Electronic Supplementary Information

Functional nanonetwork-structured polymers and carbons with silver nanoparticle yolks for antibacterial application

Yanhuan Lin,[a] Kairong Xiong,[b] Zhitao Lu,[a] Shaohong Liu,*[a] Zhaojie Zhang,[a] Yuheng Lu,[a] Ruowen Fu*[a] and Dingcai Wu*[a]

[a]Materials Science Institute, PCFM Lab and GDHPRC Lab, School of Chemistry, Sun Yat-sen University, Guangzhou 510275, P. R. China.

[b]School of Environmental Science and Engineering, Guangdong University of Technology, Guangzhou 510006, P. R. China.

E-mail: wudc@mail.sysu.edu.cn; cesfrw@mail.sysu.edu.cn;

liushh27@mail.sysu.edu.cn.

Experimental

1. Sample preparation

Materials. Styrene was passed through alkaline alumina column before polymerization. CuBr was purified by stirring over glacial acetic acid, followed by filtration and washing of the solid three times with ethanol and drying by N_2 flow overnight. The other chemicals were of analytical grade and used as received without further purification. $AgNO_3$, polyvinylpyrrolidone (PVP, M_w=55000), 3-aminopropyl triethoxysilane (APTES), N, N, N', N', N''-pentamethyl diethylene triamine (PMDETA), 2-bromoisobutyryl bromide, triethylamine, anhydrous aluminium trichloride ($AlCl_3$) and anhydrous dichloromethane were obtained from Aladdin. Tetraethyl orthosilicate (TEOS), sodium chloride, ammonia water, ethylene glycol, isopropanol, hydrofluoric acid, carbon tetrachloride (CCl_4), acetone, and tetrahydrofuran (THF) were obtained from Guangzhou Chemical Reagent Factory. Beef extract, peptone and agar were purchased from Guangzhou Institute of Microbiology.

Preparation of silver nanoparticles. 10 g of PVP was dissolved in 75 mL of ethylene glycol at room temperature, and 400 mg of $AgNO_3$ was added to this solution with continuous stirring until complete dissolution of the $AgNO_3$. Then, the system was heated at 120 °C for 1 h. After cooling down to the room temperature, the silver colloid could be separated from the solution after adding a large amount of acetone, followed by settlement overnight. The precipitate was washed three times with ethanol by centrifugation at 12000 rpm for 60 min and dispersed in 10 mL of ethanol.

Preparation of Ag@SiO_2 nanospheres. The Ag nanoparticles obtained above were dispersed in 180 mL of 2-propanol solution and ultra-sonicated for 15 min. After stirred at 40 °C for 5 min, 18 mL of H_2O and 10 mL of ammonia aqueous solution (25 wt %)

were added into the solution. Then, 0.8 mL of TEOS in 20 mL of 2-propanol solution was injected dropwise for 1 h. After reacting for 2 h, the as-obtained $Ag@SiO_2$ nanospheres were separated by centrifugation at 12000 rpm for 5 min and washed with ethanol for three times. The diameter of $Ag@SiO_2$ nanospheres could be tuned by tailoring the feed of TEOS.

Surface modification of $Ag@SiO_2$ nanospheres. $Ag@SiO_2$ nanospheres were dispersed in 110 mL of ethanol/water solution (v/v=10:1) in three-necked flask and 1 mL of APTES was added dropwise to solution for 2 h. After reacting for 12 h at room temperature and 2 h at 80 ^oC, amino group functionalized $Ag@SiO_2$ ($Ag@SiO_2$-NH_2) was obtained. Then the product was centrifuged and washed twice with ethanol and twice with dichloromethane. Subsequently, 1 g of $Ag@SiO_2$-NH_2 dispersed in 30 mL of anhydrous dichloromethane solution was placed in a single-neck flask, and then filled with N_2 for 30 min. After adding 1.4 mL of triethylamine, the reactor was transferred into an ice-water bath, and then 1.2 mL of 2-bromoisobutyryl bromide was added dropwise for 30 min. After stirred for 3 h, the reaction temperature was raised to 25 °C for 48 h. The as-obtained product was washed with THF and acetone/water (v/v=1:1) mixture for three times, respectively, and then dried in vacuum at 40 °C for 12 h.

Preparation of $Ag@SiO_2$-g-PS. A Schlenk flask was firstly charged with 0.2 g of $Ag@SiO_2$-Br, 9.8 mg of $CuBr_2$, 100 μL of PMDETA and 12.6 mL of styrene. After gently purging N_2 for 30 min, 62.7 mg of CuBr was added to the solution rapidly. The N_2 was bubbled for another 30 min to remove air completely. Then the flask was sealed and heated to 90 °C. After reacting for 24 h, the reaction was stopped by opening the flask and exposing the catalyst to air. The product $Ag@SiO_2$-*g*-PS-1, whose DP_{PS} was 930, was isolated and purified by precipitation into an excess of methanol and recovered by filtration, followed by drying. Other $Ag@SiO_2$-*g*-PS samples with different DP_{PS}

values could be obtained by controlling the reaction time.

Preparation of Ag@SiO$_2$-g-xPS. Ag@SiO$_2$-*g*-*x*PS was prepared by Friedel-Crafts hypercrosslinking reaction**.** Typically, 0.2 g of Ag@SiO$_2$-*g*-PS-1 was dissolved in 20 mL of CCl_4 in a three-neck flask equipped with reflux condenser and heated to 75 °C. Subsequently, 1 g of $AlCl_3$ was added quickly to start the Friedel-Crafts hypercrosslinking reaction. The reaction was kept at 75 °C for 24 h under stirring, and then terminated by adding the mixture of acetone and 3.7 wt% HCl solution (v/v=3:1). The product Ag@SiO$_2$-*g*-*x*PS-1 was filtered off, washed with acetone/HCl/H_2O three times and dried in vacuum at 60 °C for 12 h.

Preparation of FNNS-P-Ag. 0.2 g of Ag@SiO$_2$-*g*-*x*PS-1 was dispersed in 20 mL of 4 wt% HF ethanol/water solution（40% HF/ethanol/water = 1:3:6, volume ratio）for 15 min to remove the silica. It should be noted that the Ag nanoparticles may be etched away with longer etching time. Then the product FNNS-P-Ag-1 was dried at 60 °C for 12 h.

Other FNNS-P-Ag-x samples with different shell thicknesses and cavity sizes were prepared according to the above method, except other hairy Ag@SiO$_2$-*g*-PS nanoparticles with different DP_{PS} values of hairy PS chains as well as thicknesses of SiO_2 egg whites.

Preparation of FNNS-C-Ag. The Ag@SiO$_2$-*g*-*x*PS-2 was carbonized at 800 °C (or other temperatures) for 3 h with a heating rate of 2 °C min^{-1} in N_2 flow. After using 4% HF ethanol/water solution to remove the silica, FNNS-C-Ag was obtained. In addition, Ag/C composite was also obtained by carbonizing the FNNS-P-Ag-2 at 800 °C for 3 h with a heating rate of 2 °C min^{-1} in N_2 flow.

2. Characterization

Structural characterization. Molecular weight of Ag@SiO_2-*g*-PS was analyzed with Waters Breeze gel permeation chromatography (GPC) by using THF as an eluent. The sample was pretreated by 40% HF to obtain liner PS. The morphology of the samples was investigated by a JSM-6330F scanning electron microscope (SEM) and a FEI Tecnai G2 Spirit transmission electron microscope (TEM). 100 network units in a SEM image of FNNS-P-Ag (or 100 Ag@SiO_2 nanoparticles) were picked at random. Subsequently, a statistical analysis of the diameter distribution histograms of network units (or Ag@SiO_2 nanoparticles) was carried out, and then the thickness of network units' shells for FNNS-P-Ag was obtained by subtracting the average diameter of Ag@SiO_2 from the average diameter of network units of FNNS-P-Ag. XRD patterns were recorded on a D-MAX 2200 VPC diffractometer using Cu K radiation (40 kV, 26 mA). The thermogravimetric analysis (TGA) was performed under flowing N_2 at a heating rate of 20 °C min^{-1}. A Micromeritics ASAP 2020 surface area and porosity analyzer was utilized to study the pore structure of the samples. The BET surface area (S_{BET}) was analyzed by Brunauer-Emmett-Teller (BET) theory. The micropore surface area (S_{mic}) was determined by t-plot method. The pore size distribution was analyzed by original density functional theory (DFT) combined with non-negative regularization and medium smoothing.

Antibacterial test. The *E. coli*, Gram negative bacterial strains and *S. aureus*, Gram positive bacterial strains were pre-cultured in sterilized Luria-Bertain (LB) broth overnight at 37 °C to reach a concentration of ~10^7 CFU per milliliter.

Zone of inhibition. 200 μL of bacterial suspensions was spread uniformly over the agar plates. Three filters (diameter of 6 mm) were put into every agar plate and two of the filters were filled with 20 μL of sample solution (10 mg mL^{-1}) and another filter was filled with 20 μL of distilled water as control. In the end, the inoculated agars were kept for incubation at 37 °C. The breadth of inhibition zone was recorded after 24 h.

Minimum inhibitory concentration (MIC). Groups with specific concentration of different composites were added into 10 mL of *E. coli* suspension (OD_{600}=0.25) and incubated at 37 °C for 24 h. MIC was recorded as the lowest concentration when the OD_{600} was the same as the original suspension. After incubating for 24 h, 200 μL of the bacterial suspension was cultured on LB agar plates at 37 °C for 24 h and then the colonies were counted to investigate the bactericidal activity. The growth kinetics of *E. coli* was carried out by monitoring the value of OD_{600} in specific time on a UV-vis spectrophotometer.

The release of Ag^+ from FNNS-C-Ag. 0.04 g of the FNNS-C-Ag was immersed in 40 mL of the physiological saline solution at 37 °C, followed by divided into four tubes equally. After that, these suspensions were shaken with a rate of 150 rpm at 37 °C. One tube was taken out every day in the next four days and the concentration of Ag^+ was ananlyzed quantitatively by ICP. The obtained data were the cumulative amounts of released silver.

Long-term antibacterial performance. 0.02 g of the FNNS-C-Ag sample was immersed in 25 mL of the culture medium, seeded with 0.1 mL of *E. Coli* bacterial suspension (OD_{600}=1.424), and then shaken with a rate of 150 rpm at 37 °C. The same amount of fresh bacteria was reinoculated every day, and the total incubation time lasted for six days. At defined time intervals, the cell suspensions were withdrawn and analyzed spectrophotometrically by measuring the absorbance at 600 nm. The growth of *E. Coli* in the culture medium with Ag nanoparticles (the same content of Ag as FNNS-C-Ag), Ag@PS or with nothing as blank was also investigated as control.

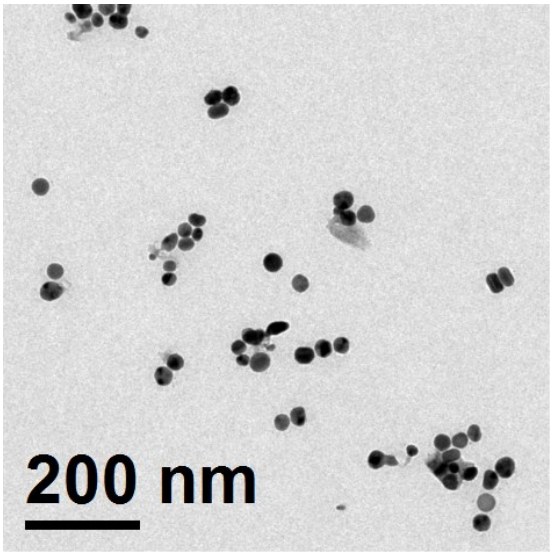

Fig. S1 TEM image of Ag nanoparticles.

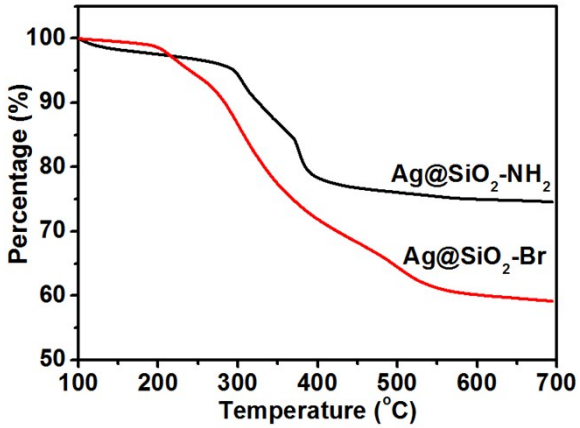

Fig. S2 TGA curves of $Ag@SiO_2$-NH_2 and $Ag@SiO_2$-Br. The content of Br on the surface of $Ag@SiO_2$ was calculated to be 0.103 mmol g^{-1}.

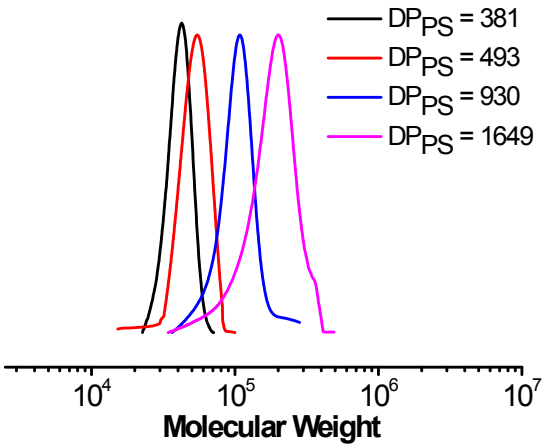

Fig. S3 GPC traces of the cleaved PS chains from the $Ag@SiO_2$-*g*-PS nanospheres.

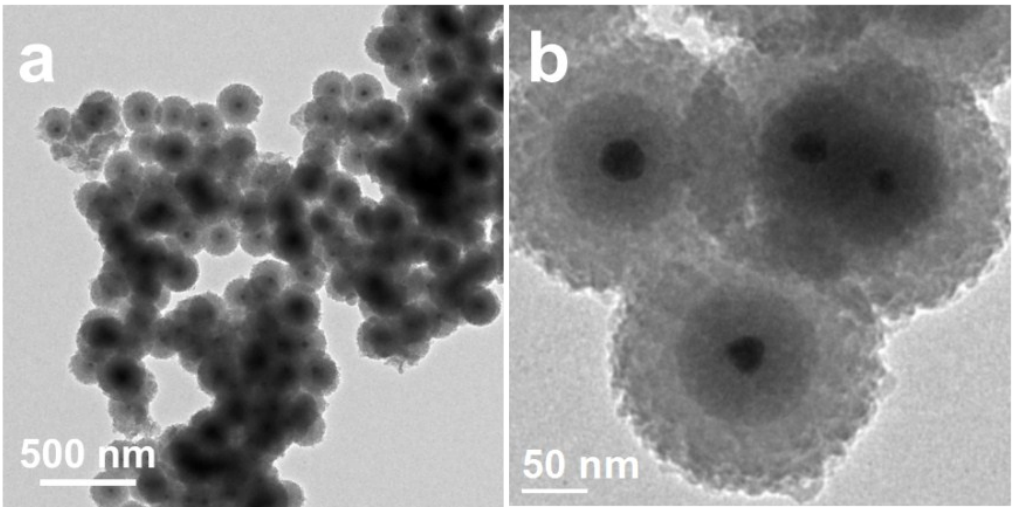

Fig. S4 (a, b) TEM images of Ag@SiO_2-*g*-*x*PS-1.

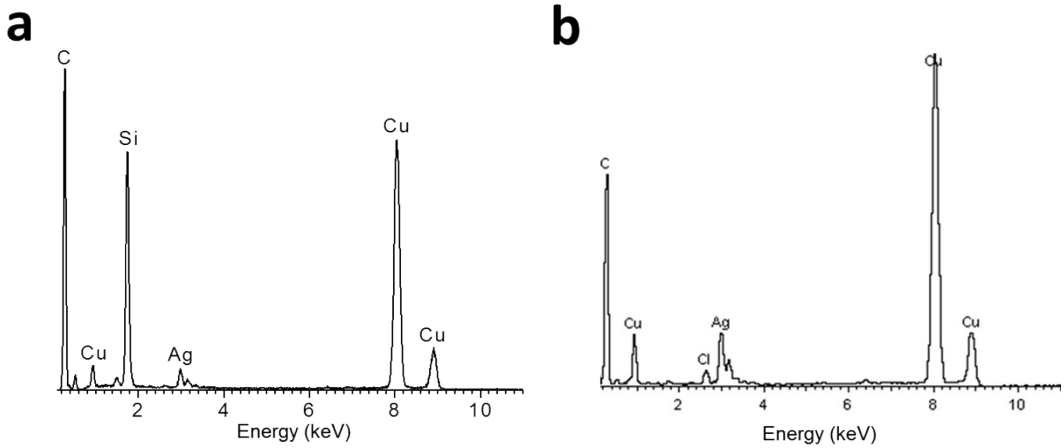

Fig. S5 EDX spectra of (a) Ag@SiO_2-*g*-*x*PS-1 and (b) FNNS-P-Ag-1.

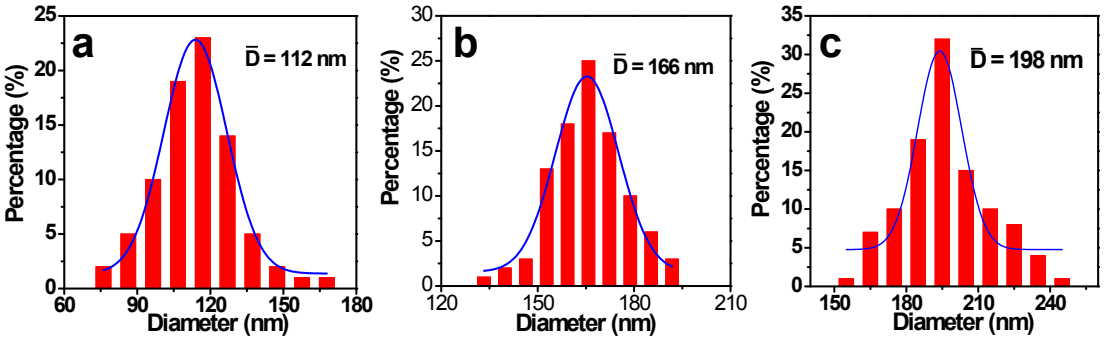

Fig. S6 Diameter distribution histograms of network units based on analysis of SEM images of (a) FNNS-P-Ag-3 with DP_{PS} of 493, (b) FNNS-P-Ag-1 with DP_{PS} of 930, and (c) FNNS-P-Ag-2 with DP_{PS} of 1649.

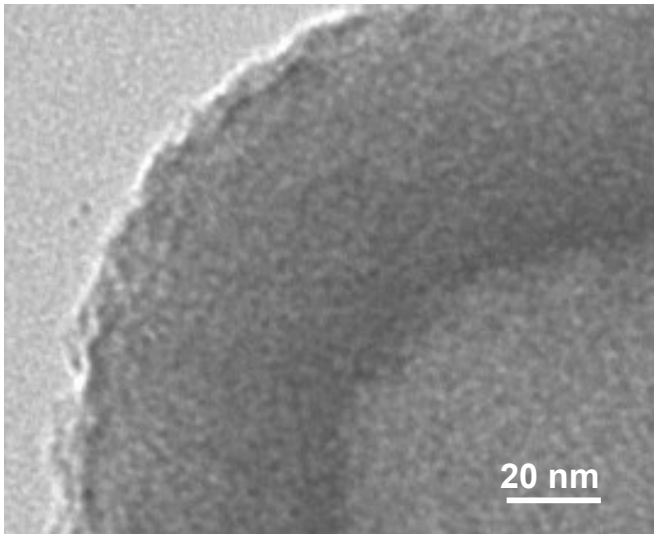

Fig. S7 High magnified TEM image of FNNS-C-Ag.

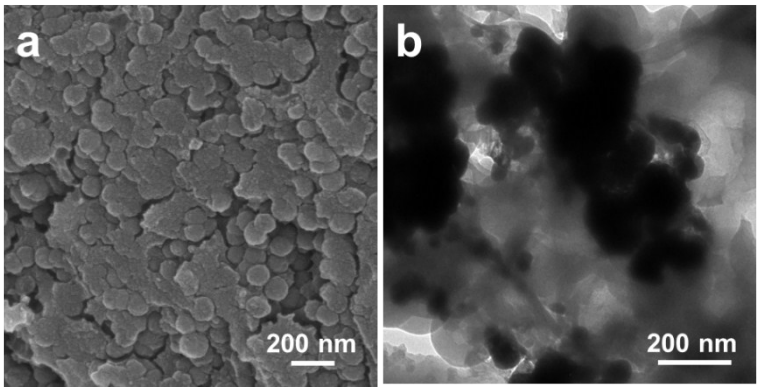

Fig. S8 (a) FESEM and (b) TEM images of Ag/C composite obtained by treating FNNS-P-Ag-2 at 800 ^{o}C.

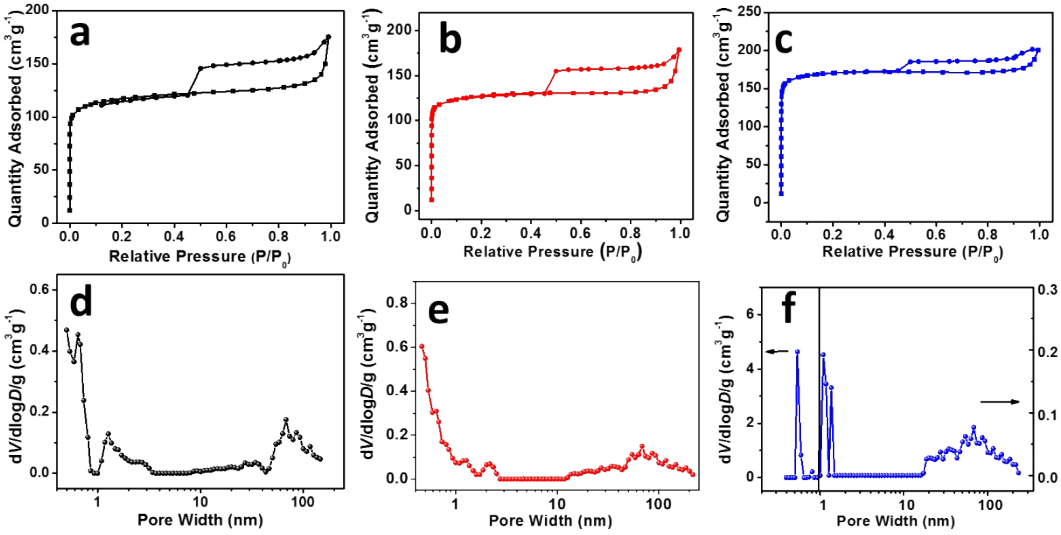

Fig. S9 N_2 adsorption-desorption isotherms (top) and DFT pore size distribution curves (bottom) of FNNS-C-Ag samples obtained at (a, d) 600 ^{o}C, (b, e) 800 ^{o}C and (c, f) 900 ^{o}C, respectively.

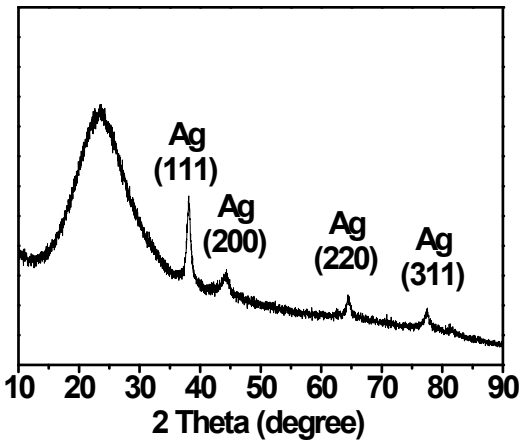

Fig. S10 XRD pattern of $Ag@SiO_2$.

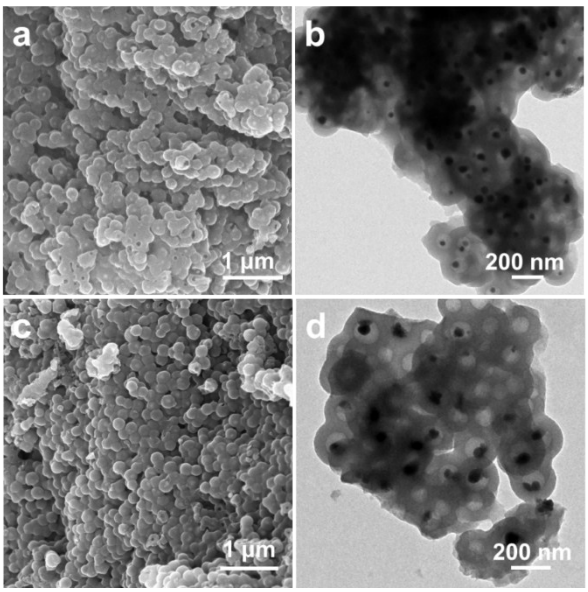

Fig. S11 FESEM (left) and TEM (right) images of FNNS-C-Ag samples obtained at (a, b) 600 °C and (c, d) 900 °C.

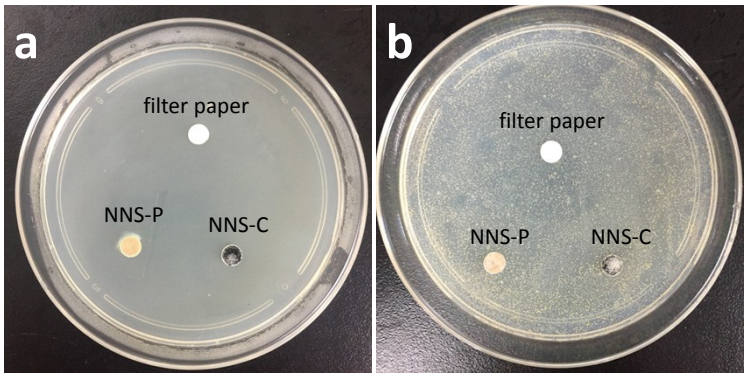

Fig. S12 Comparison of inhibition zones for filter paper, NNS-P and NNS-C towards (a) *E. coli* and (b) *S. aureus*.

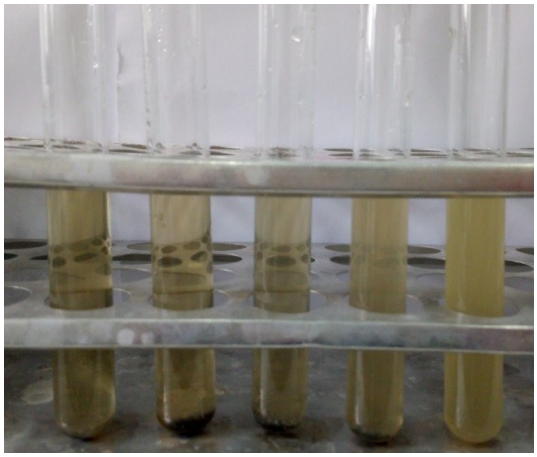

Fig. S13 Photograph of MIC results of FNNS-C-Ag towards *E. coli*. The sample concentrations were 100, 75, 50, 25, and 0 μg mL^{-1} from left to right.

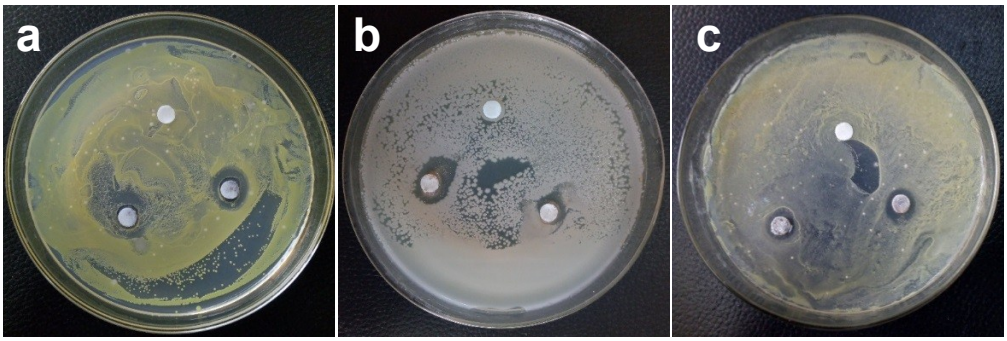

Fig. S14 Photographs of inhibition zone of (a) FNNS-C-Ag towards *S. aureus*, and FNNS-P-Ag-1 towards (b) *E. coli* and (c) *S. aureus*.

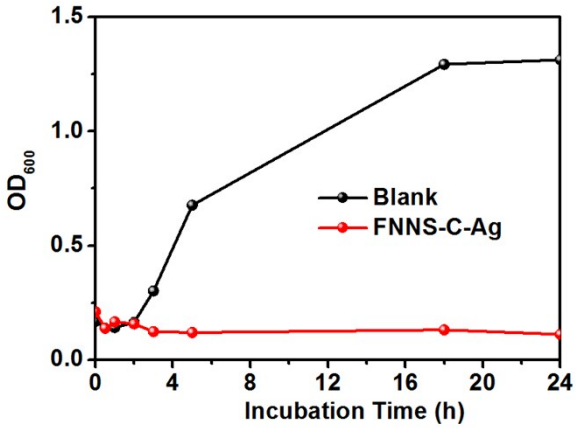

Fig. S15 Real-time OD_{600} values for *E. coli*. The concentration of FNNS-C-Ag was 125 μg mL^{-1}.

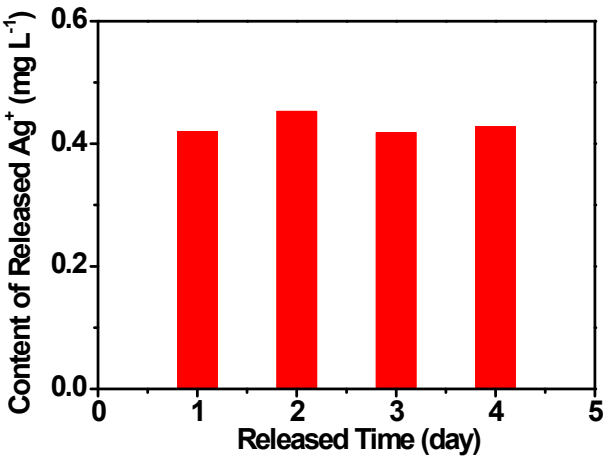

Fig. S16 Silver releasing profile of FNNS-C-Ag.

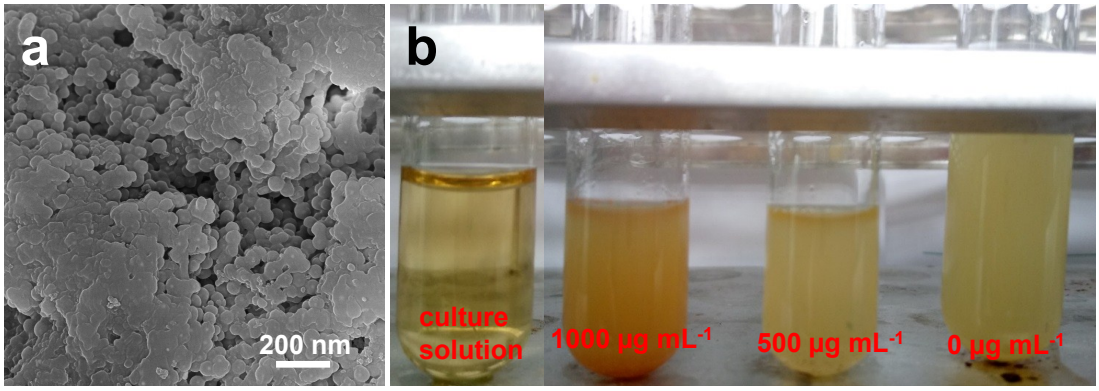

Fig. S17 (a) FESEM image of Ag@PS; (b) photograph of the MIC results of Ag@PS towards *E.coli*.

Table S1 MIC results of FNNS-C-Ag.

Sample concentration (μg mL^{-1})	Content of Ag (× 10^{-7} μg CFU^{-1})	LB broth turbidity		OD_{600}	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*
25	1.86	Turbid	Turbid	0.411	0.688
50	3.73	Clear	Turbid	0.201	0.431
75	5.59	Clear	Clear	0.206	0.187
100	7.46	Clear	Clear	0.197	0.165

Table S2 MIC results of FNNS-P-Ag-1.

Sample concentration ($\mu g\ mL^{-1}$)	Content of Ag ($\times 10^{-7}\ \mu g\ CFU^{-1}$)	LB broth turbidity		OD_{600}	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*
25	0.8	Turbid	Turbid	0.827	1.840
50	1.6	Turbid	Turbid	0.847	1.790
75	2.4	Turbid	Turbid	0.833	1.794
100	3.2	Turbid	Turbid	0.943	1.801
200	6.4	Turbid	Turbid	0.877	1.759
500	15.9	Clear	Turbid	0.258	1.838
1000	31.8	/	Clear	/	0.221