Electronic Supplementary Information:

Thiol-terminated *N*-halamine ligands to photothermal gold nanorods for synergistically combating antibiotic-resistant bacteria

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1. Materials and methods

1.1 Chemicals and materials

Sodium borohydride ($NaHB_4$), sodium chloride (NaCl), and silver nitrate (AgNO3) were purchased from Tianjin Fengchuan Chemical Reagent Company Co., Ltd. Anhydrous ethanol (C_2H_6O) and sodium hydroxide (NaOH) were purchased from Tianjin Beilian Fine Chemicals Company Co., Ltd. Cetyltrimethyl ammonium bromide (CTAB), chlorauric acid ($HAuCl_4$), and L-ascorbic acid (AA) were purchased from Sigma Aldrich (Shanghai) Trading Co., Ltd. 4,4'-Azobis(4-cyanopentanoic acid) (ACVA), ≥98.0%, Shanghai Sigma Aldrich Trading Co., Ltd; Poly(ethylene glycol) methyl ether (4-cyano-4-pentanoic acid dodecyl trithiocarbonate), M_n=5000, Shanghai Sigma Aldrich Trading Co., Ltd; n-propylamine, analytically pure, Aladdin Reagent Shanghai Co. Ltd; deuterated water (D_2O), 99.8%, Qingdao Blue Ocean Tianyue Biotechnology Co., Ltd; sodium citrate, 99.0%, Shanghai McLean Biochemical Technology Co., Ltd; sodium hypochlorite, analytically pure, Tianjin Windjammer Chemical Reagent Technology Co; All chemicals were used without further purification. Microbiological culture media, including yeast extract powder and tryptone, were bought from Guangdong Huankai Biotech Co., Ltd. Beef cream was obtained from Beijing Aoboxing Biotech Co., Ltd. Agar was provided by Beijing Kulaibo Technology Co., Ltd. The microbial culture media were biological-reagent grade. Distilled water was used in all experiments and was generated by a Millipore system (Millipore Inc.). Cell Counting Kit-8 (CCK-8) was purchased from Biyuntian Biotechnology Co., Ltd.

1.2 Synthesis of gold nanorods (GNR)

Gold seed preparation: Gold nanorod was synthesized by seed-mediated method. Firstly, 0.3645 g CTAB was accurately weighed into the bottle, and 3.5 g water was added. The solution was fully shaken and dissolved until the solution was clear. At a speed of 1050 rpm and a temperature of 25.5℃, 125 μL $HAuCl_4$ (15 mmol/L) solution was rapidly added to the CTAB solution. After mixing evenly, $NaBH_4$ (0.01 mol/L) solution after 500 μL ice bath was quickly added into the above mixed solution, stirred for 2 min, and then incubated in a constant temperature water bath at 25.5℃ for 0.5-2 h for use.

Growth solution preparation: Firstly, add 8.84 g pure water to 0.3645 g CTAB and shake well until the solution is clear. Add 400 μL $AgNO_3$ (4 mmol/L) solution to the above CTAB solution, then add 520 μL $HAuCl_4$ (15 mmol/L) solution, and shake well quickly until the solution is dark brown. Continue to add 124 μL AA (0.08 mol/L) solution, the solution becomes colorless and transparent. Under the condition that the temperature of the above solution is controlled to be 24-26℃, the prepared gold seed solution is added to 100 μL and finally incubated in a constant temperature water bath at 27.5℃ overnight.

1.3 Synthesis of *N*-halamines precursor sulfhydryl-terminated polymers (PEG-*b*-pAMPS-SH)

The synthetic route of PEG-b-pAMPS-SH was shown in Scheme 1. The specific steps were as follows[1]:0.0014 g ACVA, 0.8280 g AMPS, and 0.54 g poly(ethylene glycol) methyl ether (4-cyano-

4-pentanoic acid dodecyl trithiocarbonate) were added to a Shrank reaction flask, put into a clean magnetic stirrer, and after sealing the mouth of the flask with a rubber stopper, and after three times of Freeze-Pump-Thaw treatment, the reaction solution was placed in an oil bath at 70 ℃ and stirred at 1000 rpm for 10 h. The crude product was dialyzed in ultrapure water for 3 days to remove the unpolymerized monomer APMS, and freeze-dried to obtain 0.88 g of light yellow solid product A. 0.5 g of product A was placed in the Shrank reaction vial, and 5 mL of ultrapure water was added to dissolve the product, and the solution was placed in an atmosphere of N_2, and the N-propylamine stock solution (0.5 mL of N-propylamine stock solution was added and the reaction was carried out at 1000 rpm for 15 min. After the reaction was completed, the solution was freeze-dried again to remove the unreacted small molecules of N-propylamine, and a light yellow powder was obtained. After the reaction, 0.3 g of the light yellow powder was added into 5 mL of ultrapure water to dissolve it, and then a white insoluble substance was precipitated, and then centrifuged at 15000 rpm for 15 min, and the supernatant was freeze-dried again to obtain the final product PEG-*b*-pAMPS-SH.

1.4 Preparation of *N*-halamine polymer grafted gold nanorods (GNR@pAMPS-Cl)

The sample preparation procedure was as follows[2]: 1.0 mL of newly synthesized CTAB ligand GNR was transferred into a 1.5 mL centrifuge tube and centrifuged at 12000 rpm for 15 min at 27 ℃. Subsequently, the supernatant of centrifugation was removed, leaving about 50 μL of sample solution at the bottom, and the centrifuge tube was subjected to ultrasonic cleaning for 5 seconds to make the GNR uniformly dispersed. The GNR solution was rapidly pumped into an aqueous solution (1.0 mL, 1.0 mM) containing PEG-*b*-pAMPS-SH under ultrasonic conditions, and the solution was incubated at room temperature for 2 days to obtain a stable GNR@pAMPS solution.

By chlorinating the above *N*-halamine precursor. 1.0 mL of the solution was transferred to a 1.5 mL centrifuge tube and centrifuged at 12,000 rpm for 15 min at 27 °C. Subsequently, the centrifuged supernatant was removed, leaving approximately 50 μL of sample solution at the bottom, and the centrifuge tube was placed in an ultrasonic cleaner for 5 seconds to allow for homogeneous dispersion of the GNR. Under ultrasonic conditions, the GNR solution was rapidly pumped into the NaClO (0.1, 0.2, 0.3, 0.4, and 0.5 wt%) solution, and the solution was shaken at 25 ℃ for 2 h. The chlorinated solution was centrifuged at 12,000 rpm for 15 min at 27 ℃. Subsequent removal of the supernatant after centrifugation.

1.5 Determination of effective chlorine

Determination of effective chlorine content in *N*-halamine polymers by iodometric titration. Firstly, 2.50 g starch was weighed and diluted in 50.00 mL boiling water to make 5.0 wt% starch solution, and then $Na_2S_2O_3$-$5H_2O$ (1.00 g) was weighed and diluted to 200 mL to configure its standard solution. The 1.0 wt% KI solution was prepared from 1.00 g of solid KI to 100 mL, and the KIO_3 solution was prepared from 0.50 g of solid KIO_3 dissolved in 500 mL of ultrapure water. The $Na_2S_2O_3$ solution was calibrated before the determination, and 7.0 mL of KI solution, 2.0 mL

of H_2SO_4 solution (2 mol/L), and 10 mL of KIO_3 solution were pipetted into an iodine measuring flask, and then a few drops of starch solution were added after the solution was allowed to stand in the dark. Then using a buret, carefully drop $Na_2S_2O_3$ solution until the solution becomes exactly colorless.

Calculate the exact concentration of $Na_2S_2O_3$ according to equation (1).

$$C_{Na_2S_2O_3=} \frac{10.00\ mL \times 0.0028\ mol/L}{V_{Na_2S_2O_3}} \quad (1)$$

After calibration, add 2 mL of H_2SO_4 solution and 7 mL of KI solution to the *N*-halamine solution, mix well, and add 4-5 drops of starch solution. Titration with $Na_2S_2O_3$ standard solution until colorless is the end point of the titration. The percentage of active chlorine (Cl^+%) in the sample was calculated according to equation (2).

$$Cl^+\% = \frac{0.03545 \times C \times V}{2W} \times 100\% \quad (2)$$

1.6 Bacterial cell culture

Escherichia coli (*E. coli*, ATCC 8099, a Gram-negative bacterium) and *Staphylococcus aureus* (*S. aureus*, ATCC 6538, a Gram-positive bacterium) were used as the two model strains in antibacterial tests. *Escherichia coli pUC19* (*E. coli pUC19*, a Gram-negative bacterium) and *methicillin-resistant Staphylococcus aureus* (MRSA, a Gram-positive bacterium) were used as two MDR bacteria model strains. Briefly, a single colony was inoculated under constant shaking at an average speed of 220 rpm in 5 mL of Luria-Bertani growth medium (LB) at 37 °C for 12 h, and then the culture was allowed to expand to 10^8-10^9 colony forming units (CFU/mL).

1.7 Antibacterial test

The antibacterial activity of GNR@pAMPS-Cl was determined by plate-counting method. Before the sterilization experiments, the glassware, culture medium, and deionized water used in the experiments were sterilized at 121 ℃ for 20 min, and microbiology-related operations were carried out in a sterile environment. After the culture, 1 mL of active bacteria was centrifuged, and the centrifuged bacterial cells were washed with saline three times, and then diluted to 10^7 CFU/mL. GNR@pAMPS-Cl dispersions with different chlorination concentration gradients ranging from 0.1-0.5 (wt%) were prepared and mixed with 100 μL of the above suspension, and then contacted with each other at room temperature for 15 min, and then sequentially diluted to 10^6 CFU/mL. After contacting for 15 min at room temperature, the mixture was sequentially diluted to 10^2 CFU/mL. 1 mL of the diluted mixture was uniformly spread on LB agar plates, and incubated at 37 ℃ for 12 h or 24 h. In addition, 900 μL of sodium chloride solution was taken and mixed with 100 μL of the bacterial suspension as a blank control group, and all the experiments were repeated in parallel three times, and the number of surviving colonies on each LB agar plate was counted, and the antimicrobial rate was calculated according to the following equation Calculate the antimicrobial rate.

$$\text{Antibacterial rate \%} = (B\text{-}A) / B \times 100\ \%$$

Where B is the number of remaining colonies after contact with GNR@pAMPS-Cl and A is the number of colonies in the blank control group.

To investigate the effects of different light conditions on the bactericidal properties of GNR@pAMPS-Cl, the above experimental steps were carried out under two different conditions, namely, natural light and 808 nm excitation light (1.0 W/cm^2), and the antibacterial activities of GNR and GNR@pAMPS-Cl were determined, respectively.

1.8 Bacterial morphology observations

The morphological changes of bacteria before and after GNR@pAMPS-Cl treatment were observed by scanning electron microscopy (SEM). After the antibacterial process of the sample was completed according to the above antibacterial steps, the sample and bacteria were separated by standing, and the isolated bacterial solution was washed with sterile phosphate buffer solution (PBS) for 3 times. The obtained bacteria were fixed at 4℃ overnight with 2.5% (w/v) glutaraldehyde. The fixed bacterial solution was washed with PBS for 3 times, followed by gradient dehydration with 20%, 50%, 80%, and 100% concentrations of anhydrous ethanol, and then washed with tert-butanol for 2 times, and finally dispersed in tert-butanol. The obtained bacterial solution was dropped onto the silicon wafer for SEM observation.

1.9 LIVE/DEAD staining of bacteria

LIVE/DEAD BacLight staining kits were used to further assess bacterial viability and membrane integrity. The kit uses SYTO 9 and PI dye to quantify the number of bacteria killed and live by confocal fluorescence microscopy. First, the SYTO 9 and PI dyes are dissolved in 250 μL of sterile water, mixed evenly, and set aside. 1 mL of bacterial solution with a concentration of 10^7 CFU /mL was centrifuged at 4000 rpm for 7 min to obtain bacterial precipitation, and then re-dispersed in 1 mL of sterile water. Then, after the bactericidal procedure was completed, the sample and bacteria were separated by standing, the obtained bacterial solution was centrifuged again and the supernatant was poured away, and the bacteria were dispersed again by adding 10 μL sterile water. Next, 10 μL bacterial solution was mixed with 10 μL PI/SYTO 9 dye mixture, incubated at room temperature and dark for 20 min, and the 10 μL stained bacterial sample was dropped between the slide and the square lid plate, and determined by inverted confocal fluorescence microscopy.

1.10 Photothermal properties

The photothermal properties of the sample GNR@pAMPS-Cl were characterized by an infrared thermography system (Testo, 885-2, Germany). 1.0 mL of stabilized GNR@pAMPS-Cl solution was transferred to a 1.5 mL centrifuge tube and irradiated with 808 nm excitation light (0.8 W/cm^2), and the temperature changes were recorded by an infrared thermography camera at 1 min intervals for 10 min. The main purpose of the study was to investigate the temperature changes induced by the different optical power densities of the 808 nm laser irradiation (0.5, 0.8, and 1.0 W/

cm^2), and to determine the temperature changes that meet the experimental requirements. The main purpose is to investigate the temperature changes caused by different power densities (0.5, 0.8, and 1.0 W/ cm^2) of 808 nm laser irradiation, and to determine the optical power density that meets the experimental requirements. Finally, the photothermal stability of GNR@pAMPS-Cl was verified by studying its photothermal cycle.

The photothermal conversion efficiency (η) was calculated by the equations:

$$\theta = \frac{T - T_{Surr}}{T_{Max} - T_{Surr}}$$

$$t = \tau_s(-ln\theta)$$

$$\tau_s = \frac{\sum_i m_i C_{p,i}}{hS}$$

$$\eta = \frac{hS(T_{Max} - T_{Surr}) - Q_{Dis}}{I(1 - 10^{-A_{808}})}$$

Where T is the solution temperature (℃), T_{Max} is the equilibrium temperature (℃), T_{Surr} is the ambient temperature of the surrounding (℃), t is the time (s), τ_s is the sample system time constant (s), m is the mass of water (g), Cp is The heat capacity of water, h is the heat transfer coefficient, S is The surface area of the container, hS is The dimensionless driving force temperature (mW), Q_{Dis} is the baseline energy inputted by the sample cell, I is the incident laser power (mW), A_{808} is the absorbance of probe at 808 nm.

1.11 CCK-8 test

The biocompatibility of GNR@pAMPS-Cl was evaluated by *in vitro* cytotoxicity analysis of CCK-8 using NIH 3T3 cells as the cell model. 180 μL NIH 3T3 cells (5000 cells/well) were inoculated into 96-well plates and incubated at 37℃ and 5% CO_2 for 24 h. GNR@pAMPS-Cl were dispersed with aseptic cell medium and samples with 0.125, 0.25, 0.5, 1.0, and 2.0 mg·mL^{-1} concentrations were prepared, respectively. 20 μL of the above GNR@pAMPS-Cl sample was added to the cultured NIH 3T3 cells per well and continued to incubate at 37℃ and 5% CO_2 for 24 h. Then, PBS was washed twice, and 10% (V/V) CCK-8 solution prepared by 100 μL was added to each well, and incubated at 37℃ and 5% CO_2 for 2 h. OD value at 450 nm was determined by a microplate microscope.

1.12 Hemolysis assay

The red blood cells were collected by centrifugation of BALB/c mice fresh blood (1500 rpm 10 min), and the red blood cell suspension was incubated with GNR@pAMPS-Cl at 37℃ for 60 min. Triton X-100 and 0.9% NaCl were used as positive and negative controls, respectively. After incubation, the GNR@pAMPS-Cl were removed and the solution containing blood cells was centrifuged at 1500 rpm for 10 min to obtain the supernatant. The absorbance of the supernatant at 578 nm was determined by UV-vis spectroscopy (HITACHI U3900).

1.13 In vivo wound infection treatment of mice

The BALB/C male mice were bought from the Experimental Animal Center of Inner Mongolia University and raised in the animal laboratory for 7 days to adapt to the environment. The mice were randomly divided into five groups: (1) control (PBS) (2) GNR+laser irradiation (L) (808 nm, 0.8 W/cm^2, 10 min) (3) GNR@pAMPS-Cl (active chlorine, 0.2 wt%) (4) GNR@pAMPS-Cl +L (808 nm, 0.8 W/cm^2, 10 min + active chlorine 0.2 wt%). Mice were anesthetized by intraperitoneal injection of 10% chloral hydrate before surgery. Except for the healthy group, the other groups used a hole punch to make a 4 mm wound on the back of each mouse. We established a mouse wound model by injecting 10 μL of MRSA model bacteria with a concentration of 10^7 CFU/mL into the wound. Wound healing is observed daily by taking digital photos and measuring the area of the wound. The wound healing rate was calculated by the following formula:

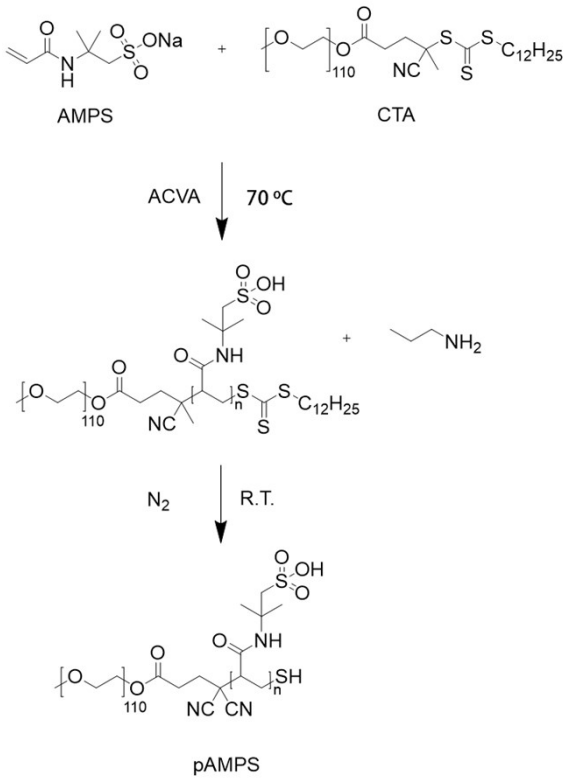
Wound healing rate (%) = $(1-A_t/A_0) \times 100\%$

where A_0 is the initial wound area and A_t is the wound area at a certain time interval.

Histology: After six days, different groups of wound tissue from the left 5 mice were harvested. Then, fixed in 10 % paraformaldehyde, embedded in paraffin, and cut into slices of about 4 μm. The tissue samples were examined under fluorescence microscopy after stained with hematoxylin-eosin staining (H&E).

The mouse experiment received ethical approval from the Animal Center of Inner Mongolia University. Experiments on infection healing in mouse epidermal wounds. Model mice (male, 4 – 6 weeks old) were obtained from the Animal Center of Inner Mongolia University, and all animal experiments were approved by the university's Institutional Review Board. Animal husbandry was performed following the Ministry of Health of the People's Republic of China's Guidelines on Animal Management and the Chinese Guide for the Use of Laboratory Animals in Husbandry. The animal experimental procedures in this study were reviewed and approved by the Ethics Committee of Inner Mongolia University (the assigned approval/accreditation number: IMU-2023/037).

2. Supplementary Scheme and figures



Scheme S1. Synthetic routes of the pAMPS.

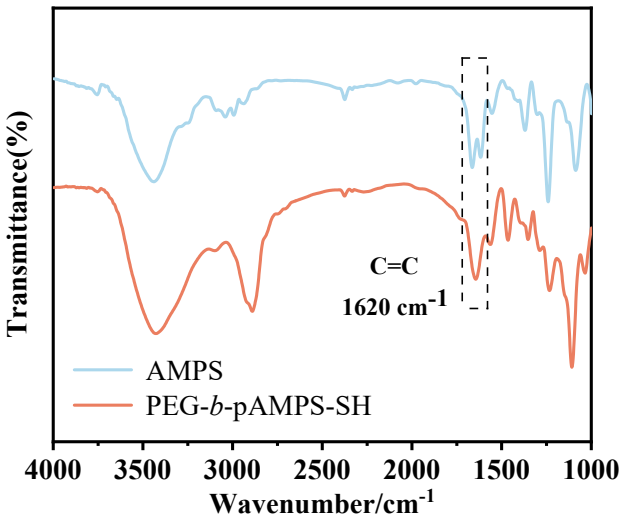


Fig. S1 FTIR analysis of the AMPS and PEG-*b*-pAMPS-SH.

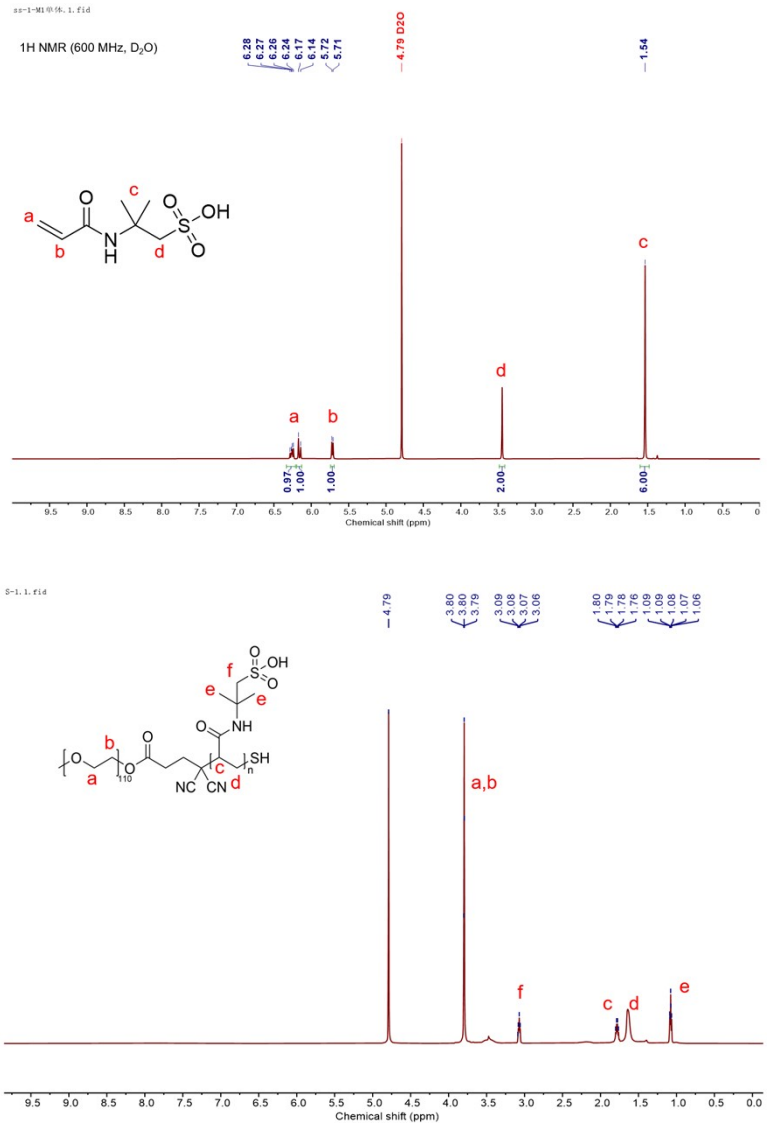


Fig. S2 ^{1}H NMR analysis of the synthesis of AMPS and PEG-*b*-pAMPS-SH.

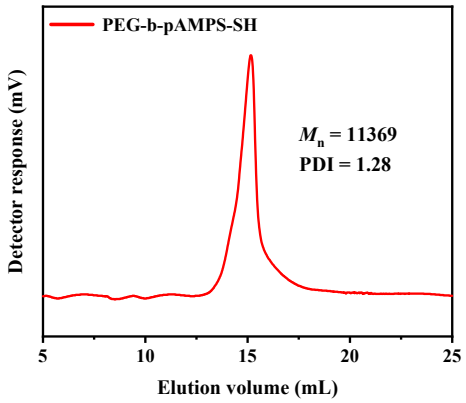


Fig. S3 GPC analysis of the synthesis of PEG-*b*-pAMPS-SH

Fig. S4 Photograph showing the color changes in pAMPS-Cl during the iodometric titration.

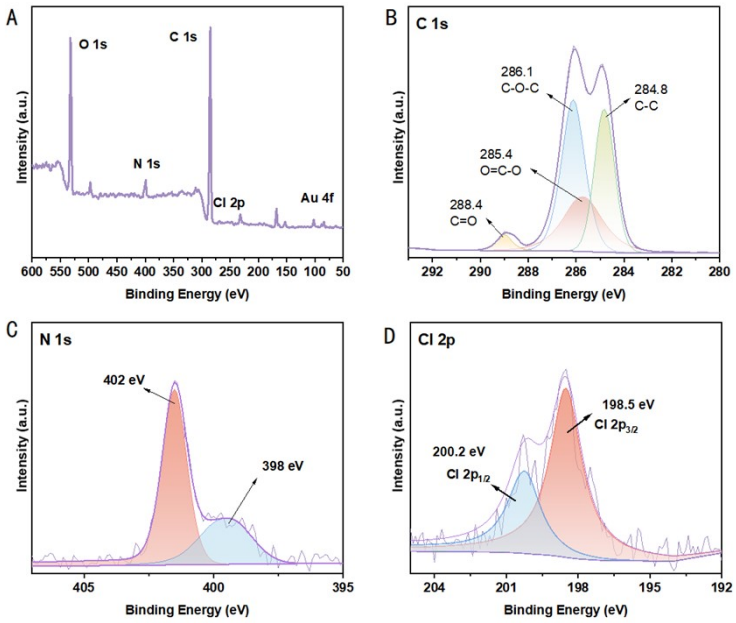


Fig. S5 (A) XPS survey spectrum for GNR@pAMPS-Cl, (B) C 1s peaks, (C) N 1s peaks, and (D) Cl 2p peaks.

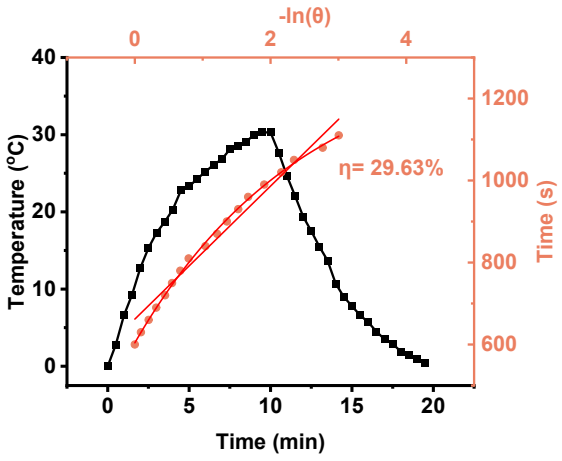


Fig. S6 Photothermal conversion efficiency of GNR@pAMPS-Cl under 808 nm laser (1.0 W/cm^2 10 min).

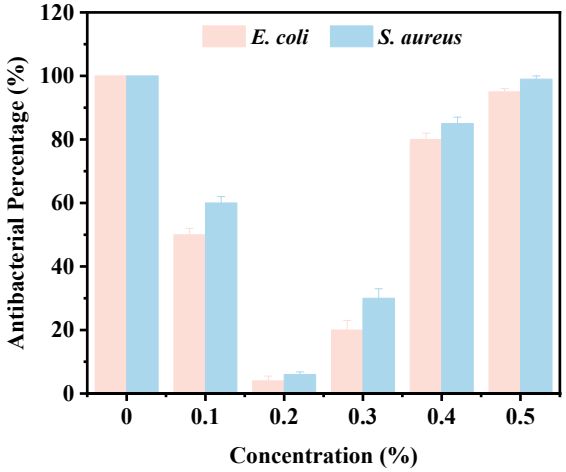


Fig. S7 Survival rates of *E. coli* and *S. aureus* treated with different chloride concentrations of GNR@pAMPS-Cl.

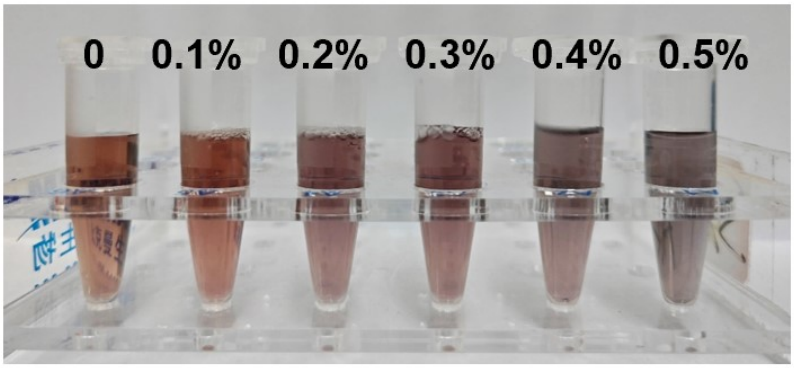


Fig. S8 Digital photos of solutions with different chloride concentrations（Chlorination for 2 hours）.

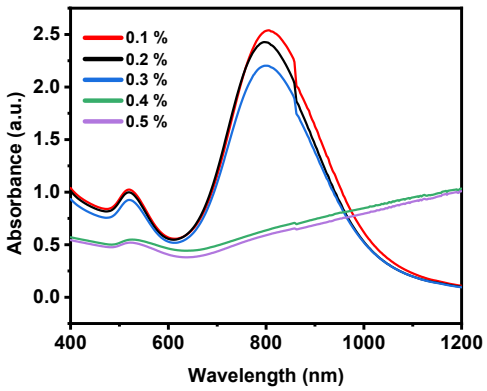


Fig. S9 UV-vis spectra of GNR@pAMPS-Cl at different chloride concentrations (0.1-0.5 wt%).

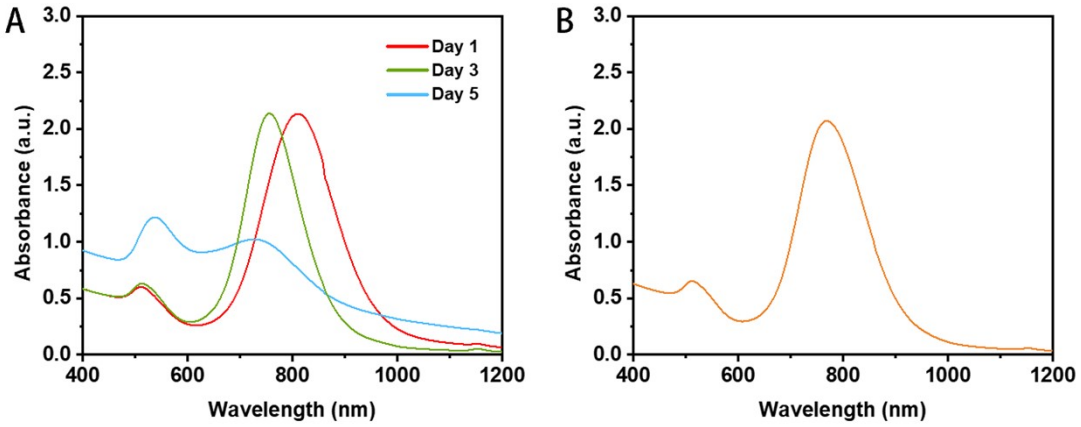


Fig. S10 (A) The UV-Vis spectra of GNR@pAMPS-Cl in PBS without free pAMPS-Cl polymer. (B) The UV-Vis spectrum of GNR@pAMPS-Cl in PBS with free pAMPS-Cl polymer for 7 days.

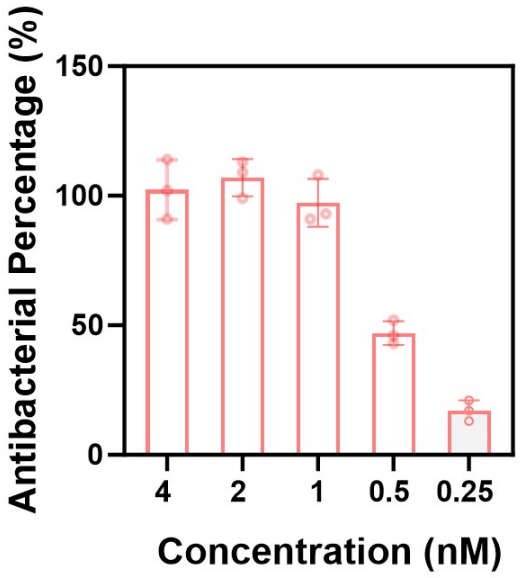


Fig. S11 Antibacterial rate of GNR@pAMPS-Cl at different concentrations.

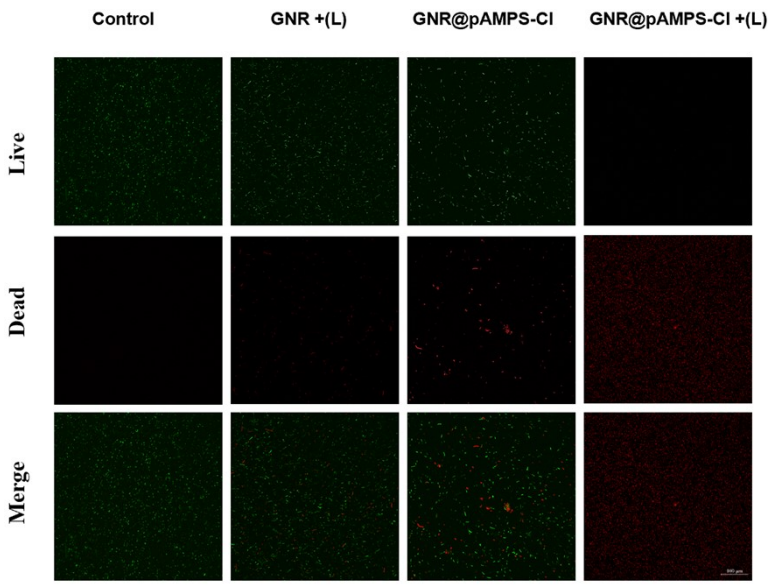


Fig. S12 Live/dead staining of *E. coli* pUC19 and MRSA bacterial strains treated with GNR@pAMPS-Cl (live bacteria are green, and dead bacteria are red).

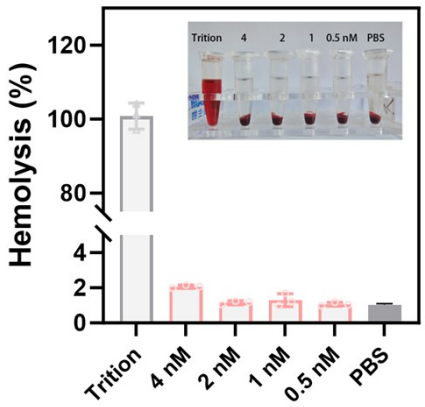


Fig. S13 Hemolysis rate of GNR@pAMPS-Cl at different concentrations

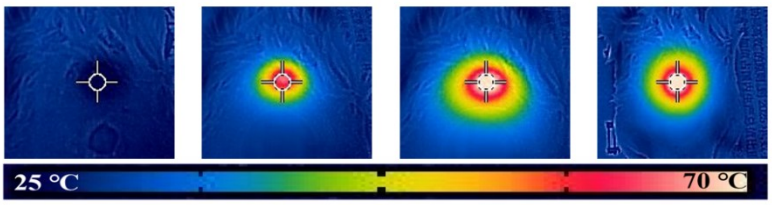


Fig. S14 Thermal images of GNR@pAMPS-Cl treatment of infected wounds before and after 808 nm NIR laser irradiation. The laser power density is 0.8 W/cm^2.

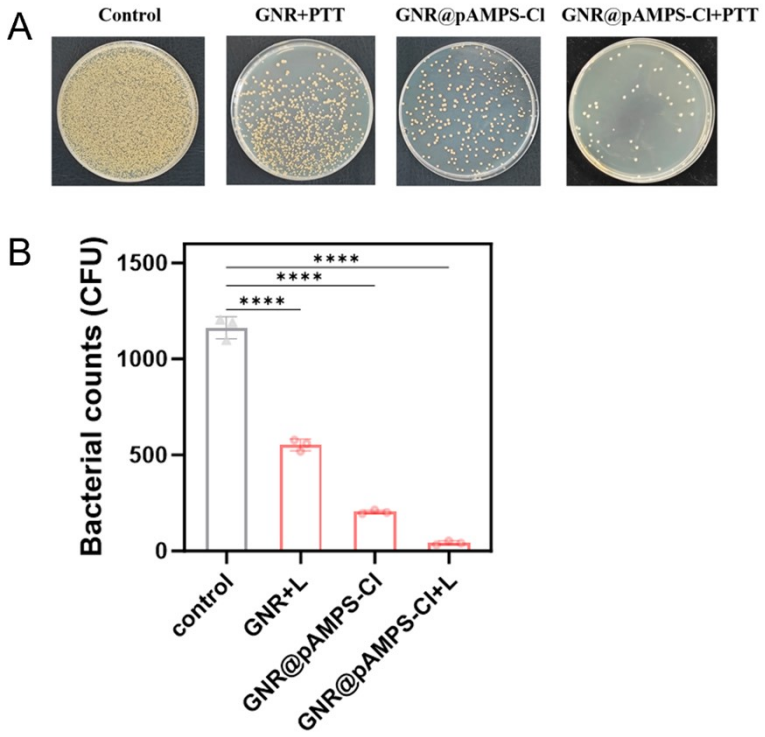


Fig. S15 (A) Corresponding bacterial survival culture plates treated with GNR@pAMPS-Cl after different conditions at day 7. (B) Bacterial counts of wounds in mice (mean ± SD, n = 3, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns denotes no significance).

References

1. G. Moad and C. Barner-Kowollik, *Handbook of RAFT Polymerization*, 2008, 51-104.
2. H. Zhu, E. Prince, P. Narayanan, K. Liu, Z. Nie and E. Kumacheva, *Chem. Commun.*, 2020, **56**, 8131-8134.