Supporting information

High-performance magnetic antimicrobial Janus nanorods decorated with Ag nanoparticles

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Experimental Section

**Reagents.** Polyacrylic acid (PAA), iron (III) chloride anhydrous (FeCl₃), diethylene glycol (DEG), tetraethyl orthosilicate (TEOS, 98%), 3-aminopropyltrimethoxysilane (APS) and cetyltrimethylammoniumbromide (CTAB) were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH), ethonal, aqueous ammonia (NH₃·H₂O, 28%), and Silver nitrate (AgNO₃) were obtained from Beijing Chemical Reagent Factory. All reagents were used as received without further purification. Peptone, Yeast extract, Agar, Sodium chloride were purchased from Shanghai Sangon Biotechnology Co. Ltd. Escherichia coli, Bacillus subtilis and the related chemical and biological agents were supplied by State Key Laboratory for Supramolecular Structure and Materials.

**Preparation of Superparamagnetic Fe₃O₄ nanoparticles.** The Fe₃O₄ nanoparticles were produced by using a high temperature hydrolysis reaction. A stock NaOH solution was prepared by dissolving 2.0 g of NaOH in 20 mL of diethylene glycol (DEG). FeCl₃ (0.8 mmol), poly(acrylic acid) (PAA, 8 mmol), and DEG (34 mL) were added into a three-necked flask, and the mixed solution was heated to 220°C for 30 min under nitrogen. The NaOH solution obtained beforehand was then dropped rapidly into the mixture with vigorous mechanical stirring for an additional 1 h. The resulting black mixture was cooled to room temperature and, thereafter, washed with deionized water and ethanol three times via centrifugation.

**Preparation of Fe₃O₄-SiO₂ JNRs:** Hydrophilic Fe₃O₄ nanoparticles were produced by using a high temperature hydrolysis reaction. 0.05 g CTAB was dissolved in 10 ml ultrapure water under ultrasonic treatment, followed by adding 1 ml (8.6 mg ml⁻¹) Fe₃O₄ NPs solution. Then, the mixture was mechanically stirred at 40 °C water bath and 0.5 ml NH₃·H₂O solution was injected into the solution, followed by a certain amount of TEOS. After 30 min, Fe₃O₄-SiO₂ JNRs were obtained followed by washing with ethanol for 3 times. In order to remove CTAB, the JNRs were firstly gradually transferred to acetone phase (60 mL) and then refluxed at 80°C for 48 h. The successful
removal of CTAB could be characterized by Fourier transform infrared spectra. (Figure S1). The aspect ratio of 2:1, 3:1, 5:1 and 7:1 can be facilely controlled by adjusting the molar ratio of [TEOS]/[Fe$_3$O$_4$NP] of 3.8, 4.9, 7.6 and 11.3, respectively.

**Preparation of Ag@Fe$_3$O$_4$-SiO$_2$ JNRs:** The surface of Fe$_3$O$_4$-SiO$_2$ JNRs were further grafted with APS by mixing the nanorods with 0.1 mL APS and 30 mL ethanol for 12 hours at room temperature. After being purified by centrifugation, the APS-functionalized JNRs in ethanol solution were added into 15 ml aqueous solution with severe stirring. 10 ml of 0.1 M AgNO$_3$ solution was first added into above stirring solution avoiding light exposure, followed by dropping 1 ml NH$_3$H$_2$O solution over a time period of 10 min. The finally product was centrifuged and washed with aqueous solution for four times, and was dispersed in 1 ml H$_2$O stored in the refrigerator at 4 °C.

**Cytotoxicity of Ag@Fe$_3$O$_4$-SiO$_2$ hetero-nanorods:** Human lung adenocarcinoma epithelial cell line A549 were cultured in RPMI-1640 medium, supplemented with 10% heat-inactivated fetal bovine serum and antibiotics at 37 °C in the humidified atmosphere with 5% CO$_2$. The cells grew overnight in 96-well plates at a density of 7×10$^3$ cells/well. Cells were incubated with fresh media containing AgNPs@ Fe$_3$O$_4$-SiO$_2$ JNRs with different concentrations for 24 hours. 10 μL of MTT solution of 5 mg ml$^{-1}$ was added to each well incubated at 37 °C for 4 h, and then were lysed with 10% acid sodium dodecyl sulfate solution. The absorbance of supernatants was measured at 570 nm by using a microplate reader.

**Antimicrobial Test and Bacterial genetic test:** Bacteria cells were grown overnight in LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl, 1.5% agar) at 37 °C and in a controlled shaking incubator. The *E. coli* or *B. subtilis* suspensions were coated on the surface of LB-agar plates. A total of 200 μL of this stock solution was plated on a single LB-agar growth plates incubated overnight at 37°C. Colonies were visualized the next day, and digital images of the plates were captured. For bacterial genetic test, bacterial suspension was added to LB medium with different concentrations of JNRs, and the mixture was shaken by a rotary shaker at 37 °C. Before bacterial colonies were counted by measuring OD600
using a UV/Vis spectrophotometer, a magnetic field was applied by a magnet to collect the AgNPs@Fe₃O₄-SiO₂ JNRs. LB bacteria suspension with no particles was as a growth control.

**Hemolysis test:** Rabbit was fixed on its back on the operation table. The common carotid artery was separated and blood was collected in centrifuge tubes. The blood was stirred gently with a glass rod to removal of fibrin, and then defibrinated rabbit blood was washed by centrifugation with physiological saline for 3 times. 1 part of erythrocytes was suspended in 49 part of 9% NaCl to obtain 2% Erythrocyte suspension used for testing. A variety of drug solution (see Table S2 in the Supporting Information) was added into the test tube with gently shaking at 37 °C, and the phenomenon of hemolysis was observed at 0.5 h, 1h, and 24 h.
**Figure S1.** Fourier transform infrared spectrum of Fe$_3$O$_4$-SiO$_2$ JNRs.
Figure S2. Square-wave curve of the changes in UV-Vis absorption peak of the bacterial solution mixed with AgNPs@Fe$_3$O$_4$-SiO$_2$ JNRs before and after magnetic separation.
Figure S3. Zone of inhibition test. Zone of inhibition of AgNPs@Fe₃O₄-SiO₂ JNRs with a 200-times dilution (1) and a 20-times dilution (2) of the original solution (3) against *E. coli* (a) and *B. subtilis* (b), showing a typical dose-dependent antibacterial effect of AgNPs@Fe₃O₄-SiO₂ JNRs.
**Figure S4.** Bacterial growth curve in LB liquid medium. *E. coli* (a) as the control and *E. coli* in the presence of AgNPs@Fe3O4-SiO2 JNRs with different lengths of 200 nm (b), 250 nm (c) and 500 nm (d).
*Figure S5.* TEM image of AgNPs@Fe₃O₄-SiO₂ JNRs of 250 nm length after killing bacteria
**Figure S6.** (a) UV-vis absorption spectra of AgNPs synthesized by chemical reduction of silver nitrate by sodium citrate in deionized water with storage time of 1 day, 4 days and 7 days. (b) Zone of inhibition of AgNPs@Fe₃O₄-SiO₂ JNRs of a freshly prepared sample (A1) and a conserved sample for one week (A2) against *B. subtilis*, and AgNPs of a freshly prepared sample (B1) and a conserved sample for one week (B2) against *B. subtilis*. 
**Figure S7.** Zeta potential of AgNPs@Fe$_3$O$_4$-SiO$_2$ JNRs.
Figure S8. SEM image of AgNPs@Fe₃O₄-SiO₂ JNRs of 500 nm length deposition on bacteria.
Table S1. Growth of gram-negative and -positive bacteria with different concentrations of AgNPs@Fe₃O₄-SiO₂ JNRs after incubation with 10⁶ per mL of *E. coli* or *B. subtilis*. ( “−” no growth; “+” growth)
Table S2. Different concentrations of test drug solution (AgNPs@Fe₃O₄-SiO₂ JNRs) and physiological saline in a test tube. The particle concentrations in test tube No.1, 2, 3, 4 and 5 are 66, 132, 198, 264 and 330 \( \mu g \) ml\(^{-1}\), respectively. Tube No.6 and No.7 are as the control. No.6 is normal physiological environment; No.7 is hypotonic solution.

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