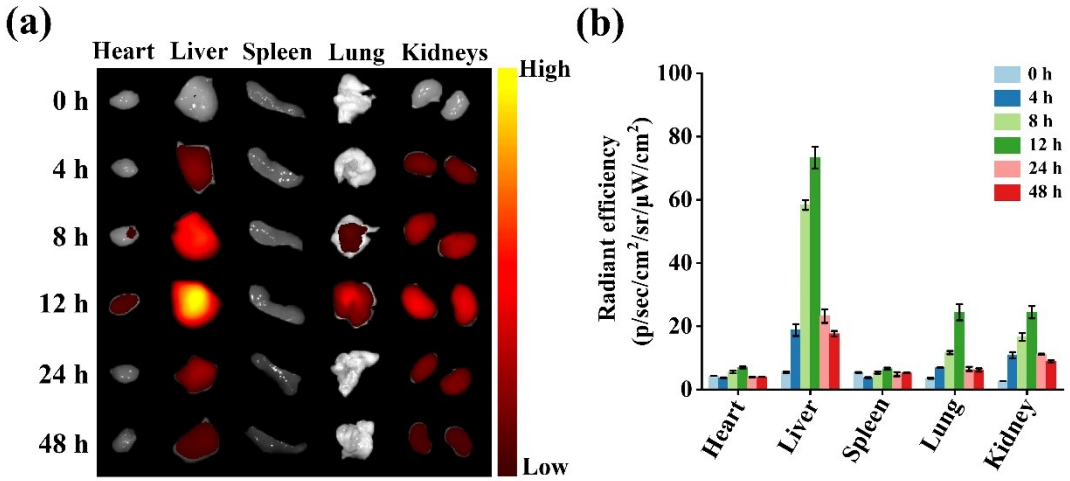

Figure S6. (a) The fluorescence images and (b) corresponding fluorescence intensity of major organs (heat, liver, spleen, lung, and kidneys) collected from mice after i.v. injected with MAPI NPs for different times (n = 3).

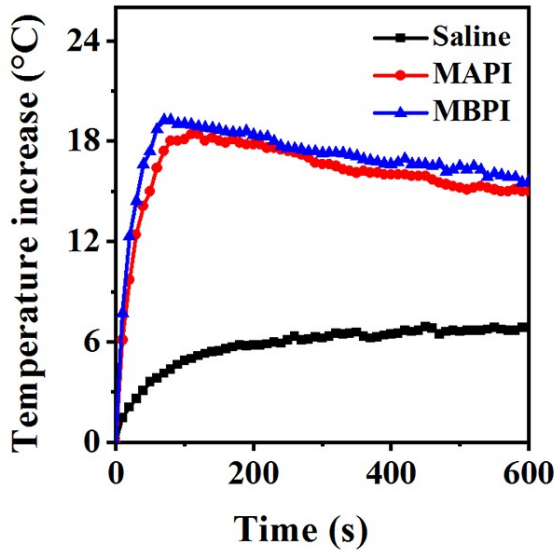

Figure S7. Temperature increase profiles of infected tissues after intravenous injection of MAPI and MBPI NSs with NIR laser irradiation at 0.6 W/cm^2.

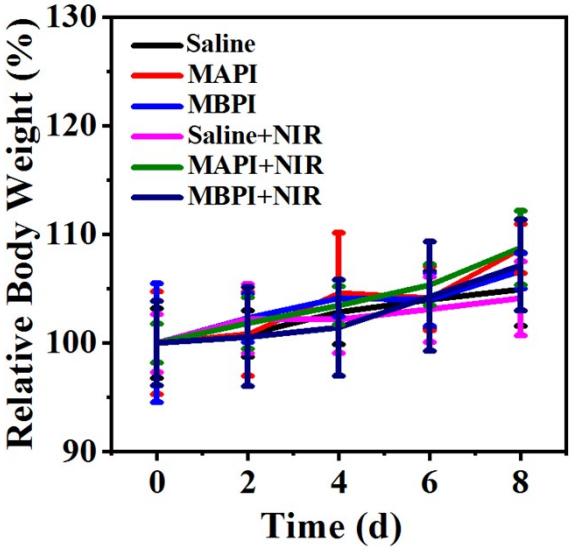

Figure S8. Relative body weight curves of the mice after different treatments at various time points.

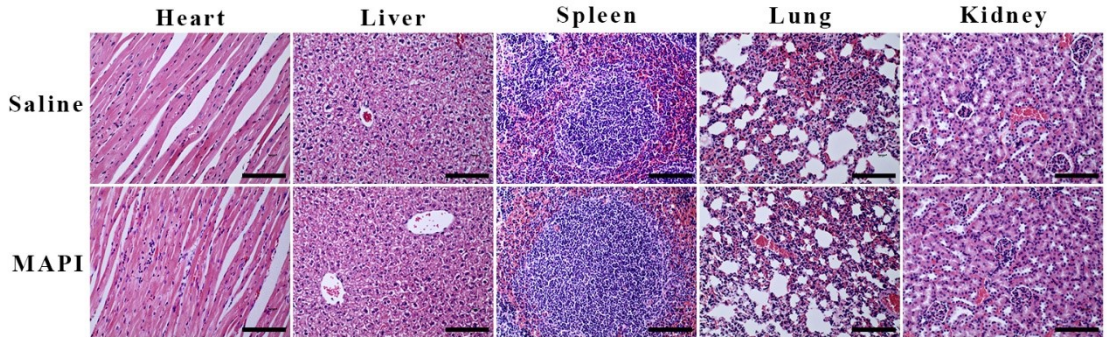

Figure S9. H&E staining images of the main organs of mice after intravenous injection of MAPI NSs for 14 days. The scale bar is 100 μm.