Support Information

Dual-mode regulation of microbial cell membrane permeability for enhanced microbial cuproptosis-like death pathway

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1. Materials and Methods

1.1 Materials

1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), potassium permanganate, hyaluronic acid (HA), phosphate buffer solution (PBS), N-hydroxysuccinimide (NHS) were sourced from Sangon Biotech (Shanghai). N-(tris(hydroxymethyl)methyl) glycine (Tris), Copper(II) chloride dihydrate, polyethyleneimine (PEI), Graphite powder, 1,3,5-trimethylbenzene (TMB), dopamine hydrochloride, phosphoric acid, purchased from Macklin. Polyoxyethylene fatty ether F 127 were purchased from Sigma-Aldrich. Co., Ltd.. Staphylococcus aureus (SA, ATCC 6538) was purchased from the Guangdong Province Microbial Strain Preservation Center. Meat extract peptone broth (LB) was purchased from SolarBio. Sulfuric acid, hydrochloric acid, hydrogen peroxide, acetone were purchased from Yantai Yuandong Fine Chemical Co., Ltd.. O-nitrobenzyl-β-D-galactopyranoside(ONPG) was purchased from Yeasen Biotechnology (Shanghai) Co., Ltd.. LIVE BacLight™ bacterial Gram staining kit was purchased from Thermo Fisher. Anhydrous ethanol was purchased from Sinopharm Chemical Reagent Co., Ltd.. 6-8 weeks of BALB/c mice were provided by Vital River. The assay kit for measuring BCA protein concentration was bought from Beyotime.

1.2 Equipment

The material morphology was characterized using a transmission electron microscope (FEI Talos F200x). An infrared spectrometer was used to record Fourier transform infrared (FTIR) spectra (Thermo Scientific Nicolet iS20). Zeta potential were conducted by mastersizer (Zetasizer Nano ZS90). Bicinchoninic Acid Assay for Protein Leakage (BCA) protein leakage assays, and O-Nitrophenyl-β-D-

galactopyranoside assay (ONPG) experiments were performed on a Microplate Reader instrument (ThermoFisher, K3 touch). The transmission electron microscopy (TEM) images were taken using both a FEI Talos F200x and a JEOL JEM 2100. Images of the bacteria were obtained through scanning electron microscopy (JEOL JSM-IT200). Using an UV-visible spectrophotometer (Agilent Cary 60), UV-visible absorption spectra were acquired. Fluorescent quantitative experiments were conducted on a fluorescent quantitative PCR machine (Pikoreal, Germany/ThermoFisher). Fluorescence images were obtained from microscope (Olympus IX83).

1.3 Synthesis of materials

1.3.1 Synthesis of Graphene Oxide (GO)

Prepared a mixture of 100 mL of H_2SO_4 and H_3PO_4 (8:2), to which 0.6 g of graphite was added and continuously stirred for 0.5 h at 25℃. An ice bath was used to keep the temperature between 0-5℃, and in this, 2.4 g of $KMnO_4$ was added incrementally and carefully. After this, the mixture was stirred for 15 h at 35℃. Following this, 240 mL of water was rapidly added, after which was continuously stirred for 1 h. Then, 7 mL of H_2O_2 was slowly added to the solution until it turned a golden yellow. After the solution had cooled to room temperature, it was centrifuged and then washed three times with 1 mol/L HCl and deionized water. The final step was freeze-drying to obtain pure GO.

1.3.2 Synthesis of Graphene Oxide Altered with Polyethyleneimine (PEI@GO)

A clear colloidal suspension of GO (20 mL of 1 mg/mL) was prepared. Thereafter, 75 mg of NHS and 50 mg of EDC were mixed into the suspension, which was then stirred in the dark at ambient temperature for 3 h. The preparation was further processed by slowly introducing 5 mL of a 2% PEI solution, while the pH was

brought to 6 with a 5% HCl solution. The altered mixture was agitated at 30℃ for two days, underwent another 4 h of sonication, and was later dialyzed against ultrapure water for 48 h. After centrifugal separation, pure PEI@GO was obtained.

1.3.3 Synthesis of Copper Ion Chelated Polydopamine (CuPDA)

Mixing 10 mL of water with 12 mL of ethanol devoid of water produced a liquid solution, into which 0.084 mL of TMB and 0.072 g of F127 were incorporated. The blend was agitated for 0.5 h. Next, the mixture was supplemented with 2 mL of an 80 mg/mL Tris solution. Then, 15 mg of $CuCl_2 \cdot 2H_2O$ was added slowly. The solution was stirred consistently, and then 3 mL of a dopamine hydrochloride solution (4 mg/mL) was slowly introduced drop by drop. At room temperature, the blend was agitated for 4 h. The solution was then subjected to centrifugal separation, and the formed sediment was rinsed thrice with a blend of ethanol and acetone in a 2:1 volume proportion. The sediment was then dried at 40℃ to obtain pure CuPDA. By following the same procedure, polydopamine (PDA) part was synthesized as a control group, excluding the addition of $CuCl_2 \cdot 2H_2O$.

1.3.4 Synthesis of Copper Ion Chelated Polydopamine Coated with Hyaluronic Acid（CPH）

10mg of CuPDA were introduced into HA solution (10 mL, 1 mg/mL) and agitated at ambient temperature for 24 h. The resultant solution was then rinsed three times with distilled water and subsequently freeze-dried to yield CPH.

1.3.5 Synthesis of the CPH and PEI@GO Complex (CPHG)

25 mL of ultrapure water, 0.025 g of PEI@GO, and 0.1 g of CPH were added. For 24 h, the mixture was stirred at room temperature. Following this, the blend was subjected to three washes with ultrapure water and then processed through freeze-drying to acquire the complex CPHG.

1.4 Photothermal Effect

In summary, a 1.5 mL centrifuge tube filled with 1 mL of the specimen was placed under an 808 nm near-infrared laser (NIR) for 10 min. Using a thermal imager, the dispersion's temperature was measured every 50 s. Plots of the irradiation time-temperature curves were used to assess the photothermal characteristics of various nanoparticles. To assess the influence of varying laser power densities on photothermal properties, samples with a concentration of 50μg/mL were subjected to irradiation of 0.25, 0.5, 0.75, 1, and 1.25 W/cm². To evaluate the concentration-dependent photothermal properties of CPHG at 0, 25, 50, 75, and 100 μg/mL, a laser was used to irradiate the samples of 0.75 W/cm².

1.5 Evaluation of Bacterial Membrane Permeability

The permeability of the membrane was evaluated using the ONPG hydrolysis test. The bacterial suspensions were kept for incubation along with the ONPG solution (pH 7.0, 0.75M NaH_2PO_4 buffer). To ascertain the extent of bacterial protein leakage, the amount of proteins that had leaked out from the bacteria was determined by employing a BCA protein assay kit.

1.6 Antibacterial Assay

The efficacy of various samples against bacteria SA was evaluated. SA was cultivated in a liquid LB broth at 37°C, agitated at 180 rpm, and allowed to grow overnight until it entered the logarithmic phase of growth. Then, 100 μL of SA (10^8 $CFU \cdot mL^{-1}$) was mixed with different nanoparticles with 808 nm radiation (or no 808 nm radiation) for 10 min and then cultured 4 h. In each group, 50 μL bacterial solution was diluted 1000 times. Subsequently, the liquid was uniformly distributed across the LB agar plate and incubated for 36 h to monitor the bacterial growth.

1.7 Live/Dead Bacteria Staining Assay

The LIVE/DEAD BacLight Bacterial Viability Kit was used to stain the bacteria in the dark for 30 min. Following this, the supernatant was removed by centrifugation at 5000 rpm for 5 min. The samples were then washed 1-2 times with 0.9% saline to eliminate excess dye. After washing, the samples were placed on glass slides and observed under an Olympus fluorescence microscope.

1.8 Infectious Mice Model Experiment

All mice were colony-bred animals free from specific pathogens, and they were adapted to the experimental environment for one week before the experiment. This work has received approval for research ethics from Science and Technology Ethics Committee, Linyi People's Hospital. The hair on the backs of the mice was shaved off, and a circular wound approximately 1 cm in diameter was created, followed by the injection of 100 μL of SA suspension (10^8 CFU mL^{-1}) to induce infection. 50 μL PBS or nanoparticle solutions were applied to the infection site, for 3 min, subjected to 10 min to an 808 nm laser's radiation. The mice's body weight and injuries were noted. On the 9th day, the experimental mice's major organs, surrounding tissues, and wound surface underwent histological examination.

2. Results and discussion

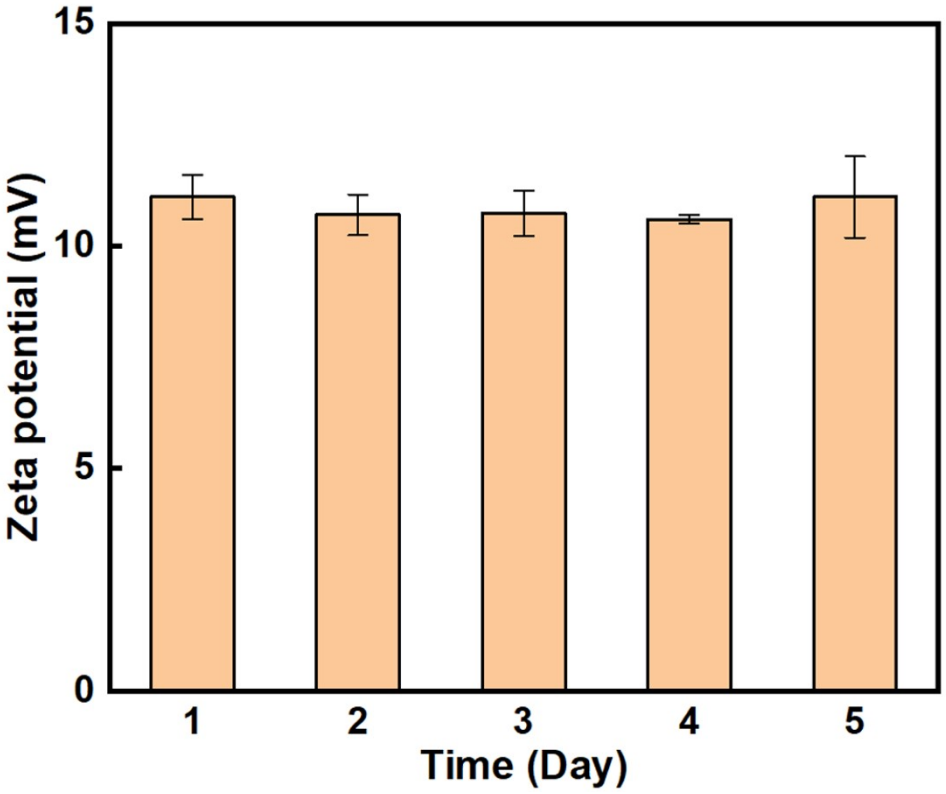

Fig. S1 The Zeta potential of CPHG on days 1, 2, 3, 4, and 5.

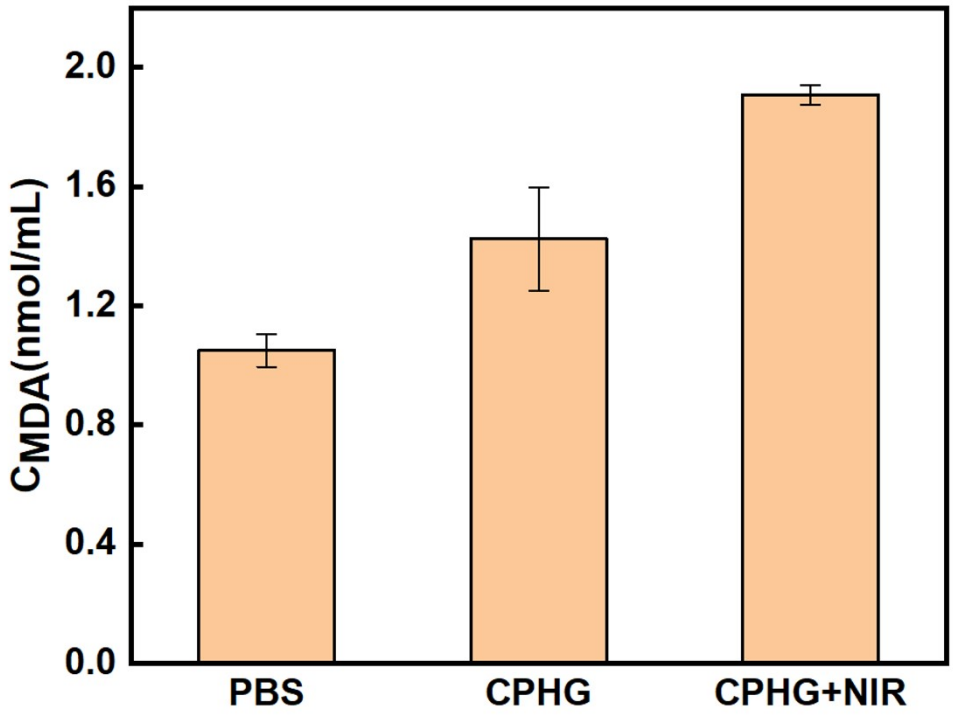

Fig. S2 MDA concentration in Staphylococcus aureus after incubation with PBS, CPHG and "CPHG+NIR" measured by the MDA assay kit.

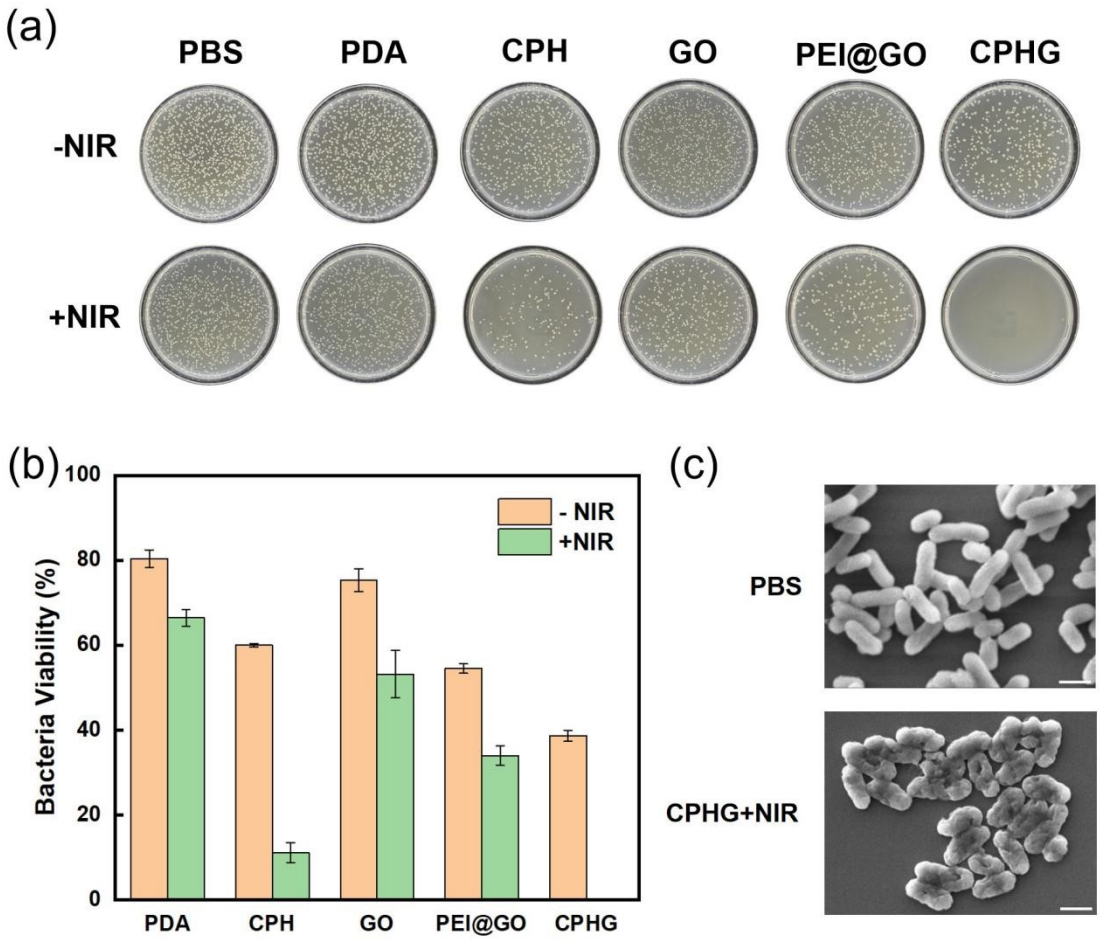

Fig. S3 Evaluation of the antibacterial efficacy of CPHG against Escherichia coli. (a) Representative images of SPM from Escherichia coli after treated with different nanomaterials. (b) Viability of Escherichia coli treated with various nanoparticles. (c) SEM images of Escherichia coli treated with PBS and "CPHG+NIR". (Scale bar: 1 μm)

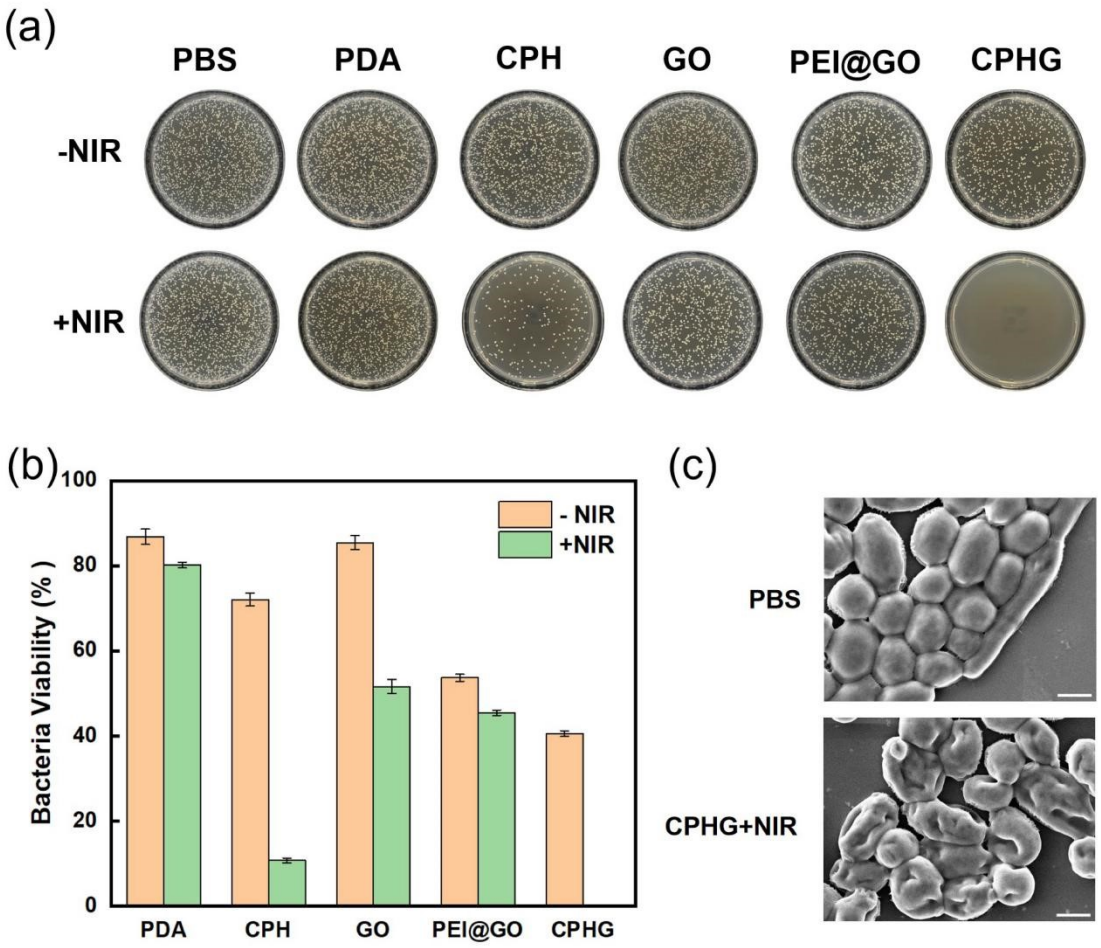

Fig. S4. Evaluation of the antibacterial efficacy of CPHG against Candida albicans. (a) Representative images of SPM from Candida albicans after treated with different nanomaterials. (b) Viability of Candida albicans treated with various nanomaterials. (c) SEM images of Candida albicans treated with PBS and "CPHG+NIR". (Scale bar: 2 μm)

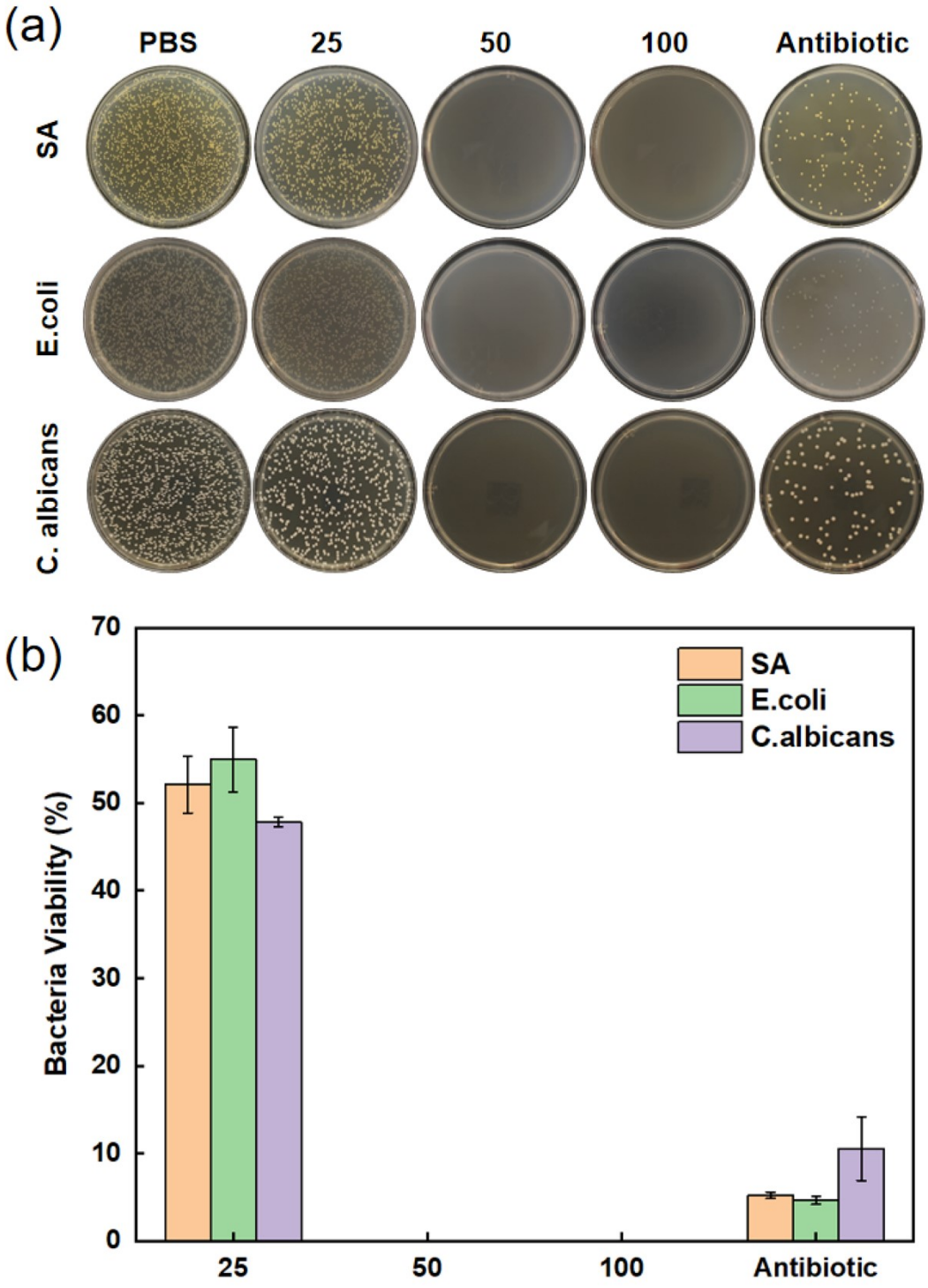

Fig. S5. Representative SPM images of Staphylococcus aureus (SA), Escherichia coli (E. coli), and Candida albicans (C. albicans) treated with different nanomaterials and antibiotics. (Staphylococcus aureus, Escherichia coli, and Candida albicans were treated with 50 μg/mL vancomycin, ciprofloxacin, and amphotericin B, respectively.)

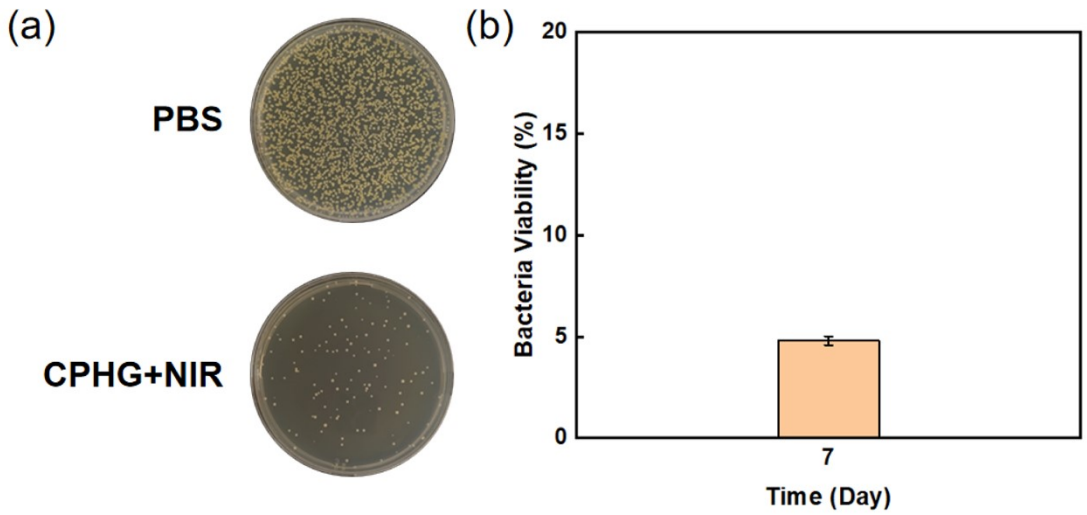

Fig. S6. The inhibitory effect of CPHG on SA after oscillating in PBS for 7 days.

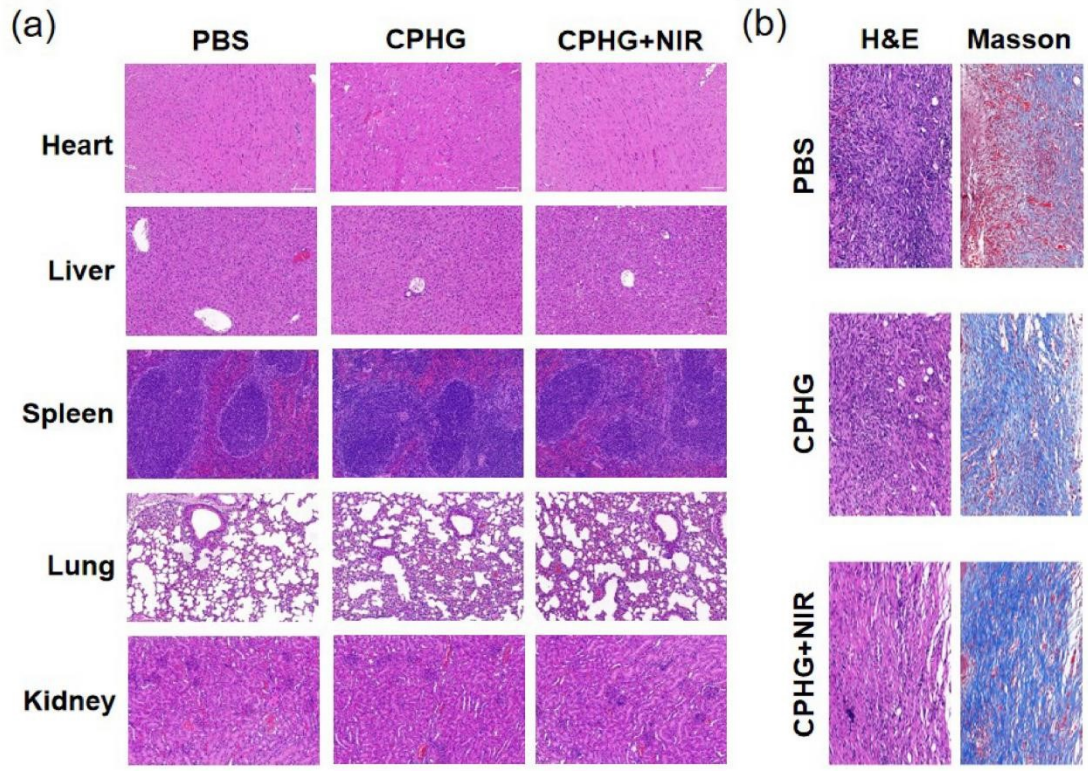

Fig. S7. H&E and Masson pathological test *in vivo*. (a) Major organs from mice treated with PBS, CPHG, and "CPHG+NIR" were examined in H&E stained images. (Scale bar: 100 μm) (b) Peripheral tissue stained with H&E and Masson after 9 days of varying treatments. (Scale bar: 100 μm)