# Supporting Information

## Kaolinite-mediated synthesis of ultra-small silver nanoparticles with high antimicrobial activity

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## Experimental section

### 1.1. Reagents and Materials

Kaolinite was sourced from LongYan Kaolinite Scientific Company (LongYan, China), while silver nitrate ($AgNO_3$) and APTES were procured from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All reagents were used without further purification. Bacterial strains *Escherichia coli* (*E. coli*, ATCC25922) and *Staphylococcus aureus* (*S. aureus*, ATCC25923) were provided by Xiangya Hospital of Central South University.

### 1.2. Preparation of kaolinite-loaded nanosilver (Ag/K)

A total of 150 g of kaolinite was initially transferred to a mixing tank, where it underwent mechanical stirring at a temperature of 80 ℃ for a duration of 30 minutes. Concurrently, a solution of silver nitrate, weighing 12.43 g, was prepared by dissolving it in a combined 40 mL solvent of anhydrous ethanol and deionized water, each contributing 20 mL. This silver solution was then incrementally introduced into the kaolinite mixture under continuous stirring at the same temperature, a process that extended over 4 hours. Following this, the blend was left to dry at 60 ℃ through the night. The subsequent heat treatment occurred at 200 ℃ within an atmosphere of Ar enriched with 10% $H_2$ for a period of 6 hours, culminating in the synthesis of the product designated as Ag/K.

### 1.3. Preparation of Ag@AT/K

In a parallel experiment, 150.35 g of kaolinite was introduced into the mixing tank for mechanical agitation at 80 ℃ over 30 minutes. Separately, 15 g of silver nitrate was

solubilized in a 40 mL mixture of anhydrous ethanol and deionized water. This solution was then combined with 1.4 mL of APTES and incrementally incorporated into the kaolinite suspension under continuous stirring at the same temperature for four hours. The resultant mixture was left to dry at 60 ℃ overnight. Subsequent thermal processing was conducted at 200 ℃ in an Ar + 10% $H_2$ atmosphere for six hours, yielding the product designated as Ag@AT/K. To elucidate the impact of Ag NP loading on the antibacterial characteristics of the composites, materials with varied Ag NP loadings were fabricated by adjusting the amounts of $AgNO_3$ and APTES. The compounds, based on their distinct Ag NP loadings, were identified as Ag@AT/K-1, Ag@AT/K-3, and Ag@AT/K-5. The specific synthetic formulations are itemized in Table S1. The process has a large one-time yield, up to 150 g in small laboratory trials. In addition, there is no need to use excess solvents and the process is simple and inexpensive. The possible reaction principle of this process is that, firstly, APTES and silver nitrate form a coordination complex [$AgNO_3$-$2H_2N(CH_2)_3Si(OC_2H_5)_3$], which is then grafted onto the kaolinite surface. The reaction can be described by Eq. S1. Finally, silver ions are thermally reduced to silver nanoparticles in an atmosphere of Ar + 10%$H_2$. During the thermal reduction process, the spatial blocking effect of APTES around the silver ions prevented the aggregation of silver nanoparticles, resulting in ultrasmall Ag.

**1.4. Preparation of Ag@AT**

The sliver nitrate (3.625g) were dissolved in 5 mL of anhydrous ethanol and 5 mL (1:1) deionized water. Then, 14 mL of APTES was added to form a coordination

complex. The solution was poured onto a glass dish and allowed to solidify and dry at 60 ℃ overnight. Finally, the obtained powders were treated at 200 ℃ in an Ar + 10% $H_2$ atmosphere for six hours. The resulting sample was labeled as Ag@AT.

### 1.5. Characterization

Silver quantification was performed via inductively coupled plasma emission spectroscopy (ICP-OES, Agilent 720ES) post-acid dissolution of the samples. TEM imagery and EDS spectra were acquired using a JEOL JEM-F200, operated at 200kV. X-ray diffraction (XRD) patterns were captured with a BRUKER D8 ADVANCE diffractometer, utilizing CuKα radiation at 40 kV/40mA, scanning from 2θ of 5-90° at a rate of 2°/min. Diffuse reflectance spectra in the UV-visible range were recorded on a Hitachi UH4150 spectrometer. Surface chemical analysis was conducted through X-ray photoelectron spectroscopy (XPS) using a Thermo Scientific K-alpha spectrometer. Surface area and porosity metrics were gauged using $N_2$-adsorption-desorption isotherms (TriStar II Plus) and analyzed via the BJH method. Zeta potential was measured with a NanoBrook 90plus PALS analyzer. Contact angle determinations were made using a Dataphysics OCA-20. Thermal stability assessments were carried out on a NETZSCH STA449 thermal analyzer, under an air atmosphere, spanning 30–800 °C at a heating rate of 10 ℃/min.

### 1.6. Antibacterial activity evaluation

The antibacterial efficacy of Ag/K and Ag@AT/K nanocomposites was assessed through growth inhibition assays against *E. coli* and *S. aureus*, using different sample concentrations. Cultures of *E. coli* and *S. aureus* were grown in Luria Bertani (LB)

broth at 37 ℃ with agitation. Kaol (6 mg) was added to 3 mL LB liquid and sonicated for 30 min. According to the ICP results (Table S1, ESI†), weigh a certain amount of Ag/K and Ag@AT/K so that they have the same mass of silver of silver nanocomposites. Subsequently, Ag/K and Ag@AT/K were added to 3 mL LB liquid at a consistent silver content (100 μg/mL) and sonicated for 30 minutes. The bacterial suspensions were diluted to achieve an optical density at 625 nm ($OD_{625}$) of 0.09 to 0.11, then further diluted 100-fold to obtain the bacterial suspension. Following this, 100 μL of the bacterial suspension was added to 10 mL LB liquid. Different volumes (15, 30, 45, 60, and 75 μL) of Ag/K suspension and Ag@AT/K suspension were added to the *E. coli* suspension, while varying volumes (120, 240, 360, 480, and 600 μL) were added to the *S. aureus* suspension. The cultures were incubated aerobically at 37 ℃. After 4 hours, 100 μL aliquots were withdrawn from each sample, and serial dilutions from $10^{-1}$ to $10^{-4}$ were prepared. Portions (50 μL) of the final 1-2 dilutions were spread on LB-agar plates, followed by incubation at 37 ℃ for 16-24 hours. Subsequently, the agar plates were observed and photographed.

**1.7. Live/Dead Staining Assay**

The viability of cells exposed to Ag@AT/K was assessed using fluorescence microscopy, employing a live/dead bacterial viability kit. For this assay, calcein and propidium iodide were the chosen fluorescent dyes; calcein emits a green fluorescence in live cells, whereas propidium iodide penetrates and marks dead bacteria red. Bacterial cultures of *E. coli* and *S. aureus*, with optical densities ranging from 0.45 to 0.55 at 625 nm, were incubated in LB medium supplemented with

Ag@AT/K at a 60 μg/mL Ag concentration. Post-incubation, the cells were stained with 2 μg/mL of each dye for 15-20 minutes, rinsed twice with PBS, and once with ultrapure water. The stained bacteria were then visualized under a laser confocal microscope.

**1.8. Morphological Analysis of Bacterial Cells**

Subsequent to a 4-hour incubation, the bacterial samples were collected, thrice washed with PBS, and fixed in 2.5% glutaraldehyde. The cellular morphology was examined using transmission electron microscopy (TEM).

## Supplementary Results

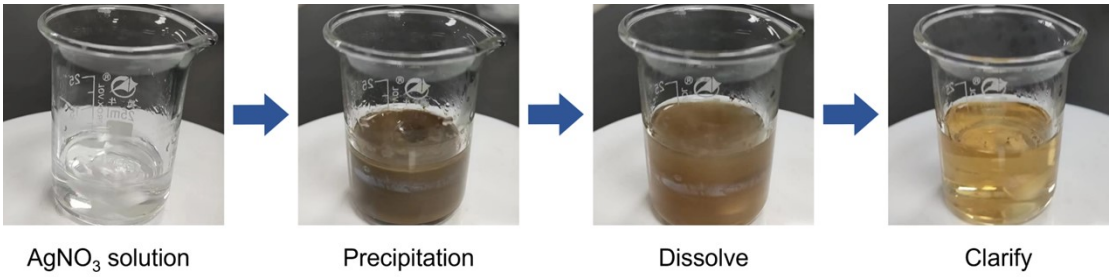


**Fig. S1.** With the addition of APTES, precipitation first appeared in $AgNO_3$ solution and then dissolved.

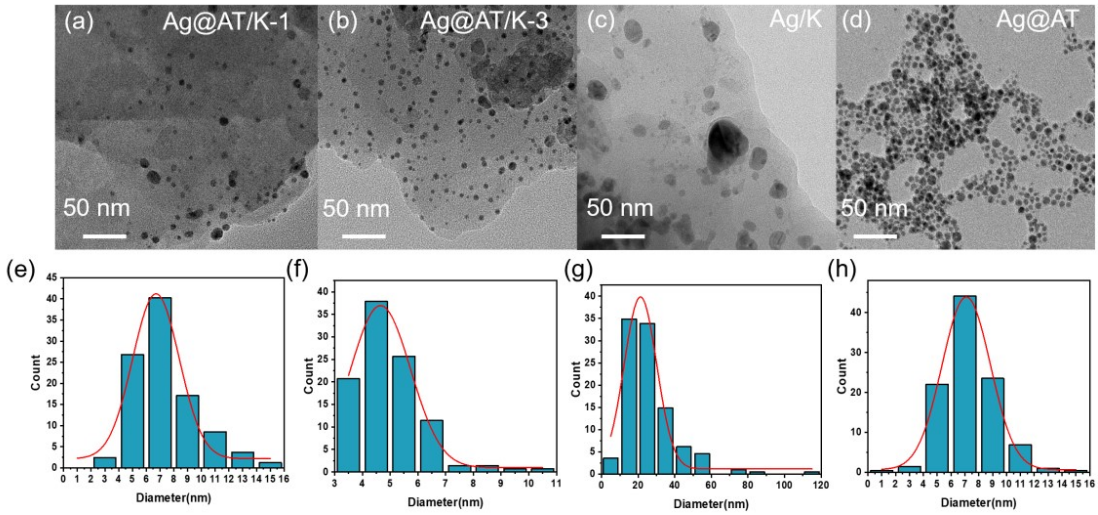


**Fig. S2.** The TEM images of (a) Ag@AT/K-1, (b) Ag@AT/K-3, (c) Ag/K and (d) Ag@AT. The statistical results of AgNPs diameter in the (e) Ag@AT/K-1, (f) Ag@AT/K-3, (g) Ag/K and (h) Ag@AT.

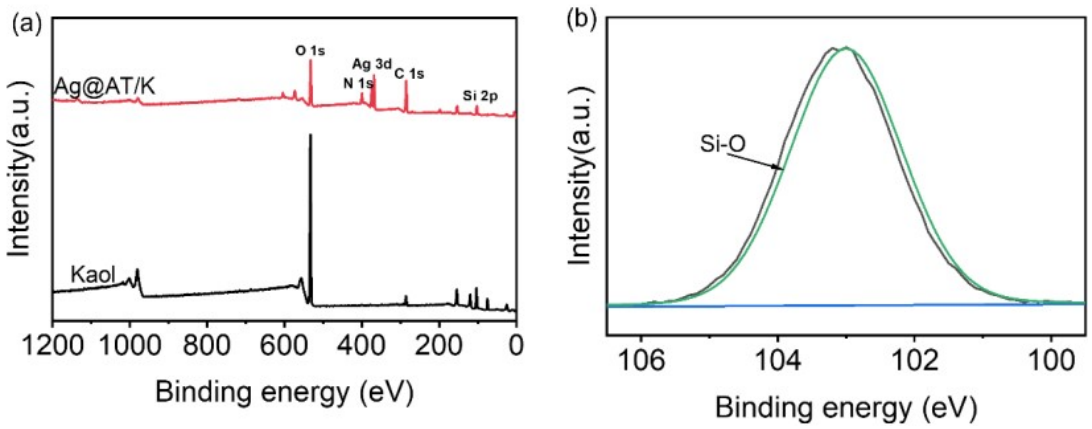


**Fig. S3.** (a) The full XPS spectrum of Ag@AT/K-5 and Kaol. (b)The high-resolution XPS Si 2p spectra of Kaol.

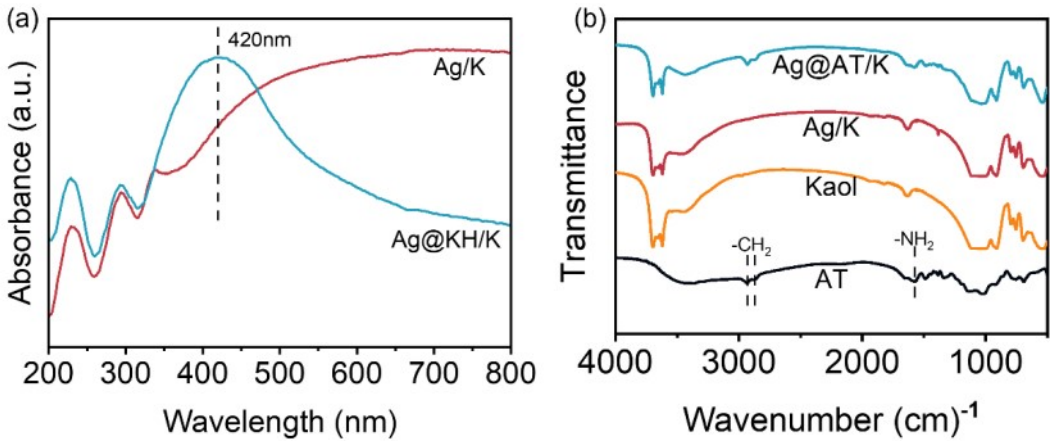


**Fig. S4.** (a) UV-vis diffuse reflectance spectra of Ag/K and Ag@AT/K-5. (b) The FT-IR spectra of AT, Kaol, Ag/K and Ag@AT/K-5.

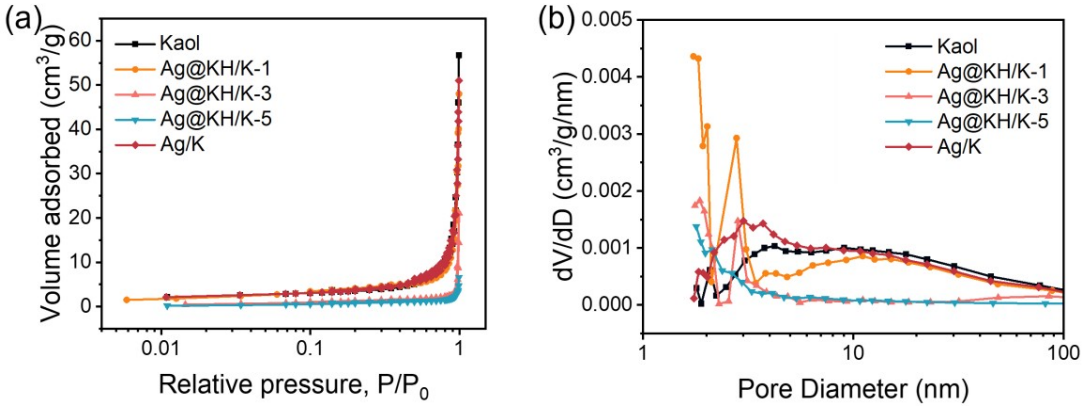


**Fig. S5.** (a) $N_2$ adsorption-desorption isotherms of Kaol, Ag/K Ag@AT/K-1, Ag@AT/K-3, Ag@AT/K-5 and (b) BJH pore size distribution of Kaol, Ag/K and Ag@AT/K.

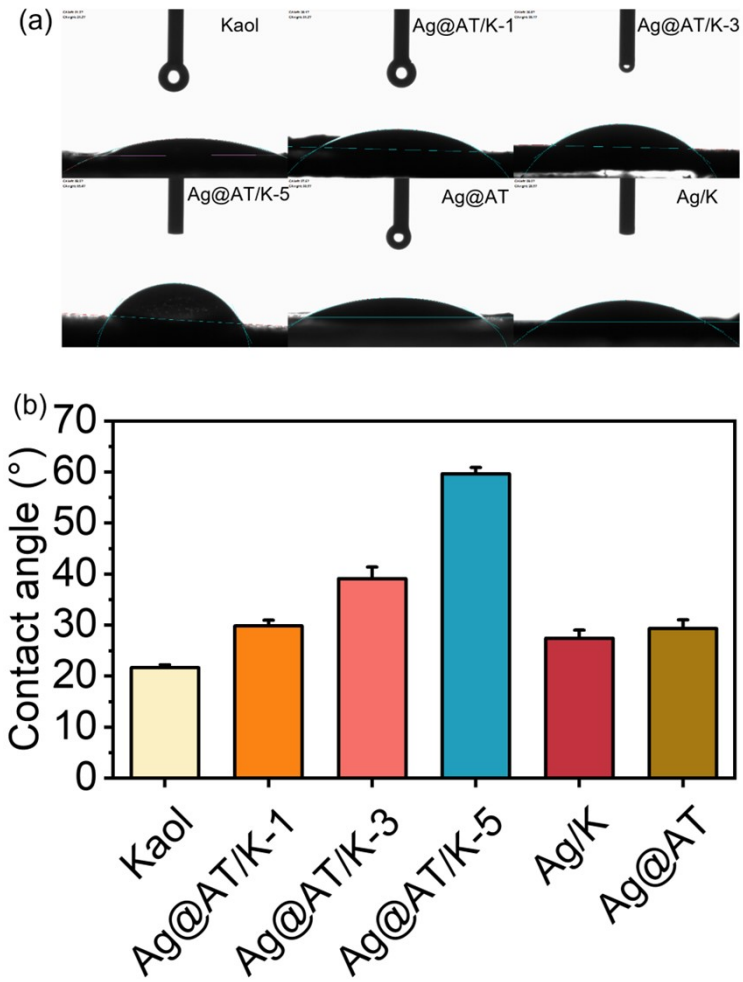


**Fig. S6.** (a and b) The contact angle of Kaol, Ag@AT/K-1, Ag@AT/K-3, Ag@AT/K-5, Ag/K-5 and Ag@AT.

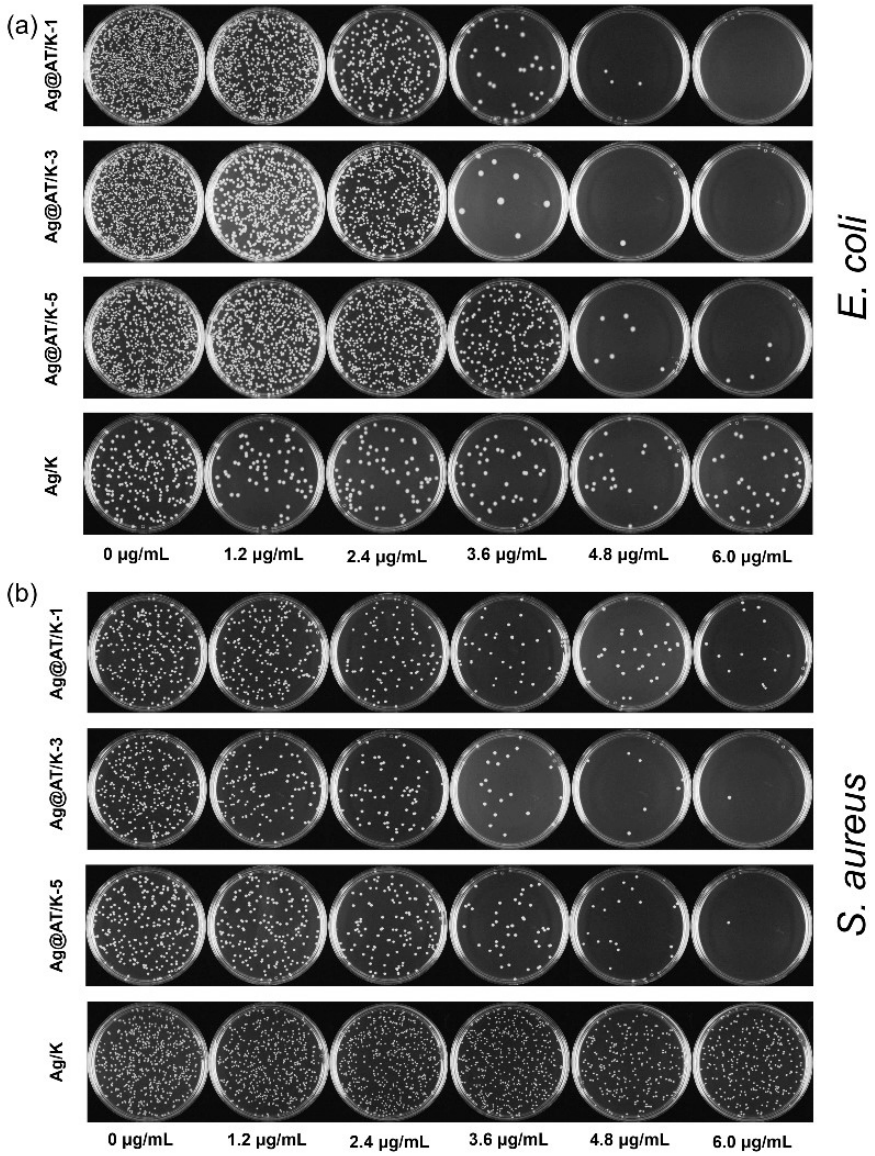


**Fig. S7.** Agar plate photographs of (a) *E. coli* and (b) *S. aureus* after treatment with Kaol, Ag@AT/K-1, Ag@AT/K-3, Ag@AT/K-5, Ag/K-5 and Ag@AT under different concentrations of Ag.

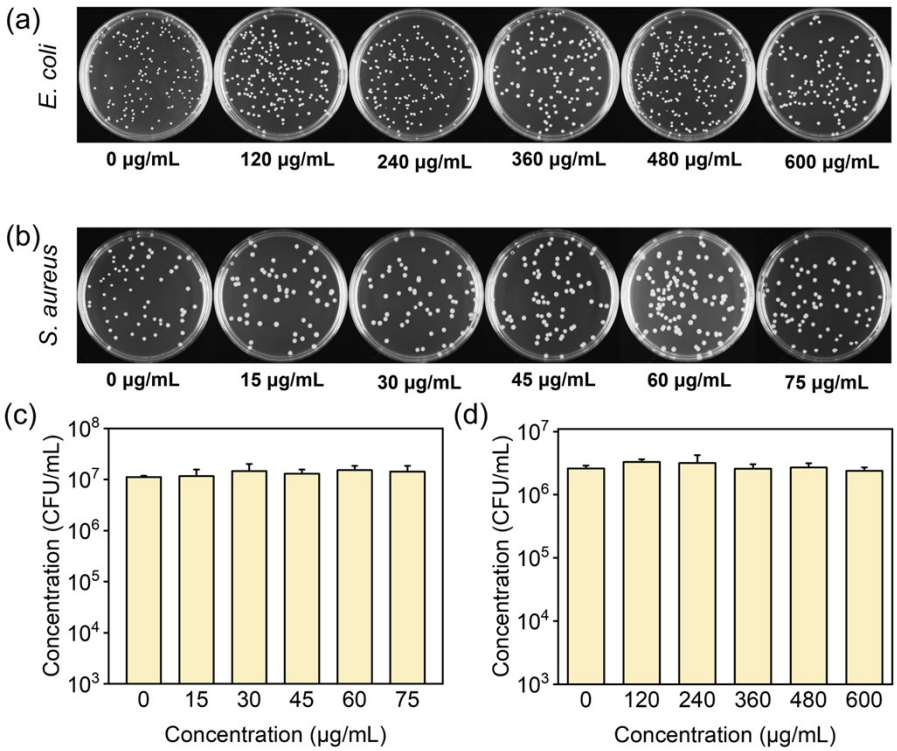


**Fig. S8.** Agar plate photographs of (a) *E. coli* and (b) *S. aureus* after treatment with Kaol under different concentrations of Kaol. Antibacterial evaluation against (c) *E. coli* and (d) *S. aureus* with different concentrations.

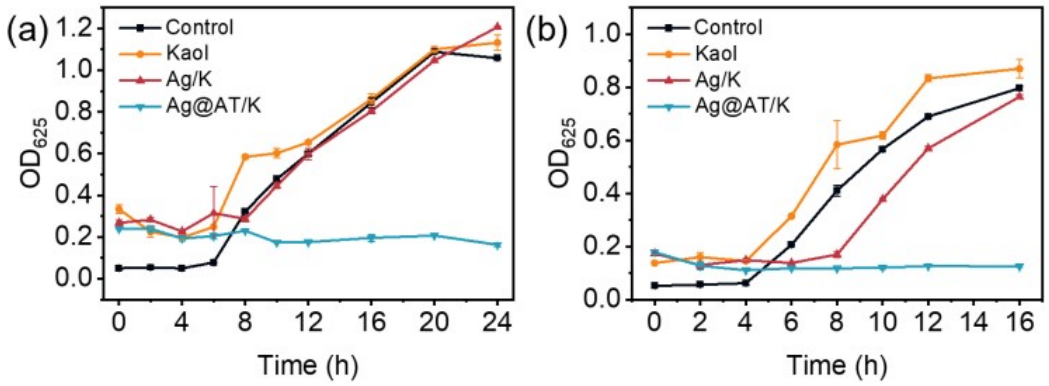


**Fig. S9.** Growth curves of (a) *E. coli* and (b) *S. aureus* in interaction with materials.

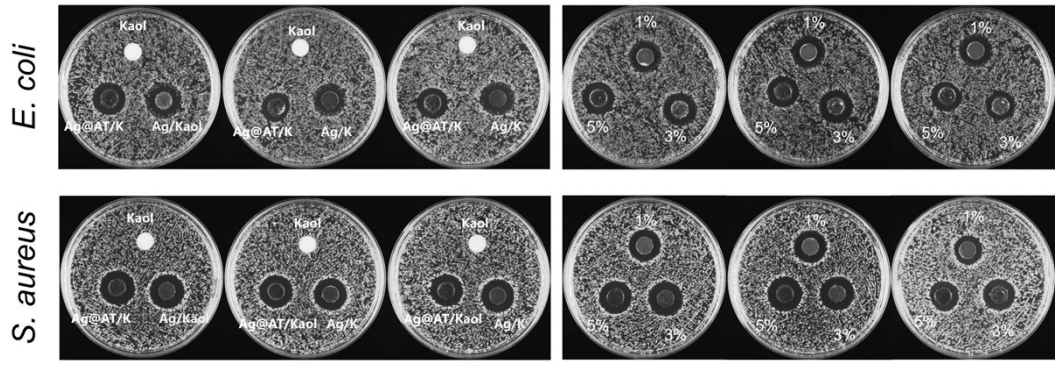


**Fig. S10.** Results of Kaol, Ag@AT/K-1, Ag@AT/K-3, Ag@AT/K-5 and Ag/K-5 against *E. coli* and *S. aureus*.

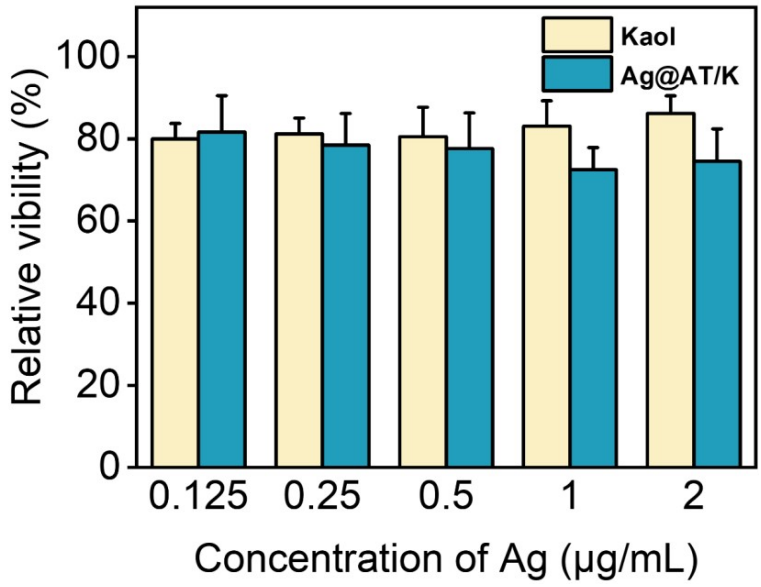


**Fig. S11.** Cell survival rate of L929 after incubation with Kaol and Ag@AT/K-3

**Table S1** The quantification of silver concentration contained in each nanocomposite.

| material | silver content (mg/kg) |
|---|---|
| Ag@AT/K-1 | 7891.83 |
| Ag@AT/K-3 | 27199.88 |
| Ag@AT/K-5 | 50397.13 |
| Ag/K | 48938.11 |

**Table S2** The statistical results of Ag NPs diameter in each nanocomposite.

| material | diameter (nm) |
|---|---|
| Ag@AT/K-1 | 6.71 ± 0.17 |
| Ag@AT/K-3 | 4.65 ± 0.05 |
| Ag@AT/K-5 | 5.26 ± 0.13 |
| Ag/K | 21.24 ± 1.22 |

**Table S3** The MIC Values (concentration of Ag) of the samples.

| material | MIC for *E. coli* (μg/mL) | MIC for *S. aureus* (μg/mL) |
|---|---|---|
| Ag@AT/K-1 | 10 | 40 |
| Ag@AT/K-3 | 5 | 40 |
| Ag@AT/K-5 | 10 | 40 |
| Ag/K | 40 | 80 |

**Table S4** The prepared formula of Ag@AT/K with different loading of Ag NPs

| material | Kaol (g) | $AgNO_3$ (g) | APTES (g) |
|---|---|---|---|
| Ag@AT/K-1 | 150.01 | 2.45 | 8.30 |
| Ag@AT/K-3 | 150.05 | 7.97 | 26.97 |
| Ag@AT/K-5 | 150.35 | 14.99 | 50.77 |

**Equation S1**

$$2\text{Si-OH} + [(C_2H_5O)_3\text{-Si-}CH_2CH_2CH_2NH_2]_2AgNO_3 \rightarrow [\text{Si-O-Si}(C_2H_5O)_2\text{-}$$

$$CH_2CH_2CH_2NH_2]_2AgNO_3 + 2\ C_2H_5OH$$