Electronic Supplementary Information (ESI)

Preparation, characterization and antibacterial properties of silver-modified graphene oxide

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1. Preparation of samples

1.1 Modification of graphene oxide with silver nanoparticles.

1.2 g of glucose was dissolved in 30 mL of graphene oxide dispersion (1.6 mg/mL) under ultrasonication for 5 min. Then, 1 mL of aqueous ammonia solution (30 wt.%) was added in 10 mL of silver nitrate solution (50 mM). While maintaining vigorous ultrasonication, the two liquid phases were mixed at room temperature. After ultrasonication for 5 min, the mixture was kept for 1.5 h without stirring. Then, solids were collected by centrifuge and washed with deionized (DI) water repeatedly.

1.2 Preparation of *E. coli* stock suspension

Before each experiment, all utensils were autoclaved at 121 ^{o}C for 15 min to ensure sterility. *E. coli* was inoculated into a Luria Bertani medium (LB) and cultured for 12 h at 37 ^{o}C on a rotary shaker at 150 rpm. Then the cells were harvested by centrifugation at 4000 rpm for 10 min, washed twice with sterile phosphate buffer saline (PBS, 0.03mol/L, pH 7.2) to remove the nutrient, and finally the pellets were suspended again in a sterile PBS buffer with a concentration of 10^{8}~10^{9} CFU/mL.

1.3 Antibacterial measurement

500 μL of the Ag-GO suspension was added to 49.45 mL of deionized (DI) water directly (or in a dialysis tubing as shown in Fig. S2) in a conical flask. Then, 50 μL of a stock cell suspension was added in the flask to obtain a cell concentration in the range of 10^5~10^6 CFU/mL. The mixture was stirred throughout the experiment to ensure well contact. After a given period of time, 100 μL of the suspension was collected and spread on agar plates, followed by incubation for 18 h to form colony units. Each dilution has three parallel samples. Control experiment was also conducted in the absence of Ag-GO composite.

1.4 Preparation of specimen for FESEM and TEM observation

A 1.0 mL of suspension was withdrew at the determined intervals from the antibacterial reactor system and centrifuged, washed three times with PBS. Then the bacterial cells were fixed with 2.5% glutaraldehyde for 30 min firstly, after washed twice with PBS buffer, the cells were fixed with 1% osmium tetraoxide for 30 min. After fixation, the *E. coli* cells were concentrated by centrifugation at 4000 rpm for 10 min, followed by washing twice with PBS buffer. Subsequently, the cells were gradually dehydrated with increasing concentrations of ethanol 50%, 70%, 80%, 90% and 100% for 5 min each. After dry, the cells were observed by using FESEM and TEM.

2. Characterization techniques

The amount of Ag deposited on the GO sheets was determined by analysis of the elemental content of the supernatant using an Agilent 7500 series inductive-coupled plasma-mass spectrometer (ICP-MS). X-ray diffraction (XRD) test was carried out by using an XRD-6000 (Shimadzu, Kyoto, Japan) instrument. X-ray photoelectron spectra (XPS) were collected on an AXIS HIS (Kratos Analytical Ltd., UK) instrument with an Al Kα irradiation source (1486.7 eV) operated at 15 kV and 10 mA. The morphologies of synthesized Ag-GO

nanosheets and treated bacteria specimen were observed by using a transmission electron microscope (TEM) (JEM 2010, JEOL, Japan) operated at 200 kV. The field-emission scanning electron microscope (FESEM) (JSM 6700F, JEOL Japan) was operated at an accelerating voltage of 10 kV to observe the morphology change of treated bacteria cells. Zeta-potential data measurements of both Ag-GO nanosheets and GO were carried out by means of a ZetaPlus Zeta-Potential Analyzer (Brookhaven Instruments Corporation, Holtsville, NY) equipped with a 570 nm laser.

3. Supporting experimental results

3.1 XPS data

The XPS data are shown in Fig. S1. Figure S1a shows the XPS survey of samples GO and Ag-GO. After deposition of Ag, the peaks due to C1s, O1s, and Ag 3d can be seen. The core-level Ag 3d XPS signal is shown in Fig. S1b. The binding energies of Ag $3d_{3/2}$ and Ag $3d_{5/2}$ were 368.1 eV and 374.1 eV, respectively. The slitting of the 3d doublet of Ag was 6.0 eV, suggesting the formation of metallic silver.[1] The core-level O 1s at 532.3 eV was attributed to the hydroxyl groups and surface adsorbed water on GO (Fig. S1c). No peak corresponding to lattice oxygen O^{2-} from Ag_2O is seen. As Ag can be easily oxidized by oxygen, it is possible that part of Ag on the surface was oxidized. However, based on the XRD and XPS data the predominant part is Ag (0). The intensity of O 1s was reduced after deposition of Ag, resulting from the partial reduction of GO during the reduction process. The C1s signal of GO is shown in Fig. S1d. The C 1s signal was deconvoluted to three peaks by curve fitting using a mixed Gaussian and Lorentzian lineshape. Except for the peak at 284.5 eV corresponding to C-C, other peaks at 286.7 and 287.9 eV were due to C-O and C=O, respectively. Fig. S1e shows the change in the C 1s signal before and after the deposition of Ag on GO. The intensity related to C-O signal decreased after the deposition of Ag. It has

been reported that GO can be reduced by glucose,[2] resulting in the lowering of surface charge (as was confirmed by the Zeta potential results in Fig. S7). However, the residual oxygenate groups on the surface of Ag-GO can still maintain a well dispersion of Ag-GO in aqueous solution. The presence of N was confirmed by the N 1s signal in Fig. S1f. The N1s signal can be deconvoluted to three parts, corresponding to pyridinic N, pyrrolic N, and quaternary N. The trace N was introduced from the ammonia during the reduction process.[3]

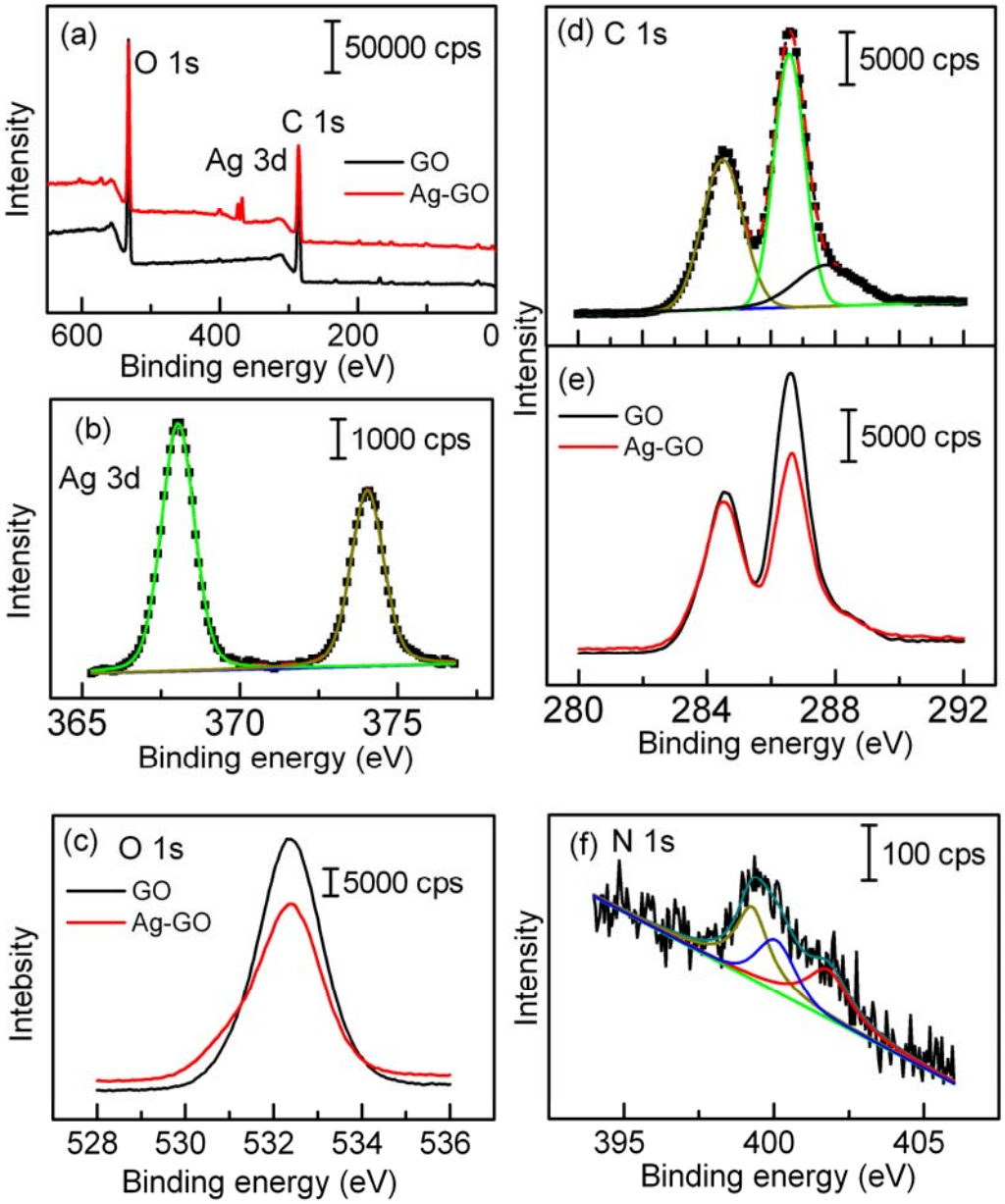

Fig. S1 (a) Wide scan XPS spectra of GO and Ag-GO. Core level XPS signals of Ag 3d (b), O1s (c), C 1s (d, e), and N 1s (f).

3.2 A partition setup

In the partition setup system, dialysis tubing was made with semipermeable membrane with the molecular weight cutoff of 12000-14000 dalton, which allowed small molecules and ions such as water and Ag^+ ions to pass through, but rejected large matters such as Ag-GO nanosheets and *E. coli* cells. Therefore, *E. coli* cells did not contact with the Ag-GO nanosheets directly.

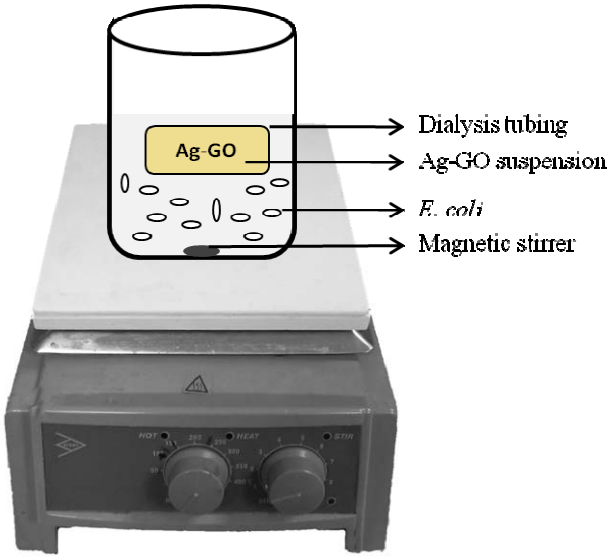

Fig. 2 A schematic illustration of the partition setup used in the inactivation experiment.

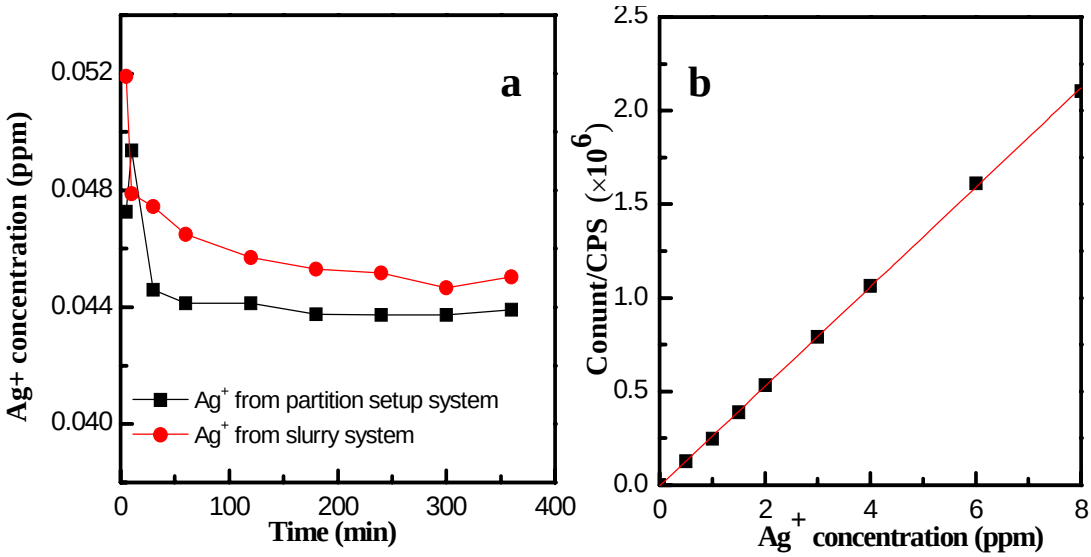

Fig. S3 (a) Silver ion concentration measured using the partition setup and slurry system. (b) A standard curve of silver ions.

3.3 Ag nanoparticles prepared using the chemical reduction method[4]

In order to investigate the antibacterial activity of pure Ag nanoparticles to *E. coli*, Ag nanoparticles were prepared by chemical reduction method reported by Chou.[4] Fig. S4 shows the morphology of the synthesized silver nanoparticles with diameters about 10 nm to70 nm.

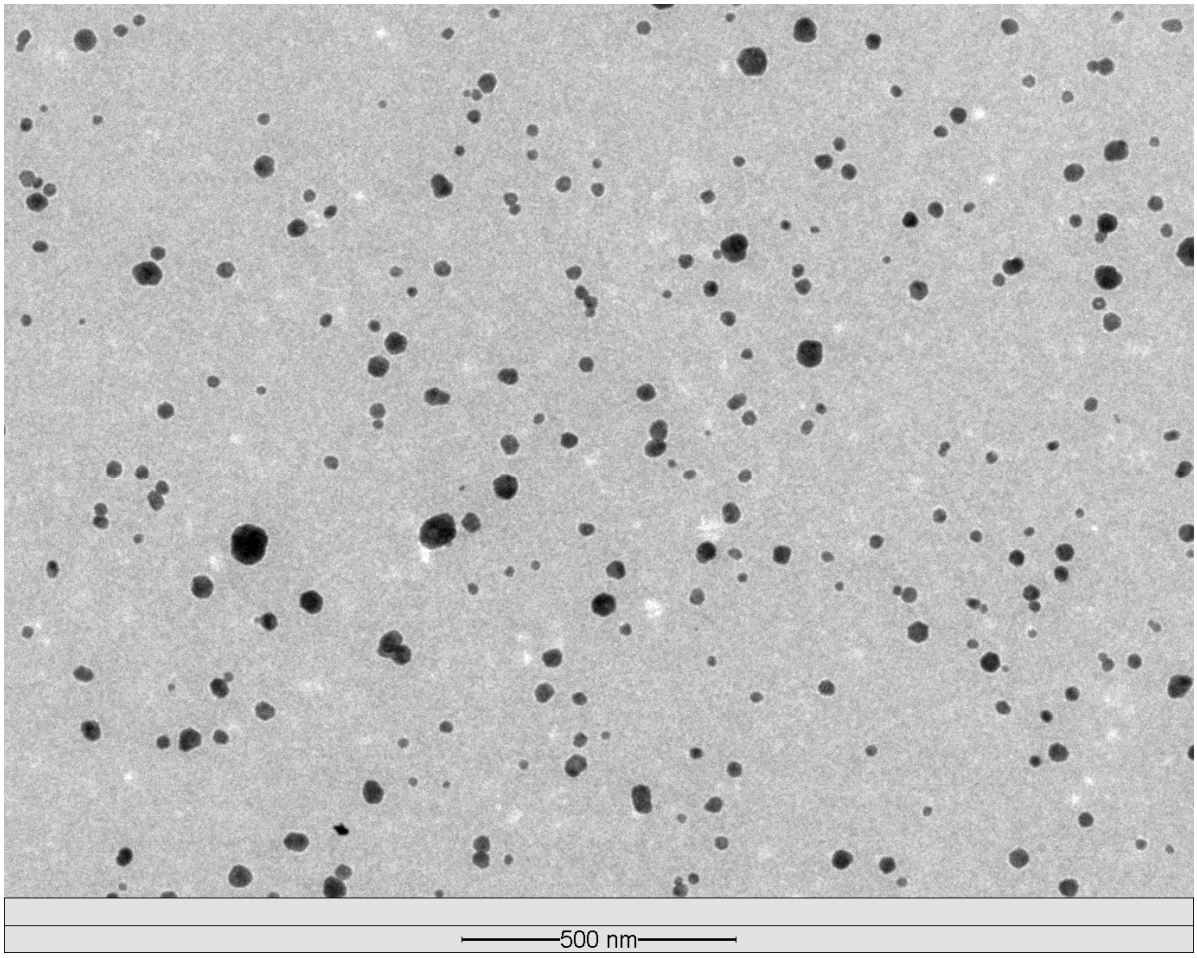

Fig. S4 TEM image of Ag nanoparticles.

3.4 Antibacterial properties of Ag nanoparticles prepared using the chemical reduction method[4]

The antibacterial activity of the Ag nanoparticles prepared above was evaluated under the same experimental conditions to that of Ag-GO nanosheets. The results are shown in Fig. S5. Compared with the control sample (Fig. S5a), there are still bacteria colonies formed after incubation with Ag nanoparticles for 10 min (Fig. S5b) and 30 min (Fig. S5c). On contrast, the Ag-GO composite exhibited a much better antibacterial activity with no colonies formed after contact for 10 min (Figs. 3c).

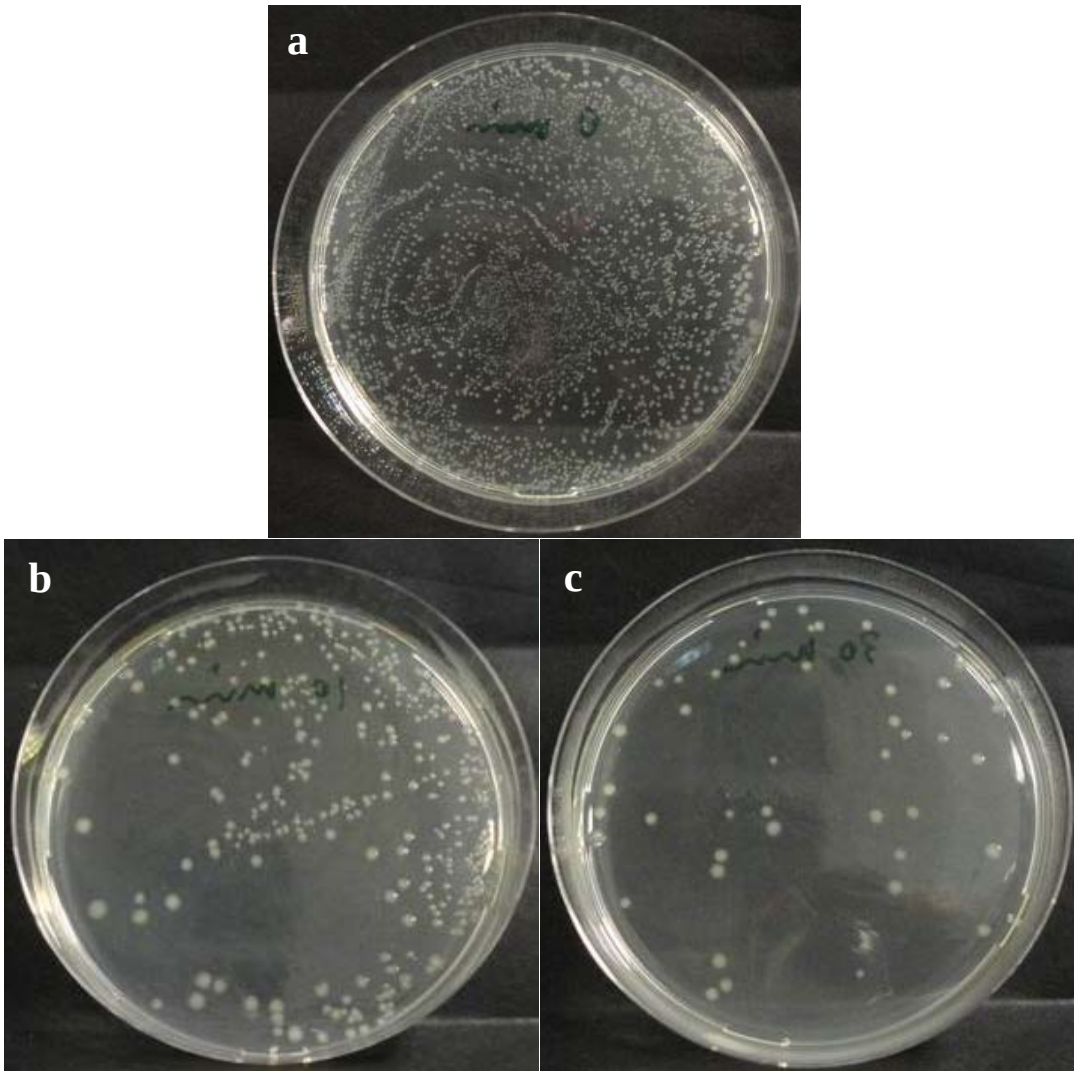

Fig. S5 Photographs of bacterial colonies formed by *E. coli* cells without treatment (the control experiment) (a) and treated with Ag nanoparticles in a slurry system for 10 min (b) and 30 min (c).

3.5 Antibacterial properties of GO

The antibacterial activity of GO was also determined by using the same experimental parameters with that of Ag-GO nanosheets. The results are shown in Figure S6. It can be seen that few bacteria cells were inactivated after contact with GO for 5 min (Fig. S6b). After contact for 30 min and 60 min, there were still plenty of colonies formed on the agar plate as is seen from Figs. S6c and S6d, showing a very poor antibacterial activity of the GO.

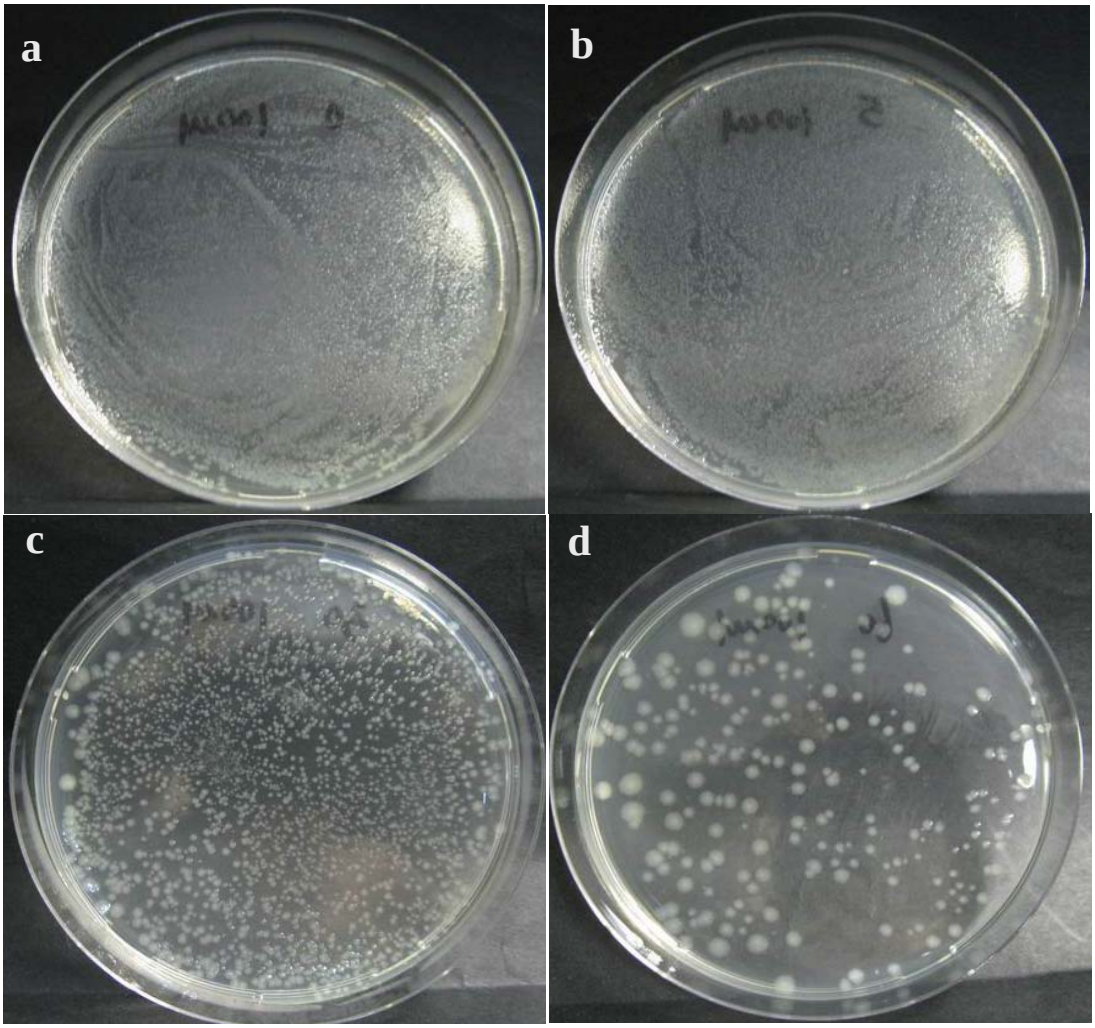

Fig. S6 Photographs of bacterial colonies formed by *E. coli* cells without treatment (control experiment) (a) and treated with GO in a slurry system for 5 min (b), 30 min (c) and 60 min (d).

3.6 Zeta potential

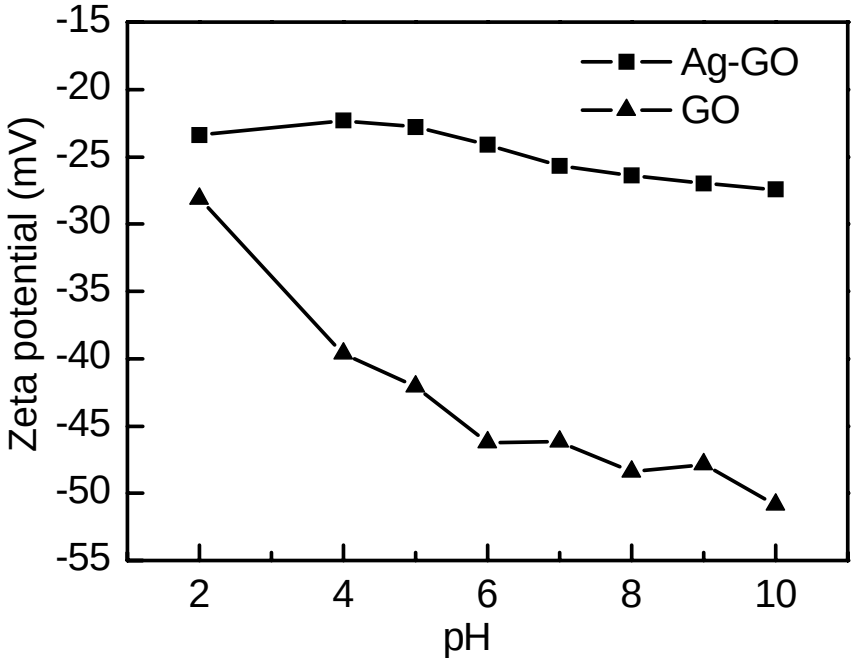

Fig. S7 Zeta potential profiles of Ag-GO and GO**.**

References:

1. C. Gunawan, W. Y. Teoh, C. P. Marquis, J. Lifia and R. Amal, *Small*, 2009, **5**, 341-344.

2. C. Z. Zhu, S. J. Guo, Y. X. Fang and S. J. Dong, *ACS Nano*, 2010, **4**, 2429–2437.

3. Y. Wang, Y. Y. Shao, D. W. Matson, J. H. Li, and Y.H. Lin, *ACS Nano*, 2010, **4**, 1790-1798.

4. K. S. Chou, C. C. Chen, *Microporous Mesoporous Mater.*, 2007, **98**, 208-213.