Supporting Information

Trap & Kill: A Neutrophil-Extracellular-Trap Mimic Nanoparticle for Anti-bacterial Therapy

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1. Materials

Dodecylthiocarbonylthio-2-methylpropanoic acid (IBADTC) and (2R,3S,4S,5S,6R)-2-(2-bromoethoxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (A_2-ManBr) were synthesized according to the related Refs [1]. 2,2-azobisisobutyronitrile (AIBN) was recrystallized twice in ethanol before used. All other chemical reagents were of analytical grade and used without further purification.

2. Preparation of NET-mimic nanoparticles

2.1 P4VP mcroRAFT agent.

IBADTC (0.08 mmol, 29.2 mg), AIBN (0.02 mmol, 3.28 mg) and poly(4-vinylpyridine) (P4VP, 40 mmol, 4.2 g) were dissolved in dry *i*-PrOH (30 mL). The reaction mixture was degassed for 15 min and refilled with N_2 and heated to 80 °C under N_2 protection for 2 h. After cooling to room temperature, the reaction mixture was poured into acetone and the precipitate was collected and washed with acetone thoroughly.

2.2 P4VP-b-PFMMA

P4VP mcroRAFT agent (~ 0.1 mmol 4VP, 877.6 mg), AIBN (0.025 mmol, 4.1 mg) and ferrocenylmethyl methacrylate (FMMA, 60 mmol, 405.2 mg) were dissolved in DMSO (3 ml). The reaction mixture degassed for 15 min and refilled with N_2. After heating to 80 °C for 12 h, the reaction mixture was dialyzed against water/methanol mixture (water:methanol = 8:2) for two days. P4VP-*b*-PFMMA was obtained by freeze-drying as brown powder.

2.3 QP4VP-b-PFMMA

P4VP-b-PFMMA (~ 0.05 mmol 4VP, 708.8 mg) and A_2-ManBr (0.15 mmol, 43.1 mg) were dissolved in 3 mL of DMSO, and this mixture was heated to 80 °C for three days. The reaction mixture was dialyzed against water for two days. QP4VP-*b*-PFMMA was obtained by freeze-drying.

2.4 NET-mimic nanoparticles

QP4VP-*b*-PFMMA dissolved in DMSO and dispersed in phosphate-buffered saline. After which, the nanoparticles were collected by centrifugation.

3. NET mimic nanoparticle characterized by Transmission electron microscope (TEM)

The NET-mimic nanoparticles in water (0.5 mg mL^{-1}) were carefully dropped onto the carbon supported copper grid and dried in air. The samples were stained by phosphotungstic acid before TEM observation (TECNAI G2 20 LaB6).

4. NET mimic nanoparticle characterized by Scanning transmission electron microscopy (SEM)

The NET-mimic nanoparticles in water were carefully dropped onto the onto a polished silicon wafer. After drying the droplet at room temperature overnight, the sample was coated with gold and then imaged by SEM (Hitachi Regulus 8100).

5. Iron concentration test

The Fe concentration in the aqueous solution of Q4PVP-b-PFMMA were measured via the phenanthroline method[2] on a microplate reader (SpectraMax M3). A calibration curve was established under standard protocol with phenanthroline spectrophotometric method (Figure S1).

6. Dnamic light scattering (DLS) measurement

The particle size of 0.5 mg/mL Q4PVP-b-PFMMA was measured by dynamic light scattering (ZS 90, Malvern).

7. Fenton reaction

With the presence of 3.2 mM (final concentration) TMB, QP4VP-*b*-PFMMA at various final concentrations (0.1, 0.2, 0.5, 1, 2, 3 mg mL^{-1}) were treated with H_2O_2 at final concentration of 50 mM. The absorbance of the mixture at 650 nm was monitored every 3

s for 30 min.

8. Biocompatibility

8.1 Cytotoxicity assay

MTT assay was used to evaluate the cytotoxicity of NET-mimic nanoparticles. Human umbilical vein endothelial cells (HUVEC) were used for cytotoxicity evaluation. HUVEC cells were cultured in 1640 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin. They were seeded into a 96-well plate (~5000 cells per well) and incubated overnight. The culture medium was replaced with fresh medium (100 μL) containing various concentrations (0.5, 0.1, 0.05, 0.04, 0.02, 0.01, 0.005 mg mL^{-1}) of NET-mimic nanoparticles and incubated for another 48 h. After which, MTT (5 mg mL^{-1}, 10 μL) was added to each well and incubated for another 4 h. Finally, the culture medium was removed carefully and DMSO was added (100 μL) to dissolve the formazan crystals. The absorbance at 570 nm was recorded with a microplate reader (SpectraMax M3). The cell viability was calculated according to the following equation: Cell viability = A*e*/A*c*, where the A*e* stands for the absorbance of groups with different treatments and *Ac* was absorbance of the control group.

8.2 Hemolysis assay

Centrifuge tubes used for blood collection were rinsed with anticoagulant before use. Fresh blood (2 mL) was collected from C57 mice. Red blood cells (RBCs) were collected by centrifugation (1200 rpm, 15 min) and washed with PBS (pH=7.4) until the supernatant was clear. Next, the obtained RBCs were added into 2 mL of PBS (pH=7.4) to prepare the stock dispersion. 100μL of the NET-mimic nanoparticles at different concentrations (0.0625, 0.125, 0.25, 0.5 mg mL^{-1}) were gently mixed with 100μL of the RBC stock dispersion. After incubated at 37 °C for 1 h, the mixed dispersions were centrifuged (1200 rpm, 15 min), and the supernatant absorbance at 540 nm was measured via microplate reader (SpectraMax M3). Deionized water was used as positive control and PBS (pH=6.5) was used as negative control. The hemolysis ratio was calculated according to the following equation: (A*e*–A*n*)/(A*p*–A*n*) × 100%, where A*e*, A*n* and A*p* refer to the

absorbance value of the NET-mimic nanoparticles, PBS and water treatment groups, respectively.

9. *In Vitro* antibacterial experiment

9.1 Bacteria agglutination observation by CLSM

FITC labeled NET-mimic nanoparticles (FITC-polymer) were used for bacteria binding activity test. FITC-polymer (1 mg/mL, 10 μL) was added to 100 μL *S. aureus* (OD_{600}=0.8) and cultured for 0.5 h, 1 h, 2 h, 3 h and 6 h in the dark. The bacteria solution was centrifuged (13000 rpm, 5 min) and the supernatant was removed. Then the precipitate was washed with PBS (pH=7.4) 2-3 times and redispersed in 100μL PBS (pH=7.4). Finally, 10μL bacteria dispersion was dropped on microscope slides and then observed by a Nikon A1R laser scanning confocal microscope (Nikon, Japan).

9.2 *Bacteria agglutination observation by microscope*

Add mannose solution (1 mg/mL) to 100μL *S. aureus* (OD600=0.8) and incubate for 3 hours. Then, Q4VP-b-PFMMA (1 mg/mL) was added to the acterial fluid and cultured for 0.5 h, 1 h. Finally, observe the bacteria under a microscope.

9.3 Bacteria agglutination observation by TEM and SEM

S. aureus bacteria (OD_{600} = 0.1, 7.5 mL) incubated with NET-mimic nanoparticles (0,0.25 mg mL^{-1}, 7.5 mL) for 1 h. Then the bacteria were collected by centrifugation and washed with PBS (pH=7.4) thrice. The bacteria acquired were fixed with glutaraldehyde solution (2 mL, 2.5%) at 4 °C overnight. The fixative solution was poured out and the sample was rinsed with PBS buffer (pH 7.4) thrice, 15 min for each time. Then the samples were treated with 1% osmium solution for 1-2 h. The osmium solution residue of was carefully removed, and the samples were rinsed with PBS buffer (pH 7.4) thrice, 15min for each time. Next, the samples were dehydrated with ethanol solution of gradient concentration (30%, 50%, 70%, 80%, 90% and 95%), 15 min for each concentration, and followed by 100% ethanol for 20 min. After ethanol treatment, the samples were treated with acetone for 20 min. Next, the samples were treated with a mixture of embedding agent

and acetone (V/V=1/1) for 1h followed by a mixture of embedding agent and acetone (V/V=3/1) for 3h. Then, the samples were stored in pure embedding agent at room temperature overnight. Finally, the embedded samples were cut into 70-90 nm thin slices using a Leica EM UC7 ultra-thin slicer (Leica, Germany). The slices were stained with lead citrate solution, followed by uranyl acetate solution. The stained slices were observed by a Hitachi H-7650 TEM (Hitachi, Japan).

For SEM observation, after dehydrated by ethanol, the samples were treated with a mixture of ethanol and isoamyl acetate (V/V=1/1) for 30min and pure isoamyl acetate for 1 h or left overnight. The samples were observed by a Hitachi SU8010 SEM (Hitachi, Japan).

9.4 Antibacterial activity

9.4.1 Antibacterial activity against S. aureus, MRSA and E.coli

Staphylococcus aureus (*S. auresu*, ATCC25923), Methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC43300) and *Escherichia coli* (*E.coli*, ATCC23716) were employed for antibacterial activity test. The antibacterial efficiency was evaluated using the plate count method. Bacteria were cultured in Luria-Bertani (LB) liquid medium at shaker (37 ℃, 260 rpm) overnight, and the absorbance at OD_{600} was measured. After, the bacterial solution was diluted with PBS (pH 6.5 or pH 7.4) to OD_{600} = 0.2, 100 μL of the bacterial solution was incubated with 100 μL NET-mimic nanoparticles at different concentrations (1, 0.5, 0.25, and 0.125, 0 mg mL^{-1}) for 1 h (final absorbance is OD_{600} = 0.1 and concentrations are 0.5/0.25/0.125/0.0625/0 mg mL^{-1}). Finally, 100μL of the treated bacteria suspension were diluted 1000 times, and 100 μL were used to spread on a solid medium and cultured at 37 °C for 12 h.

Minimum inhibitory concentrations (MICs) were assayed by plate colony counting. The MICs were defined as the minimum concentration inhibiting over 90% of bacterial growth.

9.4.2 Propidium iodide staining of bacteria agglutination

S. aureus bacteria in 100 μL bacterial solution (OD_{600}= 0.8) were collected by centrifugation. 100 μL NET-mimic nanoparticles at different concentrations (0.5, 0.25, and 0 mg mL^{-1}) of were added and incubated in 37 °C incubator for 2h. Then 0.2 μL PI (500

×，Calcein/PI Cell Viability/Cytotoxicity Assay Kit, Beyotime） were added and incubated for 45 min in the dark. Finally, 10 μL samples of each group were placed on microscope slides observed by a laser scanning fluorescence microscope (Nikon, Japan).

9.5 ROS detection

S. aureus bacteria in 100 μL bacterial solution (OD_{600}= 0.8) were collected by centrifugation. 100μL NET-mimic nanoparticles at different concentrations (0.5, 0.25, and 0 mg mL^{-1}) of were added and incubated in 37 °C incubator for 2h. Then 0.2 μL DCFH-DA (10 mM) were added and incubated for 30 min in the dark. Finally, 10 μL samples of each group were placed on microscope slides observed by a laser scanning fluorescence microscope (Nikon, Japan).

For quantitative analysis, bacteria in each group were collected by centrifugation and washed with PBS (pH=7.4) washed 2-3 times. Then bacteria were dispersed in PBS (100μL) and placed in 96-well plate (n = 3). The fluorescence intensity at 525 nm was measured by a microplate reader (SpectraMax M3). The quantitative results were calculated according to the following formula: A*e*/A*c*, where A*e* referred to the fluorescence intensity value of the groups with different treatments, and A*c* was the value of control groups.

10. *In vivo* antibacterial activity and wound healing

All animal studies were performed in accordance with the Guide for Care and Use of Laboratory Animals. All animal experiments and care were approved by the Institutional Animal Care and Use Committee at the Nanjing University of Science and Technology (ACUC-NUST-20210016). Specifically, 24 male Balb/c mice (22±2g, GemPharmatech Co., Ltd) were housed in a temperature- and humidity-controlled room under a 12 h light-12 h dark cycle. All mice were treated with isoflurane for general anesthesia. The hair on the back of each mouse was removed with an electric shaver and the skin was disinfected with 75% alcohol. The wound was made by cutting off a round skin of 1 cm in diameter on the

back, followed by inoculation of 50 μL MRSA (OD_{600} = 0.1). After 24 h (day 0), each mouse formed an infected wound and then randomly divided mice into four groups. Mice were treated with 50 μL PBS, 0.0625 mg/mL QP4VP-*b*-PFMMA in PBS, 0.125 mg/mL QP4VP-*b*-PFMMA in PBS and 0.5 mg/mL QP4VP-*b*-PFMMA in PBS 24 h after the wound creation. This treatment was conducted every day for 3 days. The wound area was monitored and photographed on day 0, 1, 2, 3, 7 ,8 and 9 after the initial treatment. Bodyweight was recorded every day for 9 days. Hematoxylin and eosin (H&E) staining was employed on day 9. The areas of the infected site were calculated by Image J software. The formula for calculating the wound healing rate is as follows: Wound healing rate = $(S_0-S_n)/S_0 \times 100\%$, where S_0 represents the wound area of day 0 and S_n represents the area of day n.

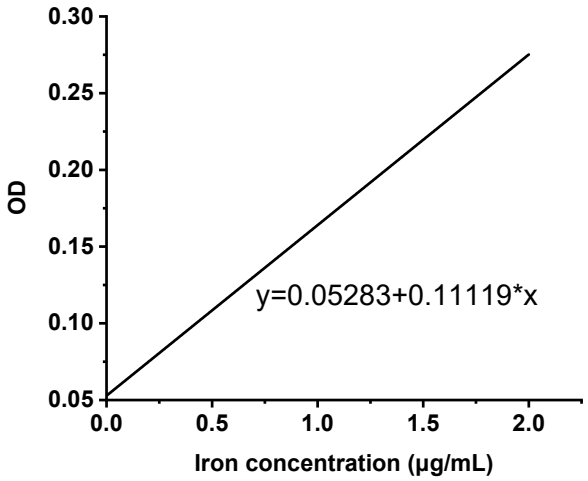

Figure S1. Iron standard curve

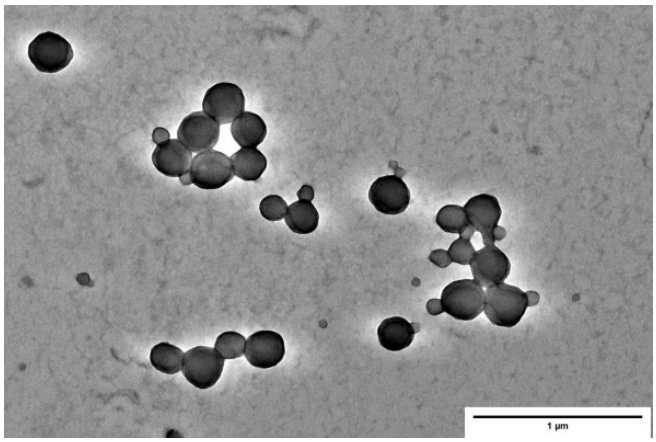

Figure S2.TEM image of NET-mimic nanoparticle. Scale bar: 1 µm.

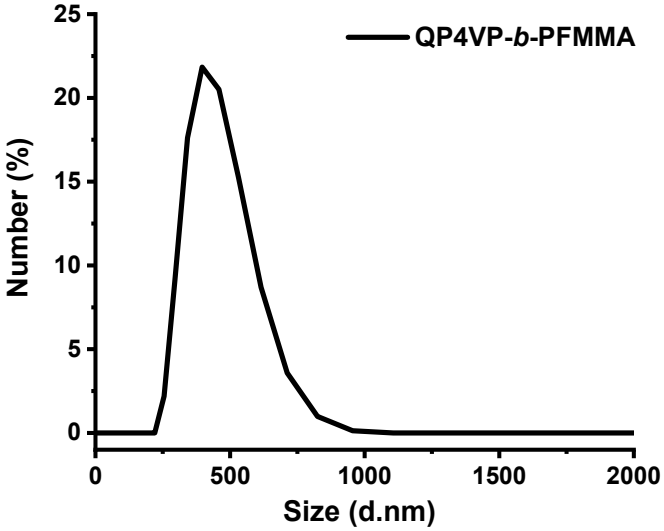

Figure S3. Hydrodynamic size distributions of QP4VP-*b*-PFMMA

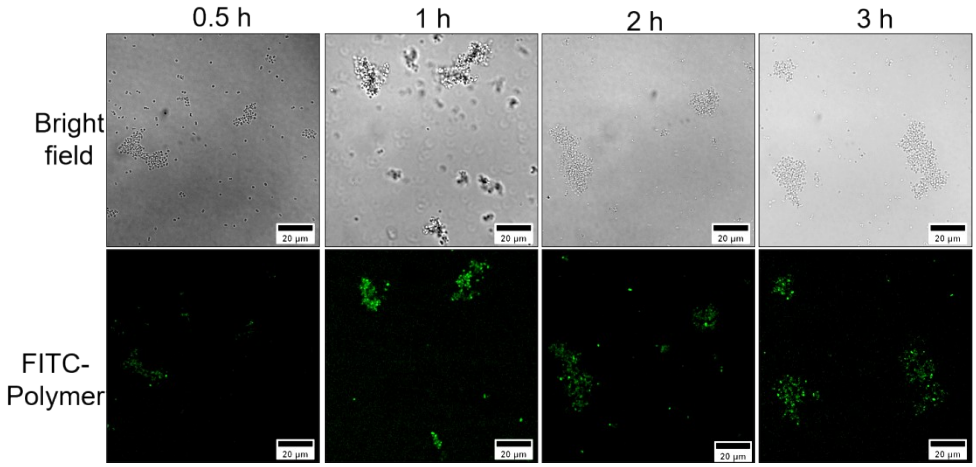

Figure S4. Fluorescent images of *S.aureus* after treated with FITC-Polymer at predetermined time. Scale bar: 20 μm.

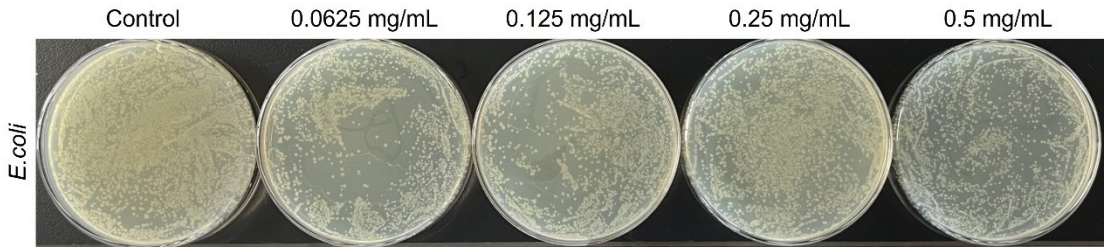

Figure S5. Photos of bacterial colonies on agar plates after the treatment of QP4VP-*b*-PFMMA nanoparticles at various concentrations. *E.coli.*

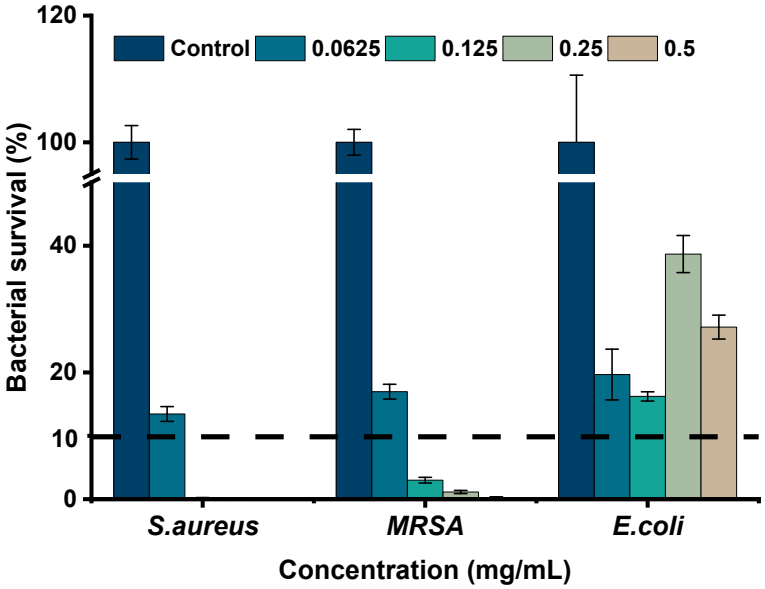

Figure S6. Statistical charts of bacterial survival rates for (A)*S.aureus*, (B)MRSA, and (C)*E. coli* after the treatment of QP4VP-*b*-PFMMA nanoparticles at various concentrations.

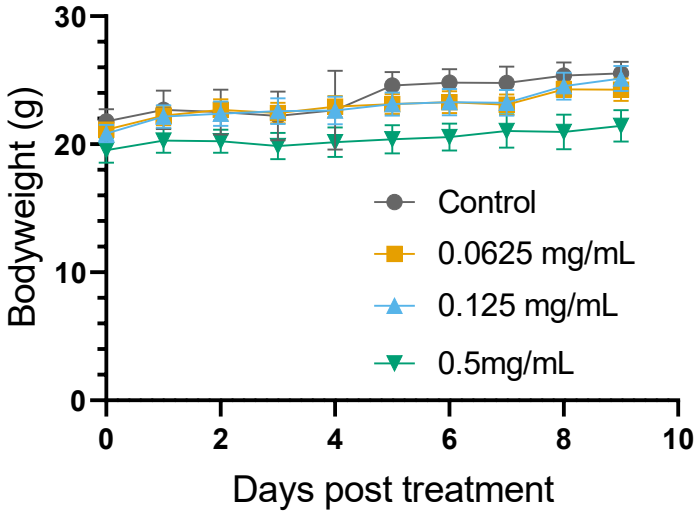

Figure S7. The body weight of infected mice during various treatments.

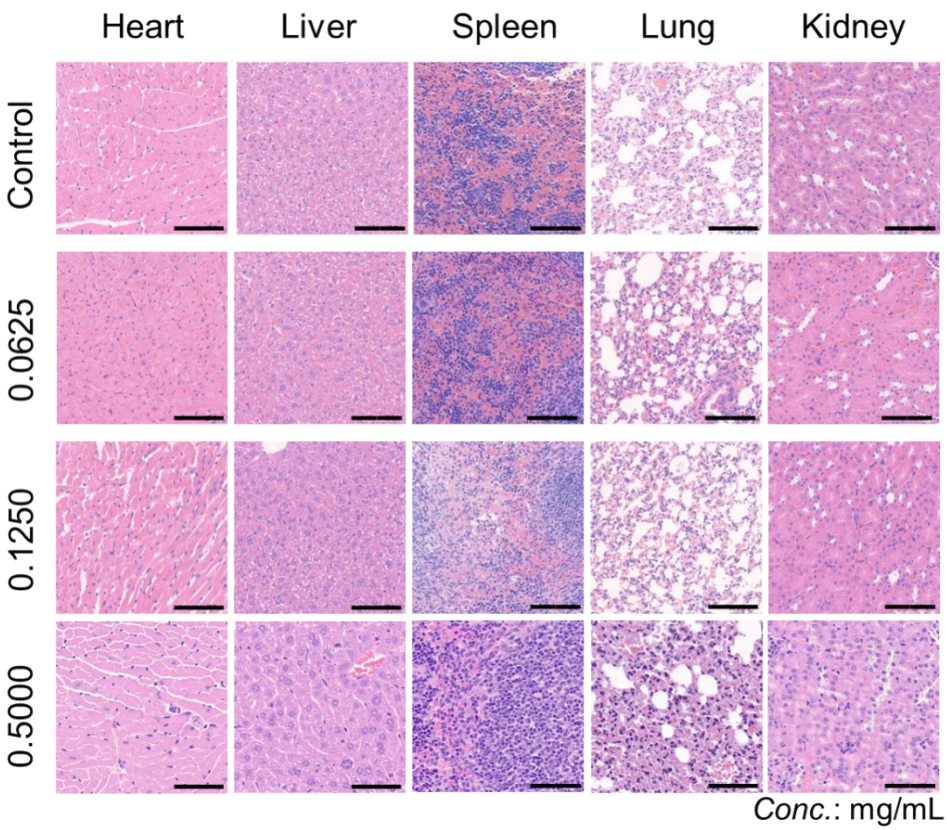

Figure S8. H&E analysis of main organs at day 9 post treatment. Scale bar: 100 μm.

Reference

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