Supporting Information

Large-scale production of Au nanoparticles as medical antibiotics

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Experimental Section

Materials

We purchased $HAuCl_4 \cdot 3H_2O$ (99.99%) from Jinke Chemical Co., Ltd., China. We purchased 4,6-diamino-2-pyrimidinethiol (DAPT) from Merck China Co., Ltd. We purchased 1,1-dimethylbiguanide (DMB) and Tween 80 from Sigma. We purchased $NaBH_4$ and Acetic acid from Beijing Chemical Reagents Co., China. We purchased

Medical gauze from Zhengde surgical dressing Co., Ltd., China. We purchased commercial AgNPs solution and AgNPs-based gauze from Changsha hairun biological technology Co., Ltd., China, which is most widely used in hospitals in China. Escherichia coli ATCC 117759 (*E. coli*), Pseudomonas aeruginosa ATCC 27853 (*P. a*) and Staphyloccocus aureus ATCC 6538P (*S. a*) were from China General Microbiological Culture Collection Center. The human umbilical vein endothelial cell line (HUVEC) was purchased from American Type Culture Collection (ATCC, USA).

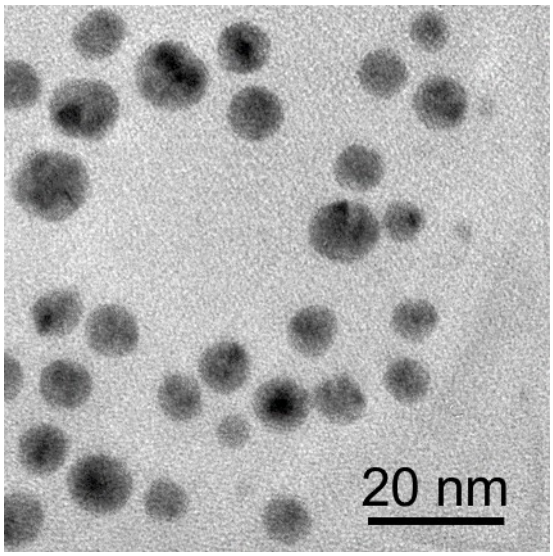

Figure S1 TEM image of the commercial AgNPs

Preparation and characterization of AuNPs

We mixed the aqueous solution of $HAuCl_4 \cdot 3H_2O$ (4 mM), DAPT (4 mM), and DMB (4 mM) in the presence of acetic acid and non-ionic surfactant Tween 80 by stiring for 10 min in the ice-water bath, then added the aqueous solution of $NaBH_4$ (12 mM) dropwise with vigorous stirring (In order to realize that producing the AuNPs in

industrial scale, we explored to accelerate the dropping speed with different time.). After an hour of gentle stirring, we purified NPs by dialysis (14 kDa MW cutoff) in deionized water for above 24 h (Dialyzing is unpractical for the industrial production, we also check the AuNPs without dialyzing by antibacterial test and cellular toxicity evaluation below.). The mass concentration of AuNPs are determined with inductively coupled plasma optical emission spectrometry (ICP-OES). We observed morphologies of NPs with transmission electron microscopy (TEM, JEM-2100F transmission electron microscope operated at 200 k V, Japan) and scanning electron microscopy (SEM, HITACHI S-4500 scanned electron microscope, Japan). UV-vis absorption spectra were recorded on UV-2450 (Shimadzu) spectrophotometer.

Antibacterial activity test

Escherichia coli (*E. coli*) and Pseudomonas aeruginosa (*P. a*) were selected as model Gram-negative bacteria and Staphyloccocus aureus (*S. a*) was selected as model Gram-positive bacteria to study the antibacterial activity of NPs. We determined the minimum inhibitory concentration (MIC) using a microbroth dilution method according to the NCCLS M7-A8 (2009) standard. Bacteria were cultured in the Luria-Bertani (LB) medium (10 g/L casein tryptone, 5 g/L yeast extract, and 10 g/L NaCl, pH = 7) at 37 ^{o}C on a shaker bed at 200 rpm until the logarithmic phase ($OD_{600\ nm}$ 0.5). We added 100 μL of AuNPs or other reagent at a gradually halving concentration in the MH broth in a 96-well microplate and 100 μL LB medium is used as a control, then added 10 μL of bacterial suspension (the final density of bacteria was 2-5 $\times 10^4$ CFU/mL) in each well,

and incubated them at 37 °C for 24 h. The MIC was the concentration at which no visible bacteria grew. Also, we evaluated the MIC of AuNPs or AgNPs by determining the optical density at 600 nm ($OD_{600\ nm}$) of bacterial suspension treated with NPs by Tecan infinite 200 multimode microplate readers (the absorbance of gold NPs is essentially zero compared to that of suspended bacteria at 600 nm). The data of $OD_{600\ nm}$ below 0.1 can be regarded as no visible bacteria grew.

In a 96-well microplate, 100 μL of a certain reagent aqueous solution (with LB medium) is added to a row of wells in a gradually halved concentration, such as $HAuCl_4.3H_2O$ at a concentration of 320 mg L^{-1} (No.1 well), 160 mg L^{-1} (No.2 well), 80 mg L^{-1} (No.3 well), 40 mg L^{-1} (No.4 well), 20 mg L^{-1} (No.5 well), 10 mg L^{-1} (No.6 well), 5 mg L^{-1} (No.7 well) and 0 mg L^{-1} (No.8 well as the control), while the amount and concentration of bacteria following being added in each well is the same (the final density of bacteria is 2~5 × 10^4 CFU/mL). So, each well along the row of the 96-well microplate has a decreasing amount of the reagent but same amounts of bacteria before incubation.

After incubation at 37 °C for 24 h, if the solution (containing reagent and bacteria) in a well is clear, it proves that the concentration of the reagent (such as $HAuCl_4.3H_2O$ at 160 mg L^{-1}, 80 mg L^{-1} or 40 mg L^{-1}) in the well can inhibit bacterial growth; but if it is turbid (such as $HAuCl_4.3H_2O$ at 20 mg L^{-1}, 10 mg L^{-1} or 5 mg L^{-1}), it proves that the concentration cannot inhibit bacterial growth, because the growth of bacteria caused the turbidity of the solution. As the result, for the reagent, the more the number of clear wells, the smaller the minimum inhibitory concentration (MIC), and the better of

antibacterial ability.

Cytotoxicity assay

HUVEC cells were cultured in the Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum. We incubated 1×10^4 cells per well of HUVEC in 96-well plates with different concentrations of samples (AuNPs without dialysis, AuNPs with dialysis, AgNPs and $AgNO_3$) in 200 μL of medium at 37 ^oC for 48 h, Cells without any treatment were used as the control, washed the cells once with PBS (0.01 M, pH 7.4), added 10% (v/v) of the CCK-8 solution in the medium, incubated the sample at 37 °C for 2 h, and determined the absorbance at 450 nm referred with 650 nm with the Tecan infinite 200 microplate reader.

Produce the AuNPs-based medical gauze from small-scale to large-scale

In order to put the antibacterial AuNPs into medical application, we choose the dressing as the first object, because the dressing is the most widely used medical products. We spent a period of time investigating the medical dressing from companies and hospitals in China, finding that the antibacterial AgNPs-based gauze was most widely used. We prepared the AuNPs-based gauze in small-scale and large-scale. In the small scale, three steps were needed, as shown in Figure 5. First, the gauze was dipped into the AuNPs solution. Second, using the padder squeeze the excessive excess solution. Third, the gauze was drying in dryer at 150 ^oC for minutes. In the large scale, the three steps were integrated into continuous process through the continuous dyeing and finishing

machine.