

Supporting information

Two dimensional BPs@AuNPs nanocomposites for photothermal/photodynamic therapy mediated wound disinfection and infectious wound healing under a single light source

Baolei Liu,[†] Yutian Su,[†] Shishan Wu,^{*,†} Jian Shen,^{*,†,‡}

[†]School of Chemistry and Chemical Engineering, Nanjing University, 163 Xianlin Avenue, Qixia District, Nanjing 210023, China.

[‡]National and Local Joint Engineering Research Center of Biomedical Functional Materials, Nanjing Normal University, Nanjing 210046, China.

Corresponding author: Jian Shen, jshen@njnu.edu.cn

Photothermal effect of the BPs@AuNPs. In order to elucidate the nanocomposites' photo-thermal effect, the as-prepared BPs@AuNPs was dilute to different concentrations with deionized water (10, 25, 50, 100 μM) and then exposed to a laser (at a power density of 1.5 W/cm^2 for 5 min. The heat maps and temperature profiles of the hydrogels were recorded using an infrared (IR) thermal camera.

Bacteria culture. As a proof-of-concept, gram negative *Escherichia coli* (*E.Coli*) and gram-positive *Staphylococcus aureus* (*S.aureus*) were employed in the experiment. Bacteria cells were prepared by inoculating a single bacterial colony from a Luria-Bertani (LB) plate and then suspended in 5 mL sterile Mueller-Hinton broth (MHB) medium at $37 \text{ }^\circ\text{C}$ and shook at 200 rpm for 12 h. The number of bacteria was estimated by measuring the medium absorbance at 600 nm using a UV-Vis spectrophotometer.

In vitro ROS generation. The cellular ROS generation in bacteria was characterized using DCFH-DA solution (20 μL , 0.01 M). Four groups of as-prepared bacterial suspensions (10 μL , 10^6 CFU ml^{-1}) were added into the following conditions: 1) 990 μL PBS buffer; 2) 990 μL PBS buffer + 650 nm laser (1.5 W/cm^2); 3) BPs@AuNPs coated 48-well plate; 4) BPs@AuNPs coated 48-well plate + 650 nm laser (1.5 W/cm^2). The indicator was co-incubated with bacteria in different groups for 30 min. Then, it was slightly washed three times with sterile PBS. At last, the fluorescence images of DCF were visualized by employing confocal laser scanning microscope.

In vitro antibiotic activity. For the in vitro antibiotic assay, four groups of as-prepared bacterial suspensions (10 μL , 10^6 CFU ml^{-1}) were added into the following conditions: 1) 990 μL PBS buffer; 2) 990 μL PBS buffer + 650 nm laser (1.5 W/cm^2); 3) BPs@AuNPs coated 48-well plate + 650 nm laser (0.5 W/cm^2); 4) BPs@AuNPs coated 48-well plate+ 650 nm laser (1.5 W/cm^2). After 2 h incubation at $37 \text{ }^\circ\text{C}$, the bacterial was re-suspended in PBS (final volume of 1 ml), the diluted bacterial solution (100 times) was taken out and plated on Luria-Bertani (LB) agar for 18 h. The viable bacteria were observed and presented by the Colony-Forming Units per mL (CFU/ml).

***In vivo* wound healing test on skin defect model.** To further evaluate the promoting effect of BPs@AuNPs on wound repair, *S.aureus* infected full-thickness skin defect model was established. Our animal experiments were approved by the institutional review board of Nanjing University. Female BALB/c mice weighting 25-35 g and 5-6-week age were used for studies. All mice were acclimatized for 1 week before surgery and then randomly divided into 4 groups including 1) PBS buffer; 2) PBS buffer + 650 nm laser (1.5 W/cm²); 3) BPs@AuNPs + 650 nm laser (0.5 W/cm²); 4) BPs@AuNPs + 650 nm laser (1.5 W/cm²). After anesthesia and shaved, skin wounds were created and 50 μL *S.aureus* (10⁸ CFU/mL) was used to establish infection. Then, control wounds were added with 50 μL of PBS and BPs@AuNPs group wounds were added with 50 μL of sample solution. For groups with laser, a 650 nm laser with different intensities were applied to irradiate the wound area for 5 min and the thermographic images of each group were recorded with a thermal infrared imager. All tissues were collected on each 3 mice in 4 groups on 14th day and stored at -80 °C before analysis. The regeneration process of wounds was assessed by wound closure ratio. For wound closure monitoring, on the 3rd, 7th, and 14th day, the mice in each group were performed standard anesthesia, then wound closure were measured by Image J. Wound area (%) were calculated using the formula below:

$$\text{Wound area} = \text{area (n day)} / (\text{area (0 day)}) \times 100\%$$

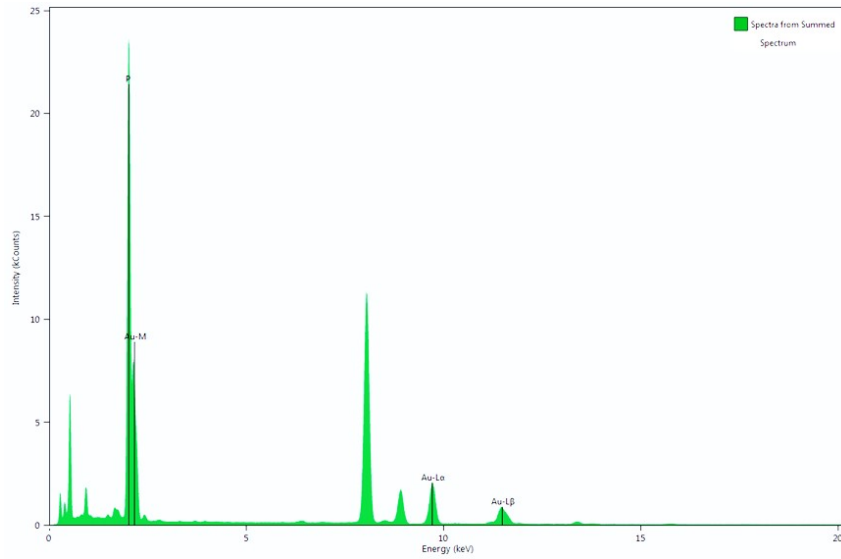


Figure S1. The EDS spectrum of BPs@AuNPs.

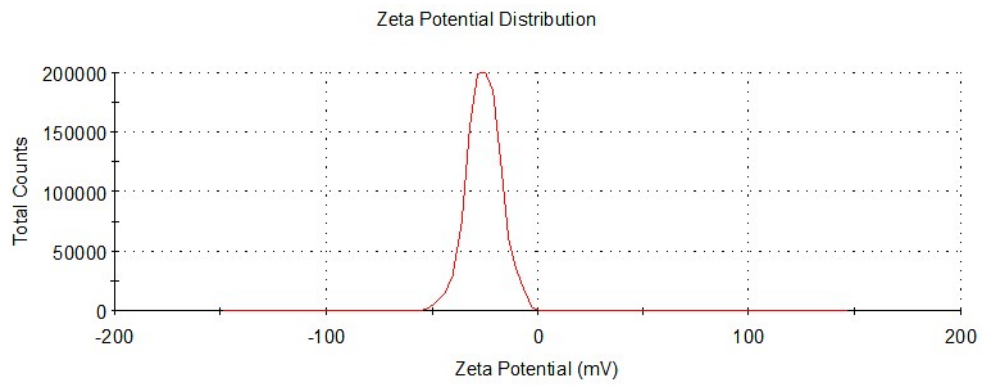


Figure S2. The Zeta potential of BPs@AuNPs.

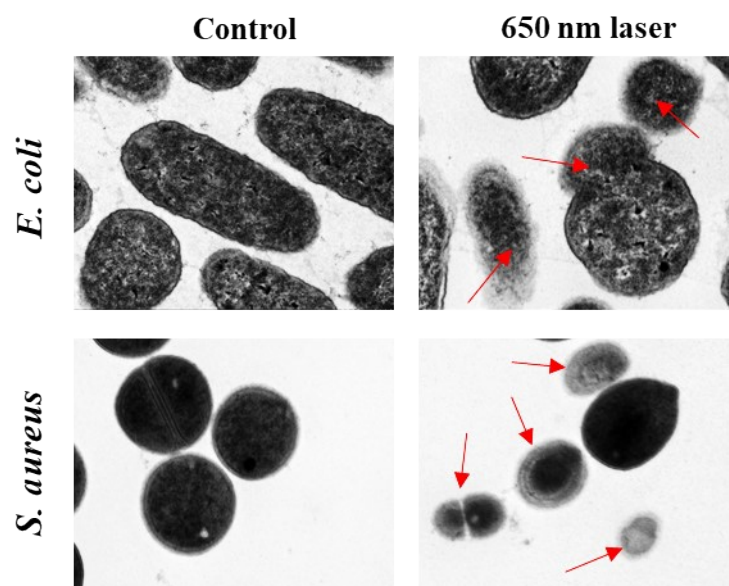


Figure S3. TEM images of *E. coli* and *S. aureus* treated with BPs@AuNPs under 650 nm light

irradiation.