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Synthesis and Molecular Dynamics Simulation of CuS@GO-CS Hydrogel for Enhanced Photothermal Antibacterial Effect

Mingqian Wang, Haomiao Zhu*, Jian Shen*

Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, Jiangsu Key Laboratory of Bio-functional Materials, Department of Materials Science and Engineering, School of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210023, P. R. China

†These authors contributed equally to this work.

*Corresponding authors: zhm@njnu.edu.cn (H. Zhu), jshen@njnu.edu.cn (J. Shen)

Tel/Fax: +86 25 83599188

Experimental Section

Preparation of Graphene Oxide (GO).

GO sheets were synthesized from the natural graphite powder with a modified Hummers' method [2]. A three-necked round-bottom flask (250 mL) containing concentrated sulfuric acid (33 mL) was put in an ice-bath. The graphite powder (1 g) and sodium nitrate (1 g) were added into the round-bottom flask and stirred. Potassium permanganate (6 g) was then added quite slowly with stirring, and the mixed solution temperature was kept at 0 °C. Subsequently, the mixture was heated to 35 °C and continuously stirred for 1.5 h. The mixture was then diluted with deionized water (40 mL) and stirred for another 15 min. The mixture was heated to 95 °C and continuously stirred for 35 min. Afterwards, additional deionized water (100 mL) was added, followed by dropwise adding of hydrogen peroxide (6 mL). The mixture was centrifuged at 1000 rpm for 5 min, and the supernatant was taken. The mixture was repeatedly washed with hydrochloric acid (1:10) at least three times to remove residual metal ions, and successively with deionized water to make the solution become neutral. After centrifugation at 4000 rpm for 5 min, the brown precipitate was freeze-dried for 48 h. Finally, the GO sheets were obtained.

Preparation of GO-COOH.

In order to improve the subsequent grafting rate of CuS, GO was modified. Chloroacetic acid (1.2 g), sodium nitrate (1.0 g) and GO (50 mg) were added to water (100 mL) and dispersed by ultrasound for 3 h. The above solution was neutralized with 25 % HNO_3 solution, and washed with acetone and water twice. GO-COOH was obtained by vacuum drying.

Preparation of GO-PEG.

For pegylation, NaOH (1.2 g) was added to GO aqueous suspension (10 mL; 1.0 mg/mL) and the suspension was bath sonicated for about 3 h to convert OH groups to COOH via conjugation of acetic acid moieties resulting in GO-COOH [22]. The resulting solution was neutralized and purified by repeated rinsing and centrifugation. Then, EDC aqueous solution (3 mL; 2.0 mg/mL) was added to the of GO-COOH

suspension (5 mL; 1.0 mg/mL), and the mixture was sonicated for another 30 min. After that, 8-arm-polyethylene glycol-amine (15.0 mg) was added to the above suspension, and allowed to react overnight. The reaction is terminated by adding mercaptoethanol (4 mL). The solution was centrifuged and washed with water to remove the unreacted PEG. GO-PEG was obtained by vacuum drying.

Preparation of CuS@GO.

The detailed procedure for the synthesis of GO@CuS nanomaterials was carried out as follows. In a three-neck flask, $CuCl_2{\cdot}5H_2O$ aqueous solution (10 mL; 1 mM) was added to GO-PEG aqueous suspension (1 mL; 1.0 mg/mL) under stirring, and kept the magnetic stirring for 30 min. Then, Na_2S aqueous solution (100 mL; 0.1 M) was added into the reaction solution under stirring. After 10 min, the reaction mixture was heated to 90 °C and stirred for 15 min until a dark-green solution was obtained. After the reaction, the resulting mixture was centrifuged and washed with water to remove the unreacted ions. CuS@GO nanomaterials were obtained by vacuum drying.

Preparation of CS Hydrogel.

The preparation of CS hydrogel was conducted following the previously reported method with slight modification [3]. To prepare CS hydrogel, CS (2.5 g) was dissolved in acetic acid solution (100 mL; 2 wt %) with continuous mechanical stirring for 1h to obtain a homogeneous viscous mixture. Then glycerinum (2 mL) and polyethylene glycol (2.0 g) were added at 50 °C for 6 min until a homogeneous mixture was obtained. Subsequently, glutaraldehyde (20 mL; 2 %) as the cross-linking agent was added with continuous mechanical stirring for 10 s. Finally, the mixture was poured into a glass Petri dish immediately and placed into the oven at 50 °C for 10 h. All the prepared samples were washed several times with hot distilled water in order to remove residual glutaraldehyde and freeze-dried for 36 h.

Preparation of CuS@GO-CS.

The obtained CS hydrogel was cut to obtain a series of wafers with regular shape and uniform size of ϕ 6 mm × 2 mm. The wafer was immersed into 2 mL of CuS@GO aqueous solution (100, 200, 400 μg/mL) for 30 min. Afterwards, these obtained CuS@GO-CS hydrogel was freeze-dried.

Photothermal effects under 808 nm laser irradiation.

The photothermal conversion efficiency of the hydrogel was measured according to the reference reported previously. The NIR laser light (808 nm) at a power density of 2.0 W/cm^2 was focused to a spot size of 1 cm. The hydrogel swelled in aqueous solution to reach equilibrium during light irradiation and the temperature was recorded at 1 min intervals for a total of 6 min using a thermal imager. This experiment was did four times.

Hemolysis Assay

The hemolytic test was used to determine the blood compatibility of CuS@GO-CS hydrogel. Fresh anticoagulated blood from human volunteers (2 mL) was centrifuged at 3000 rpm for 10 min. The supernatant was discarded and the red blood cell suspension was obtained, and then washed three times by normal saline solution. Mix red blood cell suspension and physiological saline at a volume ratio of 1:24. The different concentrations of CuS@GO-CS hydrogel (20 μg/mL, 40 μg/mL, 60 μg/mL, 80 μg/mL, 100 μg/mL) solution were prepared in normal saline solution and kept at 37 ℃ for 30 min. The diluted blood (1.0 mL) was added to the CuS@GO-CS hydrogel solution. Then the mixture was kept at 37 ℃ for 3 h and then centrifuged at 1500 rpm for 10 min. The supernatant was transferred to a 96-well plate and the absorbance was measured at 545 nm using a BioTek Synergy 2 Multi-Mode Microplate Reader. Positive controls consisted of 1.0 mL diluted blood in 1.0 mL deionized water while negative controls consisted of 1.0 mL diluted blood in 1.0 mL normal saline solution. The hemolysis degree was calculated as follows:

Hemolysis rate = [(Dt - Dnc)/(Dpc - Dnc)] × 100%

where Dt is the absorbance of the sample, Dnc is the absorbance of the negative control, and Dpc is the absorbance of the positive control.

***In vitro* antibacterial effects.**

Two bacteria, *E. coli* (Gram-negative) and *S. aureus* (Gram-positive), were used to evaluate the antibacterial activity of the hydrogels by the spread plate method. The samples were challenged with *E. coli* and *S. aureus* bacteria at a concentration of 10^7

CFU mL^{-1} and each sample was divided into two groups (irradiated for 6 min under 808 nm laser or cultured for 6 min). The antibacterial activity was first studied using a spread plate, evenly seeding 20 μL of bacterial stock suspension onto hydrogels (ϕ 6 mm × 2 mm), which were placed in 48-well plates, the tissue culture plate served as the control. For the 6 min model, the 48-well plates containing bacteria and hydrogels were then irradiated under 808 nm light for 6 min, the surface temperature was detected by using the thermal imager. Then the bacteria on the hydrogels and tissue culture plate were diluted 100-fold using Luria-Bertani (LB) broth. Afterward, the surviving bacteria were separated from the hydrogels and tissue culture plate using low-power ultrasound, and 20 μL of diluent was then collected and spread on an LB agar plate and incubated at 37 °C for 24 h to form viable colony units. The antibacterial ratio was calculated as follows in eq 1:

Kill (%) = (cell count of control group - survivor count on experimental group) / cell count of control group × 100 %

***In vitro* cytotoxicity studies.**

The *in vitro* cytotoxicity of the hydrogels was determined by the MTT assay using NIH-3T3 cells. Particularly, the NIH-3T3 cells were seeded onto the hydrogels (ϕ 6 mm × 2 mm) and tissue culture plate in 48-well plates with 350 μL of medium and cultured for 1, 3, and 7 days followed by incubation under a humidified atmosphere of 5% CO_2 at 37 °C. Tissue culture plate served as the control, and the medium was refreshed every 3 days. After days of 1, 3, and 7, the culture medium was removed from each plate, and 350 μL of the MTT solution (5 mg/mL in PBS) was added into each plate with continuous culture at 37 °C for 4 h. Afterward, the MTT solution was replaced with 350 μL of dimethyl sulfoxide, followed by shaking at a shaking table for 15 min to dissolve the formazan completely. After that, the optical density (OD) at 570 nm was measured. Meanwhile, in order to explore the safety of the samples in the presence of 808 nm light, the cells were seeded onto the hydrogels, followed by 10 min irradiation with 808 nm light. After culturing for 1, 3, 7 days, the *in vitro* cytotoxicity of the hydrogels was determined by the MTT assay. The cell viability was calculated as follows in eq 2:

Cell viability (%)

= OD in experimental group / OD in control group ×100%

Statistical Analysis.

All of the data were expressed as mean ± standard deviations (SD). Each test was repeated three or more times. Analysis of variance (ANOVA) statistics was performed, and p values less than 0.05 were considered to be statistically significant.

Figure

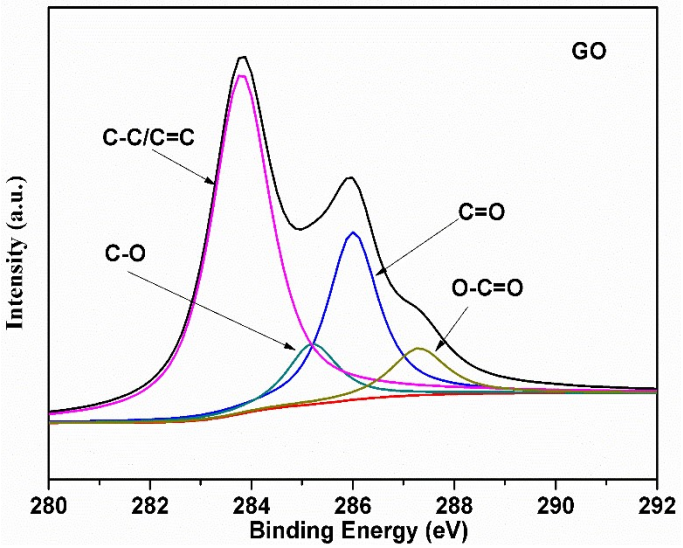

Figure S1. the C1s of GO.

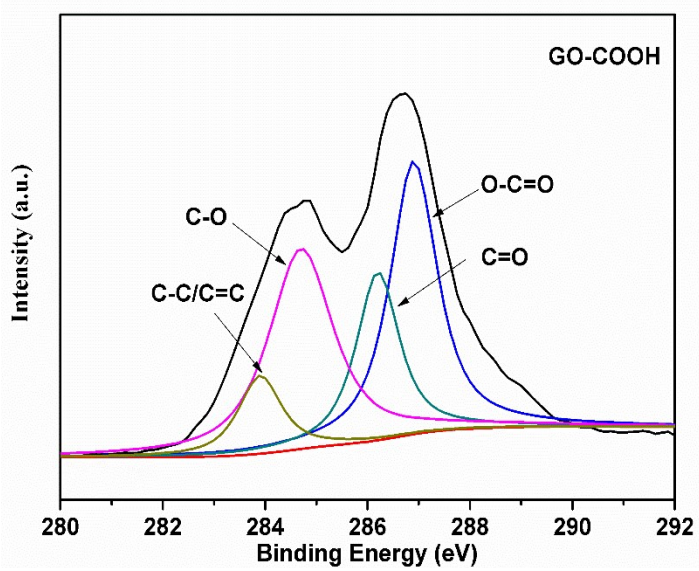

Figure S2 the C1s of GO-COOH.

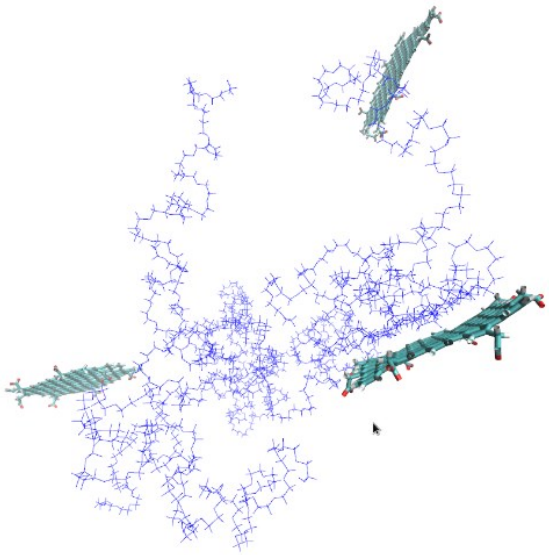

Figure S3 The interaction between carboxyl-graphite lamella and PEG.

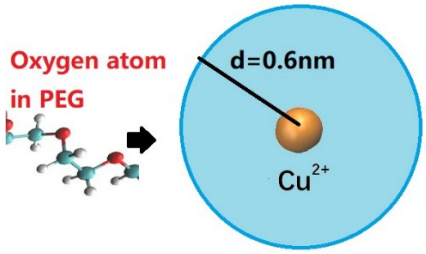

Figure S4. The definition of Cu^{2+} capture occurrence

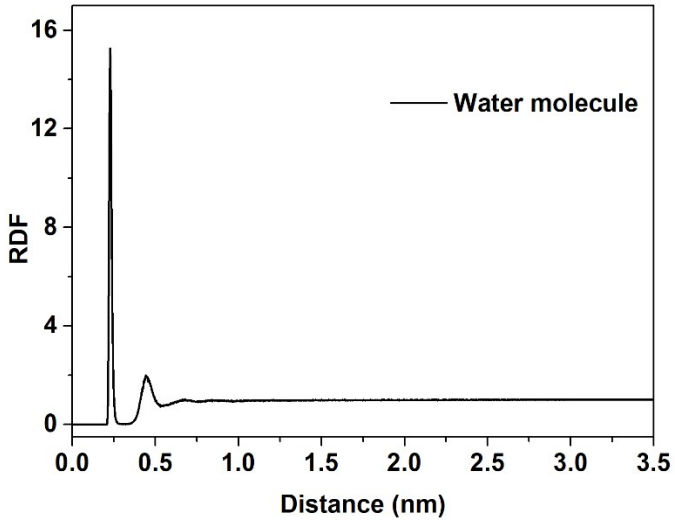

Figure S5. Radial distribution function of water molecules surrounding the Cu^{2+}. Water molecules were represented by O atoms in them.

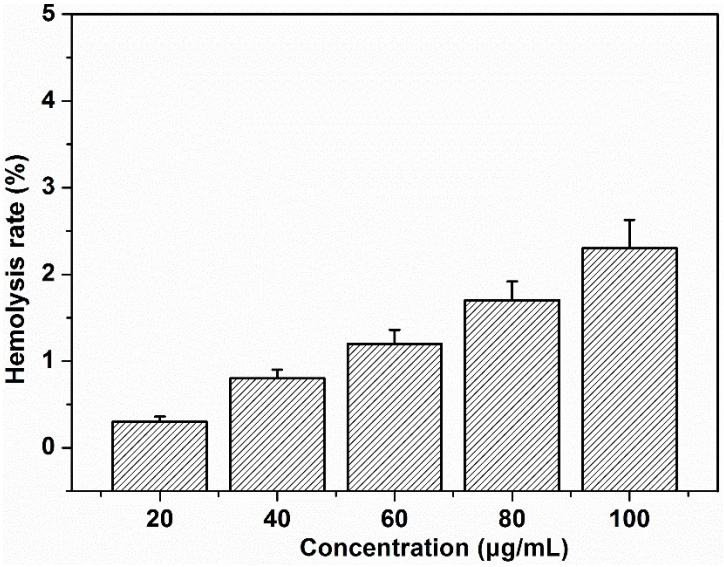

Figure S6. The hemolysis rate of the CuS@GO-CS hydrogel with different concentrations solutions.

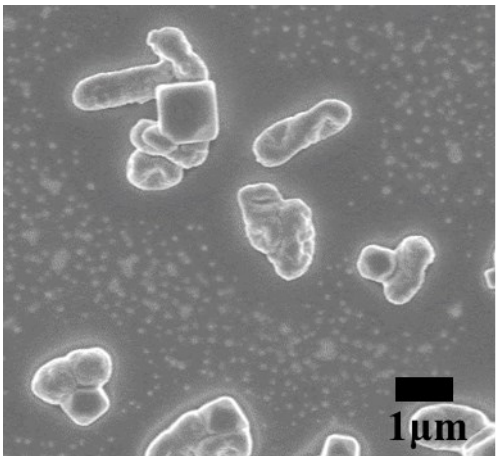

Figure S7. SEM image of *E.coli* after photothermal experiments.

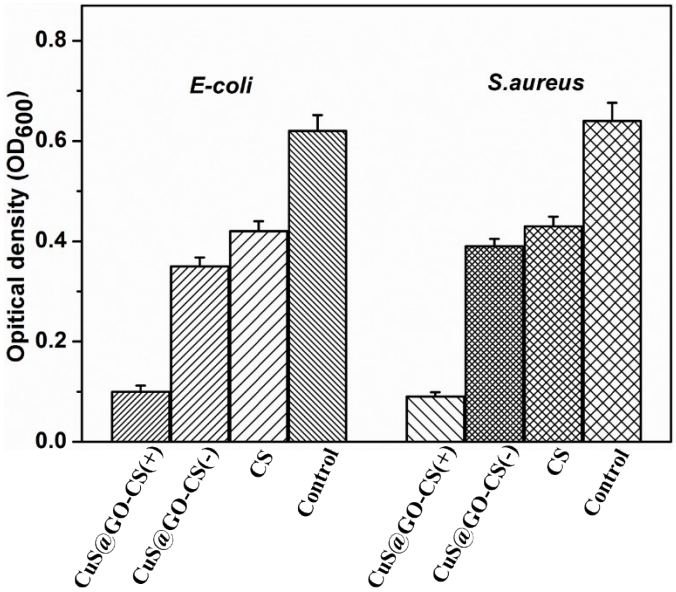

Figure S8 The O.D. 600 nm value of control, CS, CuS@GO-CS(-) and CuS@GO-CS(+) treated

with *E. coli* and *S. aureus* (5×10^6 CFU/mL) in LB broth medium for 24 h.