Robust immobilization of anionic silver nanoparticles on cellulose filter paper toward a low-cost point-of-use water disinfection system with improved anti-biofouling property

Gongyan Liu,[a,b,c] Ruiquan Yu,[a,b,c] Jing Jiang,[a,c] Zhuang Ding[a], Jing Ma,[a,b] and Ruifeng Liang[d,*]

[a]*College of Biomass Science and Engineering, Sichuan University, Chengdu 610065, China*

[b]*National Engineering Research Center of Clean Technology in Leather Industry, Sichuan University, Chengdu 610065, China*

[c]*The Key Laboratory of Leather Chemistry and Engineering of Ministry of Education, Sichuan University, Chengdu 610065, China*

[d]*The State Key Laboratory of Hydraulic and Mountain River Engineering, Sichuan University, Chengdu 610065, China*

*Corresponding Author, E-mail: lrfcy@163.com

KEYWORDS: Silver nanoparticle, cellulose filter paper, water disinfection, point of use, anti-biofouling

MATERIALS AND METHODS

Materials

N, N'-dicyclohexylcarbodiimide (DCC, 99%), Lipoic acid (99%), and 4-(dimethylamino) pyridine (DMAP, 99%) was purchased from Aladdin Technology Co. Ltd. (Shanghai, China). Cellulose filter papers were purchased from Xingya (Shanghai, China). Nutrient agar (NA) and nutrient broth (NB) were purchased from Shudu Chemical Co. Ltd (Chengdu, China). The bacterial strains of *Staphylococcus aureus* (ATCC6538) and *Escherichia coli* (ATCC25922) were purchased from BeNa Culture Collection (Beijing, China). LIVE/DEAD BaclightTM bacterial viability kit (L-7007, Invitrogen) was purchase from Thermo Fisher Scientific (China) Co. Ltd (Shanghai, China). Ethanol and glutaraldehyde 25% were purchased from Kelong chemistry company (Chengdu, China).

Synthesis of gallic acid protected silver nanoparticles (GA@AgNPs)

Gallic acid protected silver nanoparticles (GA@AgNPs) were prepared according to our previous work. Briefly, aqueous $AgNO_3$ solution (17 mM) was mixed with an equal volume of GA solution (18 mM). Then, the above mixture was added into $NaBH_4$ solution (37 mM) and reacted for 2 h to obtain GA@AgNPs with average diameter of 6 nm and concentration of 400 ppm.

Characterization

The surface morphologies of the synthesized GA@AgNPs-LA-FP samples were accomplished using scanning electron microscopy (SEM, Quanta250, FEI) operated at an accelerating voltage of 15 kV. The elemental compositions of the GA@AgNPs-LA-

FP samples were determined by an EDS spectrometer coupled with the SEM. The chemical states of the element on the paper surface were investigated by X-ray photoelectron spectroscopy (XPS, Thermo 250Xi, Thermo scientific). The surface zeta-potential value of GA@AgNPs-LA-FP was detected using the streaming potential method *via* an electrokinetic analyzer (Mütek SZP-10, BTG, Germany). CLSM images were captured using a Nikon N-SIM equipped with a 60× objective.

The silver loading content and the silver loading stability of GA@AgNPs-LA-FP

Briefly, 100 mg of dry GA@AgNPs-LA-FP sample was nitrated by 5 mL of HNO_3/H_2O_2 mixture solution (7:3, v:v). Then the solution was diluted by DI water to 100 mL for ICP-AES analysis to determine the silver loading content. The silver loading stability of GA@AgNPs-LA-FP sample was determined by measuring the leaching amount of silver under static and dynamic conditions, respectively. For testing stability under static condition, 50 mg GA@AgNPs-LA-FP sample was immersed into 50 mL DI water with continuous shaking (150 rpm) at room temperature and the water was replaced with 50 mL fresh DI water every 24 h. The silver content in DI water was measured by ICP-AES to determine the leaching of silver from GA@AgNPs-LA-FP sample. For evaluating the dynamic filtration stability, 2 L DI water was filtered through circular GA@AgNPs-LA-FP sample by gravity-driven. During the gravity-driven filtration, the depth of the DI water above GA@AgNPs-LA-FP sample was maintained at 10 cm to provide constant hydraulic pressure, and the filtrate was collected and the silver concentration was measure by ICP-AES.

Bacterial anti-adhesion ability

Briefly, 0.02 mL of bacterial suspension (10^8 CFU mL^{-1}) was dripped onto the filter paper and incubated in a 12 well tissue culture plate for 2 h at 37 °C (*E. coli* and *S. aureus*). After that, unadhered bacteria on filter paper surface were removed by five times PBS (pH 7.4) washing. Then, the filter paper samples were immersed in 1 ml PBS solution for ultrasonic treatment for 4 min to separate the strong adhesive bacteria on the surface of the filter paper. This operation was repeated five times, and the above solution was collected and mixed. The solution was cultured with the 0.02 mL isolated bacteria for 24 h at 37 ℃ on NA plate, and the viable bacterial colonies on plate were counted. The bacterial anti-adhesion rate can be estimated by:

Bacterial anti-adhesion rate (%) = ($CFU_{control}$ mL^{-1}- CFU_{sample} mL^{-1})/$CFU_{control}$ mL^{-1} * 100%

Bactericidal ability assay

According to ASTM E 2149-2001 standard procedure, GA@AgNPs-LA-FP was used to evaluate the antimicrobial activity against *E. coli* and *S. aureus*, respectively. 0.5 g dry filter paper specimen (sliced) was added to 20 mL PBS (pH 7.4) with bacterial concentration of 10^6 CFU·mL^{-1}, placed in a conical flask at 37℃ (*E. coli* and *S. aureus*), and oscillated continuously at 150 rpm. At 0 min and 10 min, 0.02 mL bacterial solution was collected from flask and inoculated in NA agar dish. This dish was cultured for another 24 hours at 37 °C. Colony-forming units were then counted:

%Kill = (CFU_{0min} mL^{-1} − CFU_{10min} mL^{-1})/ CFU_{10min} mL^{-1} ×100%

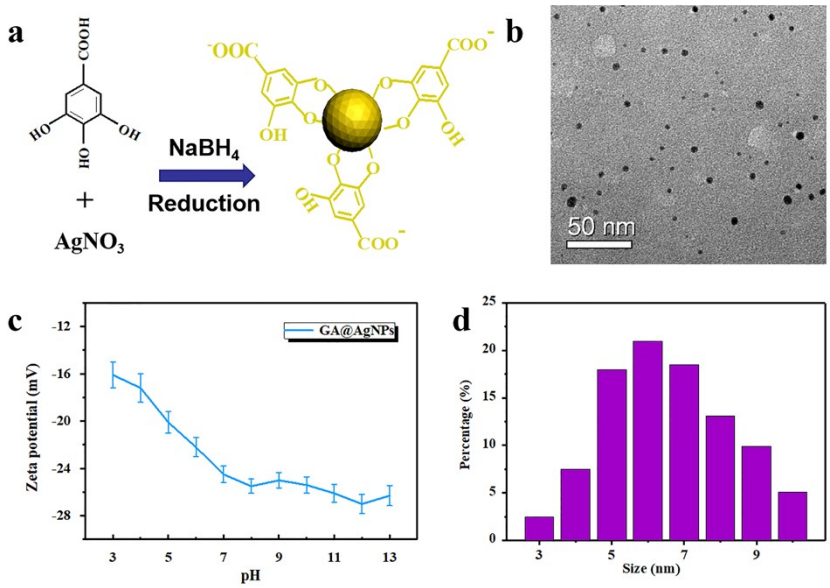

Fig. S1 (a) Schematic illustration of the synthetic route of GA@AgNPs; (b) HRTEM image of GA@AgNPs; (c) zeta potential of GA@AgNPs at different pH values; and (d) particle size histograms of GA@AgNPs calculated by Nano Measurer from HRTEM image.

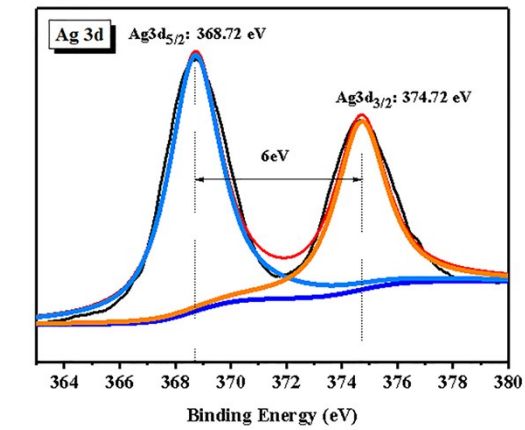

Fig. S2 Ag 3d core-level XPS spectra of the GA@AgNPs-LA-FP.

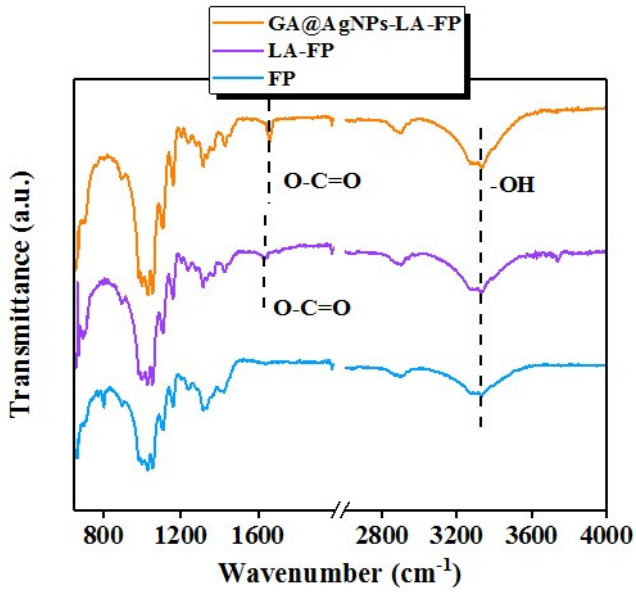

Fig. S3 ATR-FTIR spectra of pristine FP, LA-FP and GA@AgNPs-LA-FP.

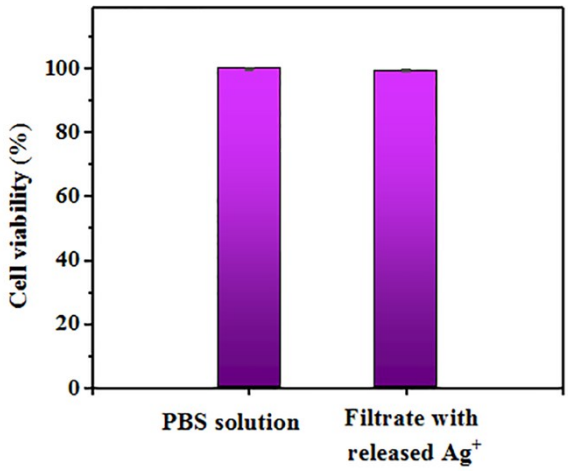

Fig. S4 Cell viability of L929 cells cultured with PBS solution and filtrate of testing release rate of Ag^{+} after 24 h.

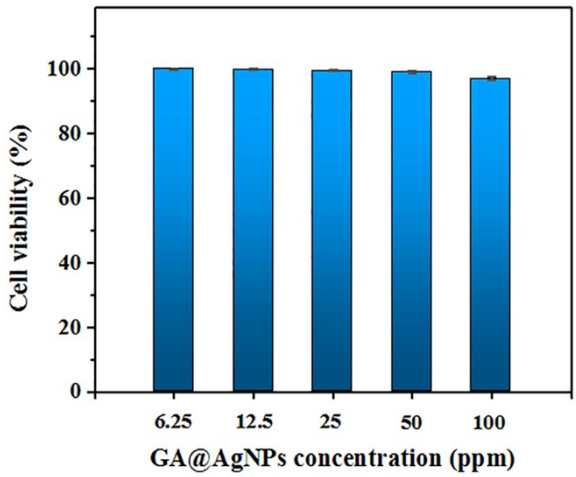

Fig. S5 The cytotoxicity of GA@AgNPs with different concentrations.

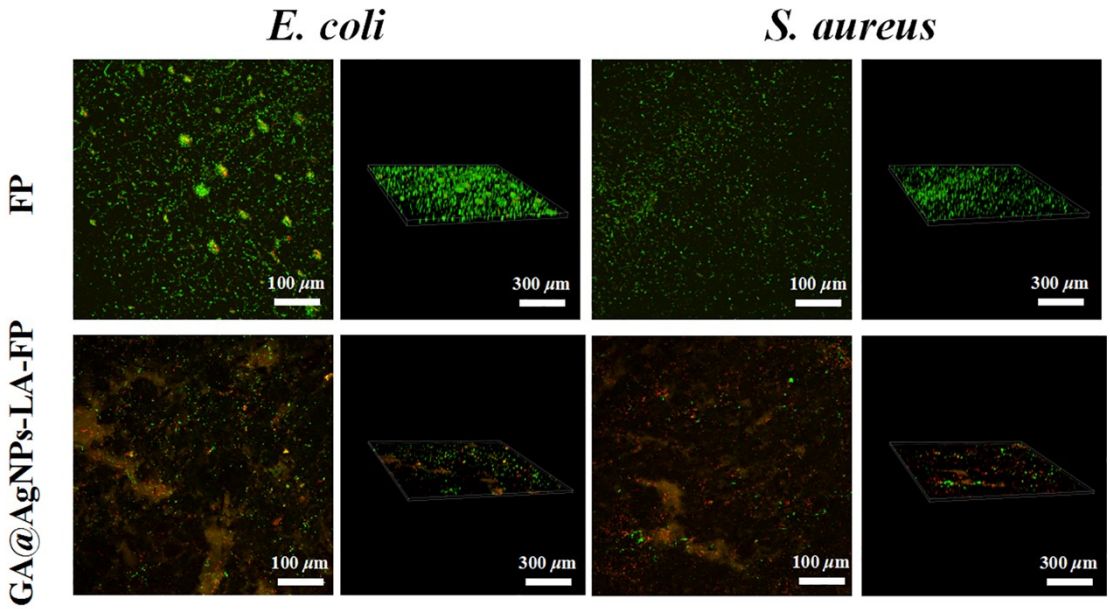

Fig. S6 CLSM images of biofilm structures developed on FP and GA@AgNPs-LA-FP after biofouling for 2 weeks. The thickness of biofilm on FP and GA@AgNPs-LA-FP were calculated from CLSM images by NIS Viewer software.

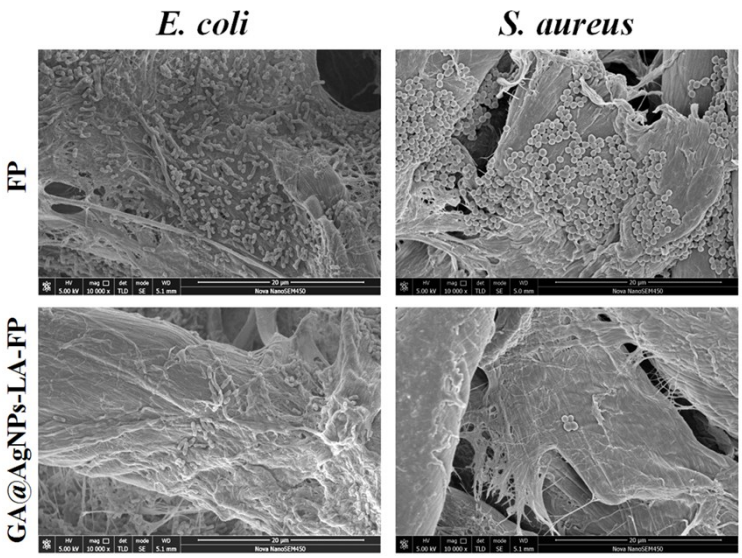

Fig. S7 FESEM images of microbial stain treated with FP or GA@AgNPs-LA-FP.

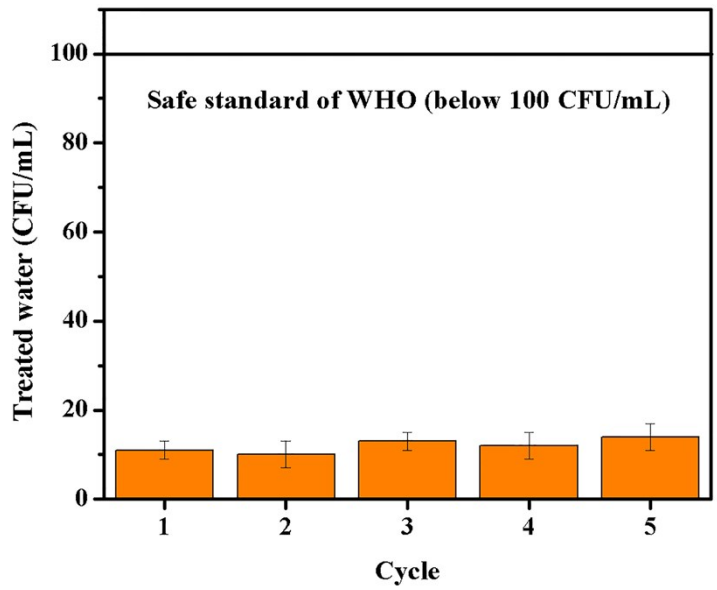

Fig. S8 Bactericidal stability test of the GA@AgNPs-LA-FP. Note that the experiment was done by analyzing every 200 mL of water. The total volume of the disinfected water was 1 L.

Table S1. Percentages of aliphatic C-C, C-O, C=O, and O-C=O bonds from XPS C1s peak fitting for FP, LA-FP, and GA@AgNPs-LA-FP samples.

Simples	Bond percentage (%)			
	C-C	C-O	C=O	O-C=O
FP	39.7	50.4	9.9	-
LA-FP	28.6	43.8	20.9	6.7
GA@AgNPs-LA-FP	25.3	44.2	20.3	10.2

Table S2. Bacteria counts and coliforms in river water before and after treatment with the FP and GA@AgNPs-LA-FP.

	Original	Treated by FP	Treated by GA@AgNPs-LA-FP	Inactivation rate
Total bacteria (CFU/mL)	2.1×10^3	5.1×10^2	11.0 ± 1.0	＞99.5%
Coliform (MPN/100mL)	1.5×10^4	5.2×10^3	negative	100%