Electronic Supporting Information

Stable gold graphitic nanocapsules doped hydrogel for efficient photothermal antibacterial applications

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1. Experimental section

1.1 Instrumentation

A UV-2450 spectrophotometer (Shimadzu, Japan) was used for UV-Vis spectra measurement. The average hydrated diameter and ζ-potential were measured by a Zetasizer Nano ZS90 DLS system (Malvern Instruments Ltd., England). Raman spectroscopy was measured on a Raman imaging microscope system (Renishaw, England) with the laser wavelength of 633 nm. Fluorescence imaging was performed on an Olympus IX71 fluorescence microscope (Olympus, Japan). A DSA100 Optical Contact Angle Measuring Device was used for the contact angle measurements (Kruss, Germany). The prepared materials were characterized by a JEM-2100F field-emission scanning electron microscopy (JEOL Ltd., Japan) and a JSM-6700F scanning electron microscope (JEOL Ltd., Japan). Oscillatory rheology experiments were performed on a rotational rheometer AR2000ex (TA, USA). Infrared spectroscopy was measured on a TENSOR27 Fourier transformed infrared spectroscopy (Bruker, Germany).

1.2 Reagents and Samples

Chitosan (CS, BR, μ = 50~800 mPa.s), methanol (CH_3OH), sodium hydroxide (NaOH), glutaraldehyde (GA, 25 %), hydrochloric acid (HCl) were purchased from China National Medicines Co., Ltd. (Shanghai, China). Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), sodium borohydride ($NaBH_4$) and silver nitrate ($AgNO_3$) were obtained from Sigma Aldrich (USA). Poly(vinyl alcohol) (PVA) was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Anhydrous chloroauric acid ($HAuCl_4$), o-nitrophenyl-β-D-galactoside (ONPG)

and fumed silicon were purchased from Aladdin (Shanghai, China). Dulbecco's modified Eagle medium (DMEM), RPMI 1640 medium, penicillin-streptomycin (PS) solution and fetal bovine serum (FBS) were obtained from Invitrogen (USA). Propidium iodide (PI) and acridine orange (AO) were purchased by Thermo Fisher Scientific (USA). Human embryonic kidney 293 (HEK293) and human LO2 hepatocytes (LO2) cells were obtained from Guangzhou Cellcook Biotech Co., Ltd. (Guangzhou, China). *Escherichia coli* (*E. coli, ATCC 25922*) and *Staphylococcus aureus* (*S. aureus, ATCC 6538*) were obtained from Shanghai Luwei Science and Technology Co., Ltd. (Shanghai, China). Analytically pure reagents and deionized water with resistance of 18.2 MΩ/cm were used throughout the experimental process.

1.3 Synthesis of PVA-AuNR@G

Gold nanorod (AuNR) and AuNR@SiO_2 were prepared by the seed-mediated growth method[1] and a modified Stöber method[2], respectively. AuNR graphitic nanocapsules (AuNR@G) was produced in a chemical vapor deposition (CVD) system with a flow of 50 cm^3/min methane for 12 min at 1000 ℃. After that, the PVA functionalized AuNR@G (PVA-AuNR@G) was obtained by etching the silicon from the resultant products using HF in PVA solution. Finally, PVA-AuNR@G was purified by centrifugation at 10000 rpm for 25 min.

1.4 Synthesis of AG-PC hydrogel

At first, 6 g PVA and 1 g CS were added in acetic acid solution (80 mL, 0.175 M)

with stirring in 90 ℃ oil bath until the solid substances were completely dissolved. Next, PVA-AuNR@G solution (2.5 mg/mL, 20 mL) was added to the mixture of PVA and CS (PVA/CS solution), then glutaric dialdehyde (GA, 800 μL, 2.5 %) was injected into the mixture above with stirring. After the solution cooled to room temperature, the AuNR@G doped PVA/CS hydrogel (AG-PC hydrogel) was finally formed. Besides, the PVA/CS hydrogel (PC hydrogel) was obtained with the same method for AG-PC hydrogel preparation without the addition of PVA-AuNR@G.

1.5 ICP-OES measurements

A. Determination of Au in 1g AG-PC hydrogel：Five AG-PC hydrogel samples with quality of 1 g were placed in centrifuge tube containing deionized water for overnight. The samples were treated with repeated high-speed oscillation to break them completely, followed by heating them in boiled aqua regia for several times. Inductively coupled plasma optical emission spectrometer (ICP-OES) analysis was executed to measure the concentration of Au.

B. Leakage of AuNR@G in 0.5 g hydrogel: 0.5 g AG-PC hydrogel warped with white nylon gauze (internal mesh aperture 0.074 mm) was put into a wide-neck flask containing 400 mL phosphate buffer solution (PBS, 0.1 M, pH 7.4). Then, the wide-neck flask was put on the shaking bed with a speed of 200 rpm at 37 ℃ for 2 weeks. The sample solution was collected every 24 h followed by heating it in boiled aqua regia for several times, ICP-OES analysis was finally executed to measure the concentration of Au^{+}.

114

115 1.6 Thermodynamics analysis

116 8 mg AG-PC and PC hydrogel were put in an Al_2O_3 sample pan of the
117 simultaneous thermal analyzer (STA 409 PC/4/H), respectively, then the differential
118 scanning calorimetry (DSC) and thermogravimetric (TG) curves were obtained by
119 heating the samples to 800 ℃ at the heating rate of 10 ℃/min and carrier gas flow rate
120 of 30 mL/min.

121

122 1.7 Swelling ratio tests

123 At first, newly prepared AG-PC and PC hydrogel sample were immersed in
124 deionized water for 24 h to obtain swollen hydrogels. Next, the excess surface-
125 adhered water was removed by filter paper rapidly and the swollen hydrogels were
126 weighed. The swelling ratio (*SR*) of the hydrogel was calculated using the following
127 equation, where *Wt* and *Wd* stand for the weight of initial hydrogel and swollen
128 hydrogel, respectively.

129 $$SR = \frac{Wt - Wd}{Wd} \times 100\,\%$$

130

131 1.8 *In Vitro* cell culture and cytotoxicity tests

132 The human LO2 and HEK293 cell lines were cultured in 1640 and DMEM
133 culture medium, respectively, and supplemented with 10 % FBS and 1 % PS solution
134 at 37 ℃ in a 5 % CO_2 incubator. Measurement of cell viability was carried out by
135 reduction of CCK-8 relative to control cells incubated with the same volume of

Dulbecco's phosphate buffered saline (DPBS). Cells were seeded into 96-well plates in 100 μL DMEM or 1640 culture medium containing 10 % FBS and 1 % PS at a seeding density of 10000 cells each well and then incubated for 12 h at 37 °C to permit cell adhesion. To extract the infiltration of AG-PC hydrogel, 0.5 g sterilized hydrogel were washed with DPBS repeatedly and the immersed in 5 mL DMEM/1640 at 37 ℃ on a shaker bed at 150 rpm under sterile conditions over 48 h. The infiltration was diluted with DMEM/1640 to 100 %, 75 %, 50 %, 25 %. Cells were then maintained in 100 μL culture medium without or with four different diluted infiltration for 12 h and 24 h. After removal of the supernatant, 90 μL fresh cell culture medium and 10 μL CCK-8 were added to each well and the plates were incubated for another 2 h at 37 ℃ in the dark. The absorbance was measured at 450 nm, and the assay was repeated over three times.

1.9 Bacterial culture

E. coli and *S. aureus* were inoculated into nutrient agar by streak plate method, and cultured in a constant temperature incubator for 24 h. Colonies were transferred to the nutrient broth culture medium at 37 ℃ and grown to mid-log phase. After centrifugation and washing for three times, PBS was used to prepare a series of bacterial suspensions with different concentration.

1.10 Bacterial adsorption performance test

At first, crystal violet (CV)-stained *S. aureus* with concentration of 10^6, 10^7 and

158 10^8 CFU/mL obtained according to gram's staining method.[3] Next, the stained
159 bacteria solution was added into the screw-mouth bottle with AG-PC hydrogel at the
160 bottom and kept for 24 h, the hydrogel slice at the depth of 0, 1, 2 mm were observed
161 under an inverted fluorescence microscope. Absorption of the bacterial solution at 600
162 nm were measured, the corresponding values before and after adsorption were
163 denoted as OD_b and OD_a, respectively. Bacterial adsorption rate of the hydrogel was
164 calculated according to the following equation:

165 $$\text{Adsorption rate} = \frac{(OD_b - OD_a)}{OD_b} \times 100\,\%$$

166

167 1.11 Bacteria dyeing

168 The fluorescent dyes were prepared by dissolving 10 mg AO and 10 mg PI in 10
169 mL PBS. 1 mL bacterial solution (10^8 CFU/mL) was added into three 24-well plates
170 covered with (*AG-PC hydrogel group*) and without (*control group*) hydrogel, then
171 each plate was irradiated with 0, 1 or 2 W/cm^2 laser for 10 min, respectively. Then
172 removing the bacteria solution and washing with PBS for three times, the obtained
173 bacteria were dispersed in 1.5 mL PBS. After that, the bacteria were stained with 100
174 mL fluorescent dyes for 15 min followed by washing with PBS. Finally, the obtained
175 samples were finally observed under a fluorescence microscope, in which bacterial
176 cells in green/red stand for live/dead bacteria with intact/damaged membranes.

177

178 1.12 Plate colony counting experiments

179 1 mL bacterial solution (10^8 CFU/mL) was added into three 24-well plates

covered with (*AG-PC hydrogel group*) and without (*control group*) hydrogel, then each plate was irradiated with 0, 1 or 2 W/cm^2 laser for 10 min, respectively. After that, the bacteria solution was diluted 10^4 times to 10^4 CFU/mL using 0.1 M PBS (pH 7.4), then 50 μL diluted bacterial solution with the number of ~500 CFU was spread onto Mueller-Hinton agar plates and incubated at 37 ℃ for 24 h to form viable colony units. The number of survival bacteria was finally recorded. The tests were repeated 3 times. The bacterial viability was calculated according to the following equation, where CUF_o stands for colony forming units in *control group* without laser exposure and CUF_h stands for colony forming units measured in other five plates with different laser exposure, respectively.

$$\text{Bacterial viability} = \frac{CUF_h}{CUF_o} \times 100\%$$

1.13 SEM characterization of bacteria

E. coli and *S. aureus* (10^8 CFU/mL) were added to the 24-well plate covered AG-PC hydrogel at the bottom of the plate, respectively. Each plate was irradiated with 2 W/cm^2 laser for 10 minutes, then the bacteria solution was fixed in 2.5 % glutaraldehyde for 4 h. Subsequently, the bacteria solution above was centrifugated and washed with PBS three times, the dehydration process was performed through treating with 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 100 % gradient ethanol for 10 min. Finally, the morphology of the freeze-dried both *E. coli* and *S. aureus* were observed on a SEM, respectively.

1.14 Photothermal antibacterial mechanism study of AG-PC hydrogel

The cytoplasmic membrane permeabilization was analyzed by determining the release of β-galactosidase from the cytoplasm of *E. coli* or *S. aureus* using ONPG as the substrate.[4] *E.coli* and *S. aureus* were grown to mid-log phase in Mueller-Hinton medium containing 2 % lactose at 37 ℃. The bacteria with concentration of 10^9 CFU/mL before and after freeze drying were added to a 24-well plate with AG-PC hydrogel at its bottom, respectively. Every plate was irradiated with 1 or 2 W/cm^2 laser for 2.5, 5, 10 or 20 minutes. The extracted bacterial solution, 1 mL 20 mmol/L ONPG and a certain volume of PBS were added in the test tube to obtain a 10 mL mixed solution. The test tube was oscillated for 10 min under a 37 ℃ shaker, and the absorbance at 420 nm was detected by UV-Vis spectrophotometer.

2. Results and discussion

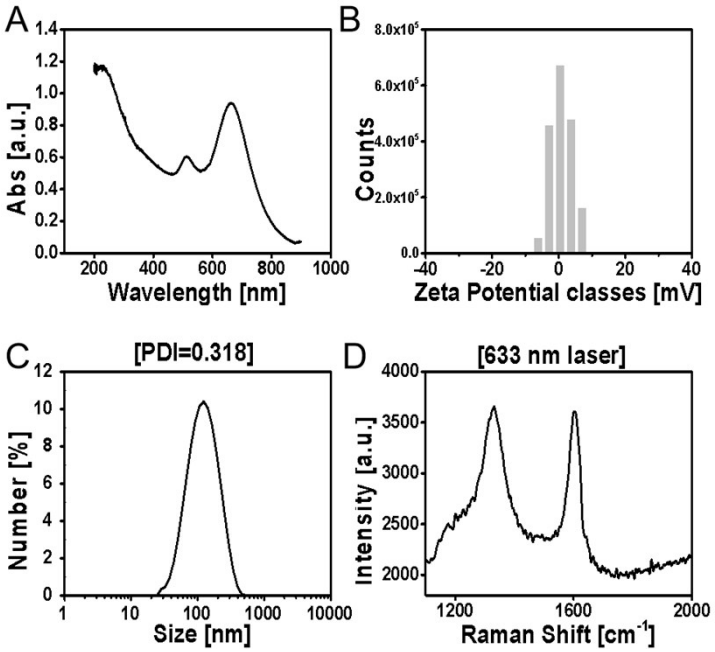

Fig. S1. Characterization of AuNR@G. (A) UV-Vis spectrum, (B) ξ-potential curve, (C) Dynamic light scattering (DLS) characterization of the size distribution and (D) Raman spectrum.

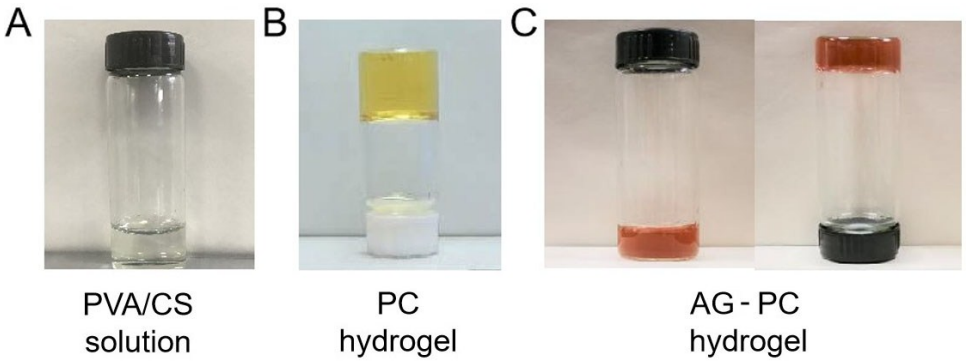

Fig. S2. Photographs of the prepared materials. Pictures of (A) PVA/CS solution, (B) PC and (C) AG- PC hydrogel.

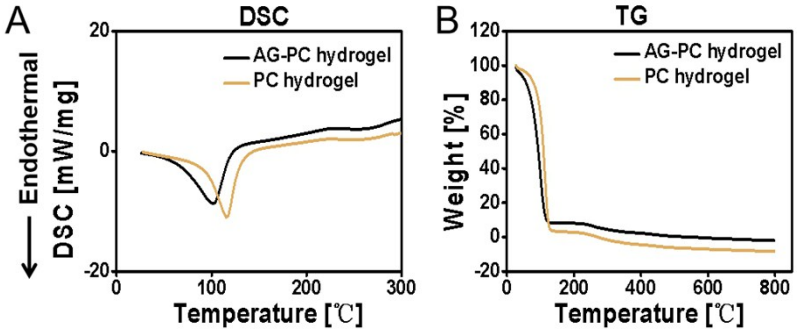

Fig. S3. DSC and TG analysis of the hydrogels. (A) Differential scanning calorimetry (DSC) and

(B) thermogravimetric (TG) curves of AG-PC and PC hydrogel.

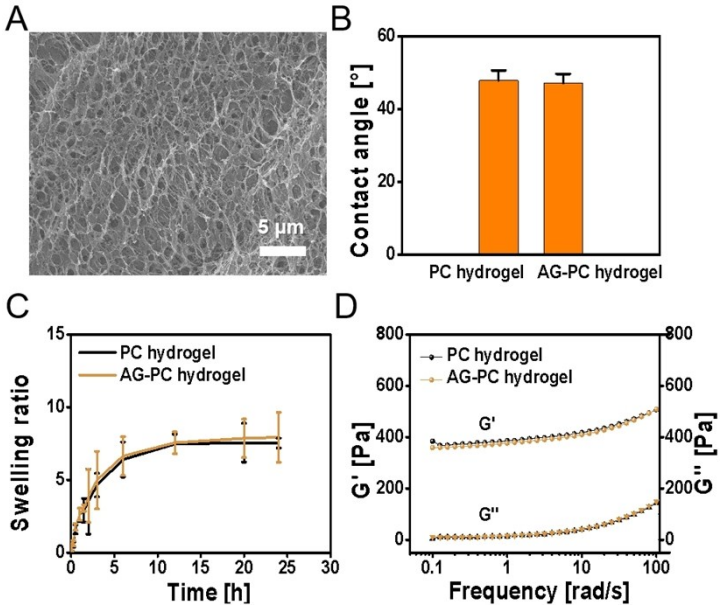

Fig. S4. Physical properties of the hydrogels. (A) SEM image of PC hydrogel. (B) Contact angle, (C) swelling ratio and (D) tensile property measurements of AG-PC and PC hydrogel.

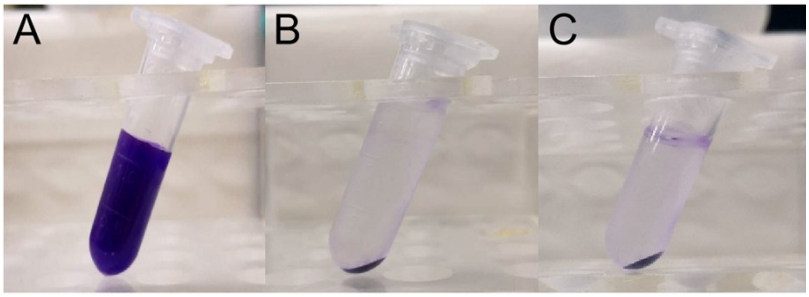

Fig. S5. Photographs of CV-stained *S. aureus*. Pictures of newly stained bacterial solution (A) before and (B) after centrifugation, (C) stained bacterial solution stored for one day after centrifugation.

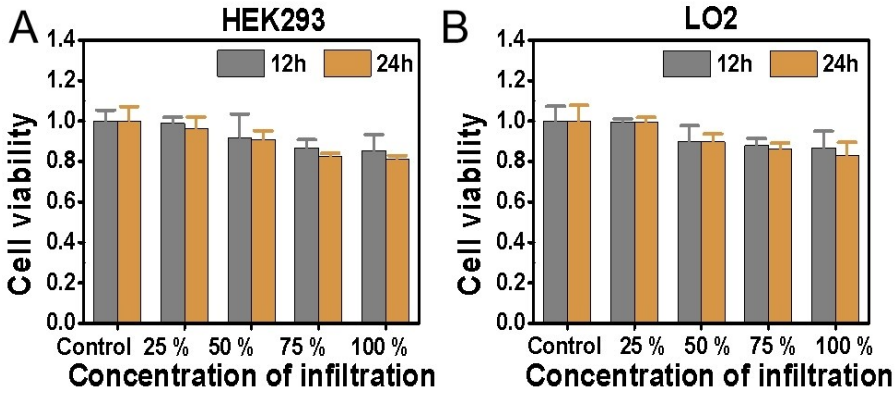

Fig. S6. Cytotoxicity tests. Cytotoxicity test of AG-PC hydrogel on (A) HEK293 and (B) LO2 cell lines.

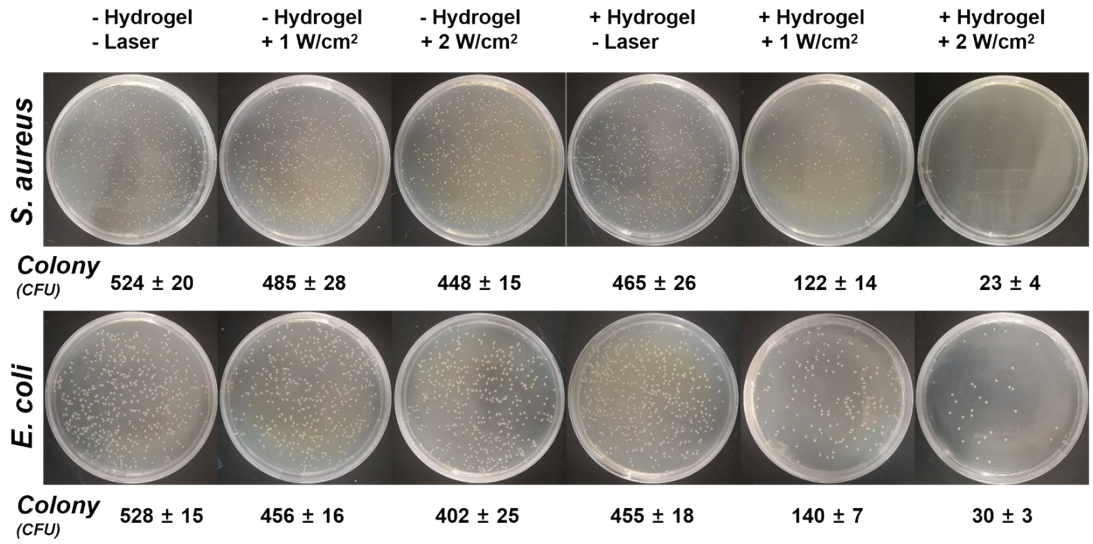

Fig. S7. Bacterial viability tests. Bacterial viability tests of both *S. aureus* and *E. coli* (~500 CFU) with or without 10 min exposure of different power lasers.

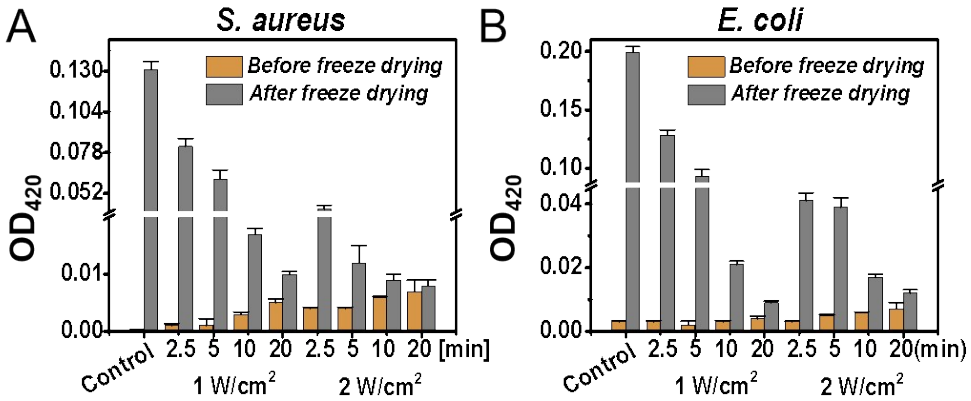

Fig. S8. ONPG tests. O-nitrophenol absorbance at 420 nm of (A) *S. aureus* and (B) *E. coli* at different times.

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