Supporting Information

Phenylboronic Acid-Functionalized Silver Nanoparticles for Highly Efficient and Selective Bacterial Killing

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Experimental section

Materials

$AgNO_3$ (98%, Macklin), $NaBH_4$ (98%, Macklin), cysteamine (Cys 97%, Macklin), 2-(bromomethy)phenylboronic acid (98%, J&K) were used as received. Live/Dead fluorescence probes (SYTO-9 and PI (propidium iodide)) were obtained from Molecular Probes (Shanghai, China). Mueller–Hinton (MH) broth and tryptic soy broth (TSB) was obtained from Qingdao Hope BioTechnology (Qingdao, China).

Fetal bovine serum (FBS), dulbecco's modified Eagle's medium (DMEM), and phosphate buffer saline (PBS) were purchased from Hyclone. *Escherichia coli* (*E. coli*, ATCC25922), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC27853), *Staphylococcus aureus* (*S. aureus,* ATCC25923), *Bacillus subtilis* (*B. subtilis*，ATCC6051) were obtained from ATCC (USA). All other reagents were obtained from Sinopharm Chemical Reagent Company (Shanghai, China).

Synthesis of silver nanoparticles

Cysteamine-capped silver nanoparticles (AgNPs) were synthesized by reduction of silver nitrate in the presence of cysteamine as capping agent, and DMF as reaction medium. In a typical preparation, 1 mL of an aqueous solution of cysteamine (1.4 mmol) and 1 mL of an aqueous solution of $AgNO_3$ (0.468 mmol) were sequentially added to 40 mL of DMF under stirring in an ice bath. Afterwards, 5 mL of an aqueous solution of $NaBH_4$ (421 mM) were added dropwise. The reaction mixture was stirred for 20 min. To remove the untreated chemicals, we sequentially dialyzed the solution with a dialysis bag (3500 Da *M*w cut-off, Solarbio) for 24 hours. The resulting nanoparticles were then lyophilized to obtain the AgNPs as a dark brown powder.

Surface functionalization of AgNPs by 2-(bromomethyl)phenylboronic acid (AgNPs-PBA$_n$).

The AgNPs (10 mg) were dispersed in 10 mL of methanol, then 2-(bromomethyl)phenylboronic acid dissolved in the methanol was added at various

weight ratios to result in AgNPs-PBA_n with different conjugated number of phenylboronic acid. The mixture was stirred for 24 h, then washed and centrifuged three times with methanol at 10 000 rpm for 10 min.

We characterized the morphologies of different nanoparticles by transmission electron microscopy (TEM, H-H7650). The dynamic light scattering (DLS) and Zeta potential analysis of the samples were measured using dynamic light scattering (DLS, 90Plus PALS). We used X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi), Fourier transform infrared spectroscopy (FT-IR, Thermo Nicolet 8700), and elemental analysis (Optima 7300 DV) to characterize the chemical element of the AgNPs composed of phenylboronic acid with different ratios, respectively.

Bacterial plate-killing assays. The bactericidal activities of different nanoparticles were evaluated by plate bacterial killing assays. *E. coli* and *P. aeruginosa* were used as representative Gram-negative bacterial strains, while *S. aureus* and *B. subtilis* were used as representative Gram-positive bacterial strains. For each bacterial strain, 3–5 individual colonies were inoculated into fresh TSB and incubated at 37 °C for 16–18 h to stationary phase. A 40 μL culture was diluted in 4 mL fresh TSB and regrown at 37 °C to mid-log phase (OD_{600} = 0.4–0.7). Bacterial cells were then washed once with PBS via centrifugation (10,000 g for 5 min at 4 °C), adjusted with PBS to ~1.5×10^6 CFU mL^{-1}. 100 μL nanoparticles were mixed with 50 μL adjusted bacteria suspension, and then the mixture was added into 96-well microplate. The final concentrations of nanoparticles are in the range of 0.25–128 μg/mL. The microplate was then incubated

at 37 °C for 3 h. Serial 10-fold dilutions were subsequently obtained by adding the mixture of nanoparticles and bacteria into PBS buffer. 20 μL dilution was then plated onto MH agar and incubating at 37 °C overnight to form visible colonies. Each trial was performed in triplicate. The reported minimum bactericidal concentration (MBC) values are defined as the minimum concentrations of nanoparticles that can kill 99.9% of bacteria.

To assess the effect of phenylboronic acid on antibacterial activity of AgNPs-PBA_n, we mixed the AgNPs-PBA_n with 10 μg mL^{-1} glucose to block phenylboronic acid. Then, the killing efficiency of the mixtures against Gram-negative *E. coli* were tested according to the methods mentioned above.

SEM characterizations on the bacterial morphology. *E. coli* strain was used as representative strains for Gram-negative bacteria, while *S. aureus* stain was used as representative Gram-positive bacteria. The bacterial density was adjusted with PBS to ~1.5×10^7 CFU mL^{-1}. Then the obtained bacterial suspensions were mixed with nanoparticles at concentration of 16 μg mL^{-1} and 32 μg mL^{-1} for *E. coli* and *S. aureus*, respectively. The resulting mixtures were then incubated at 37 °C for 3 h and subsequently subjected to centrifuge at 10,000 g for 5 min to remove the supernatant, followed by fixation with 2% glutaraldehyde at 4 °C for 12 h, and then dehydration successively with a series of graded ethanol solutions (30, 50, 70, 90, and 100%) via centrifuge (10,000 g for 5 min). The obtained bacteria were dripped on a silicon wafer and dried overnight, and then sputtered with gold for 40 s for imaging under a scanning electron microscope (SEM, GeminiSEM 500).

Bacterial Dead/Live viability assays. Bacterial live/dead viability assays were performed using SYTO-9 and PI, and examined under fluorescence microscopy (Olympus). SYTO-9 and PI, two nucleic acid stains with strikingly different spectral characteristics and abilities to permeate healthy and disrupted bacterial membranes, were used to label all and dead cells, respectively. The density of different bacterial strain was adjusted with PBS to ~1.5×10^7 CFU mL^{-1}. The nanoparticle dispersion (20 μL) was added into 980 μL as-adjusted bacteria suspension, to achieve final nanoparticle mass concentration of 16 μg mL^{-1} and 32 μg mL^{-1} for *E. coli* and *S. aureus*, respectively. The resulting mixing suspension was then incubated at 37 °C for 3 h. The as-treated bacteria were subsequently stained with SYTO-9 (192 μM in PBS, 5 μL) and PI (250 μM in PBS, 5 μL) via incubation in the dark for 20 min, and then centrifuged at 10,000 g for 5 min to remove the supernatant. The bacteria were washed with 500 μL PBS for twice and separated in 100 μL PBS. 10 μL of the resultant bacterial suspension was dripped onto a coverslip, and imaged under fluorescence microscopy.

Cytotoxicity assessments. Murine fibroblast 3T3 cells were used as representative mammalian host cell lines. Briefly, approximate 8000 cells were seeded into each well of a 96-well microplate, cultured in DMEM with 10% FBS at 37 °C (5% CO_2) for 24 h to ~80% confluency. The cells were rinsed with PBS and then incubated with fresh DMEM containing AgNPs or AgNPs-PBA_n with different concentrations (5–80 μg/mL) for another 24 h. The medium in each well was replaced with fresh culture medium containing 0.75 mg mL^{-1} MTT. After 4 h incubation allowing viable cells to

reduce the yellow tetrazolium salt into dark blue formazan crystals, the medium was replaced by 200 μL DMSO to dissolve the formazan crystals. The absorbance was detected at 490 nm using a microplate spectrophotometer.

Antibiofilm activity assessments. The bacteria dispersion was diluted to 1.5×10^6 CFU mL^{-1} and added into 6-well plate with sterile coverslips on the bottom. The 6-well plate was incubated at 37 °C for 1 day to obtain the biofilm on the coverslips. After incubated with different samples at predetermined concentration (16 μg/mL) at 37 °C for 6 h, the medium was discarded and coverslips with biofilms were washed with PBS for three times to remove planktonic cells. The biofilm treated with PBS was used as control. Then, the biofilms received different treatments were fixed with 2% glutaraldehyde at 4 °C. After 12 h, the glutaraldehyde was removed and the biofilms on the coverslips were washed with PBS for three times following dehydrated by a series of ethanol solutions (30%, 50%, 70%, 90% and 100%, each for 5 min) and dried overnight. Then the coverslips with biofilms were sprayed with gold and SEM images were taken.

The crystal violet staining tests were also used to investigate the efficiencies of different nanostructures on *P. aeruginosa* biofilms. To each well of a 96-well plate, 20 μL bacterial culture (1.5×10^6 CFU mL^{-1}) was added into 200 μL fresh MH media. Plates were incubated for 24 h at 37 °C to establish *P. aeruginosa* biofilms. After 24 h, the wells were carefully emptied and washed with PBS. Then different nanoparticles (8–64 μg/mL) were added to each well. The plates were incubated at 37 °C. After 6 h,

the medium from each well was removed, biofilms were washed three times with PBS to remove planktonic bacterial cells. Subsequently, the biofilms were fixed with 100% (v/v) methanol and then stained with 100 μL of 0.5% (v/v) crystal violet solution. After 20 min of treatment, the wells were rinsed with PBS to remove unbound dye. Then, 200 μL of 95% (v/v) ethanol was added to dissolve the dye. Finally, the resultant solution was quantified by measuring the absorbance at 590 nm using a microplate reader.

Animal models. To evaluate the antibacterial effect and potential safety of AgNPs-PBA_n *in vivo*, we established a mouse skin wound model. All animal experiments were conducted in compliance with the guidelines for the care and use of research animals established by the Animal Care and Use Committee at the University of Science and Technology of China. *E. coli*-infected full-thickness skin wound model was constructed as following: BALB/c mice (20±3 g, 6–8 weeks) were anesthetized by peritoneal injection of 1% (w/v) pentobarbital (0.1 mL per gram body weight). After successful anesthesia, the dorsal fur was shaved. Next, on each side of the back, a full-thickness wound with a diameter of ~1 cm was created with scissors. The skin wound was inoculated with a 50 μL aliquot of bacterial suspension (*E. coli*, OD_{600} =0.5). After 24 h of treatment, the wound was successfully infected.

For *in vivo* antibacterial therapy, mice were randomly divided into three groups (6 mice per group), which were subsequently treated with PBS (control, 20 μL), AgNPs (50 μg/mL, 20 μL), and AgNPs-PBA_2 (50 μg/mL, 20 μL), respectively, for 9 days.

The wounds were photographed and measured every two days throughout the whole treatment period. The mouse weights were recorded every two days. On the 9th day, all mice were sacrificed and their wound tissues were collected for H&E staining and Masson's trichrome staining. The colony-forming units (CFUs) in the wound were determined via a plating method. Briefly, the wound tissues from randomly selected mice in each group were placed into sterile PBS (0.2 mL) and then homogenized using a homogenizer (Precellys Evolution, Bertin Instruments) for 4 min, followed by 10-fold serial dilution with sterile PBS. 20 μL of the resulting dilutions were plated onto MH agar plates, followed by incubation at 37 °C overnight to form visible colonies.

All animal experiments were conducted in compliance with the guidelines for the care and use of research animals established by the Animal Care and Use Committee at USTC (Approval Serial: 201902191612000555725).

Additional figures

Table S1. Chemical components of the silver nanoparticles with different phenylboronic acid contents analyzed by elemental analysis.

Samples	**AgNPs-PBA$_{0.6}$**	**AgNPs-PBA$_1$**	**AgNPs-PBA$_2$**
S:B (molar ratio)	**1:0.6**	**1:1**	**1:2**

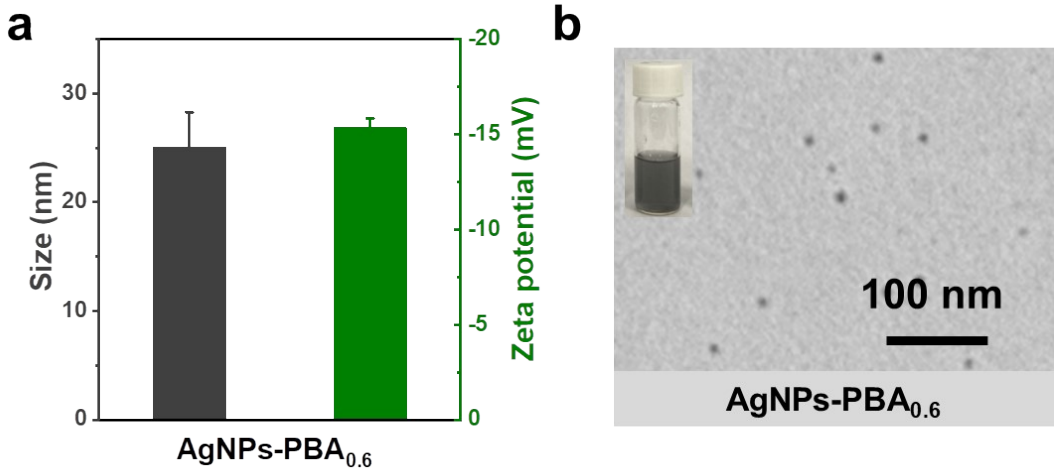

Fig. S1 (a) The size and Zeta potential of AgNPs-PBA$_{0.6}$. (b) Representative TEM image and the pictures of AgNPs-PBA$_{0.6}$.

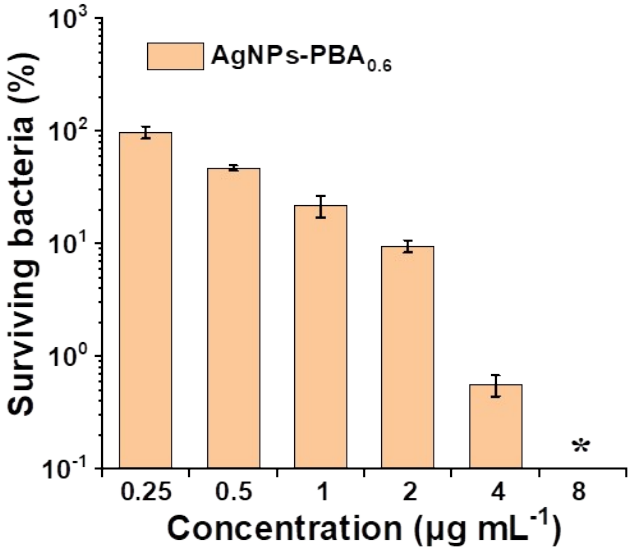

Fig. S2 Killing assessments of *E. coli* in the presence of AgNPs-PBA$_{0.6}$. Asterisk (*) indicates no detection of bacteria.

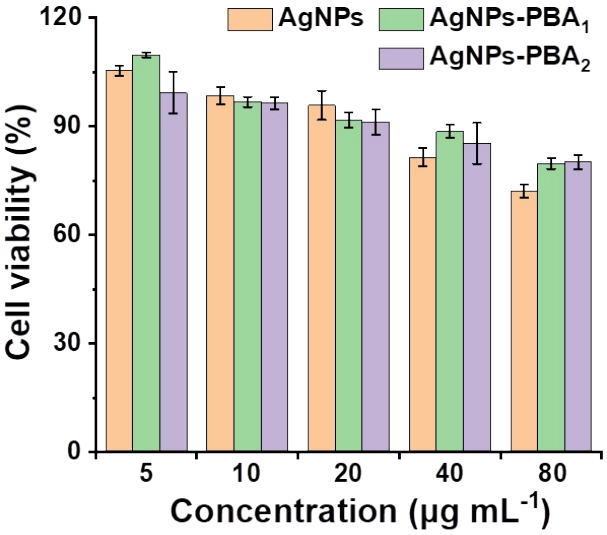

Fig. S3 Toxicities of AgNPs, AgNPs-PBA_1, and AgNPs-PBA_2 on 3T3 cells for 24 h incubation.

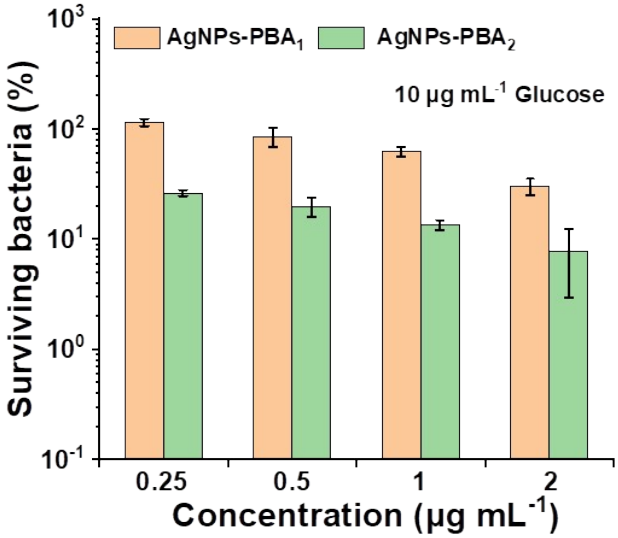

Fig. S4 Killing assessments of *E. coli* in the presence of the mixtures of AgNPs-PBA_n and glucose (10 μg mL^{-1}).

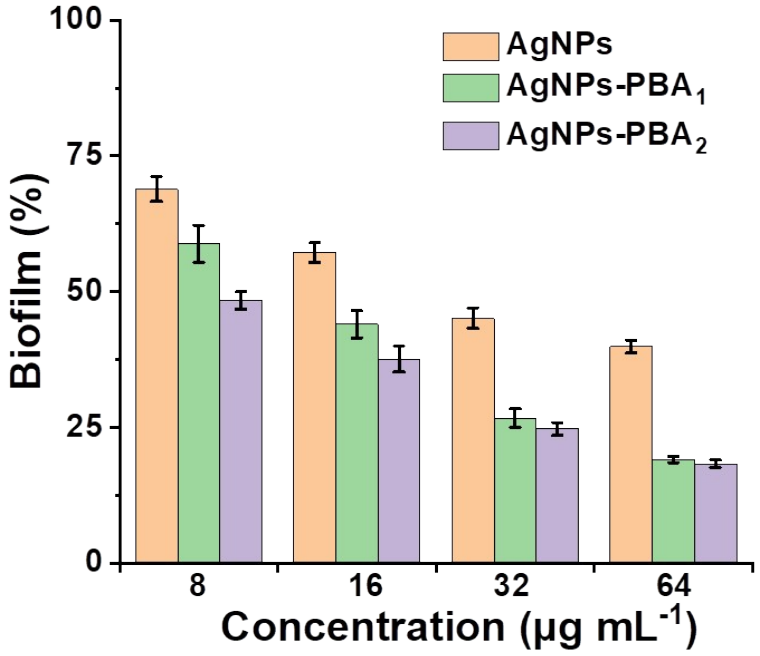

Fig. S5 Evaluation of the activity of AgNPs, AgNPs-PBA_1, and AgNPs-PBA_2 against *P. aeruginosa* biofilms at different concentrations.

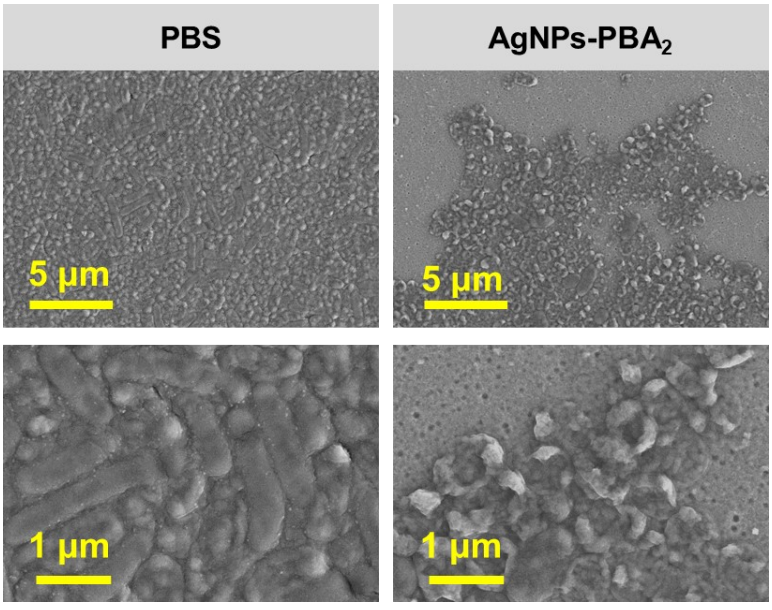

Fig. S6 SEM images of *P. aeruginosa* biofilms incubated with AgNPs-PBA_2 at 16 μg mL^{-1} for 6 h. *P. aeruginosa* biofilms treated with PBS as a control.

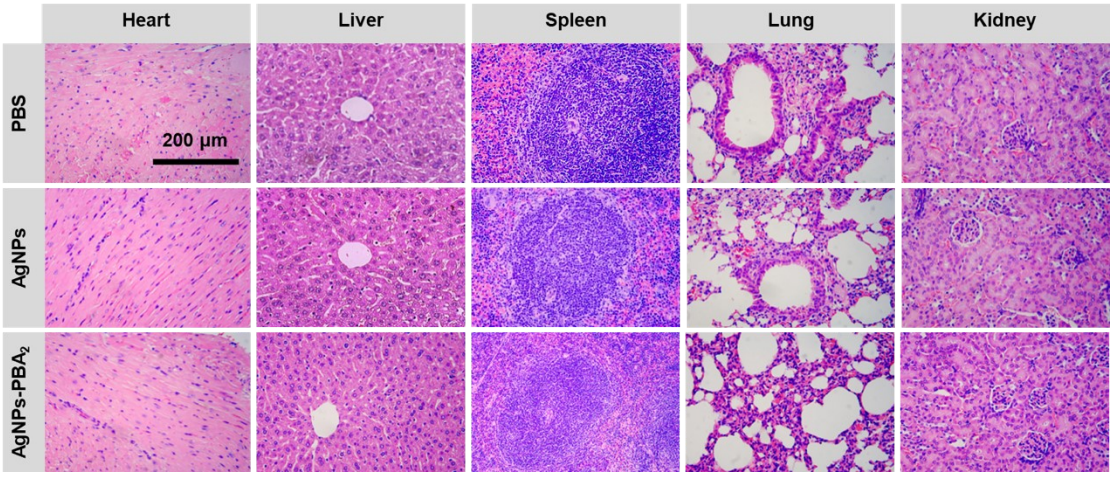

Fig. S7 H&E staining images of the major organs (heart, liver, spleen, lung, and kidney) from the mice after 9 days of treatment with PBS, AgNPs, and AgNPs-PBA_2, respectively.