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Preparation of graphene oxide–silver nanoparticle nanohybrids with highly antibacterial capability

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ABSTRACT

A simple method based on electrostatic interactions was utilized to assemble silver nanoparticles (AgNPs) to graphene oxide (GO) sheets. This method allows conjugation of AgNPs with desired morphologies (densities, sizes and shapes) onto GO. In this process, poly(diallyldimethylammonium chloride) (PDDA) was introduced as an adhesive agent. The as-prepared graphene oxide–AgNPs composites (GO–AgNPs) have enhanced colloid stability and photo-stability than that of AgNPs. After conjugating to GO sheets, the antibacterial activities of AgNPs against Gram negative (G−) bacterial strain (*Escherichia coli, E. coli*) and Gram positive (G+) bacterial strain (*Bacillus subtilis, B. subtilis*) have been improved significantly. The antibacterial activity of GO–AgNPs is dependent on the size of AgNPs, i.e. the small AgNPs modified GO sheets show more effective antibacterial capability than that of large AgNPs modified GO sheets. Compared with AgNPs, the enhanced antibacterial activity of GO–AgNPs might not only be due to high stability of AgNPs anchored on GO sheets, but also the positive charged surface of hybrids which increases the electrostatic interaction of bacterial cell membrane with nanohybrids.

1. Introduction

Bacterial species have been identified as important pathogens in many severe pathogen-related diseases among humans. And bacterial pathogens are enabling to distribute in a wide range of substrates, such as soil, contaminated food and animals excrements [1–4]. One of the most effective strategies for the prevention of microbial colonization is to develop a functional material with highly antimicrobial property. Recently, the antimicrobial efficacy of engineered nanoparticles (NPs) including metal and carbon-based NPs has been widely studied [5–8]. Among the great variety of antibacterial materials, silver NPs (AgNPs) are marked out as antimicrobial reagents with high capability due to their large surface area and slow release properties [9–12]. Although the bactericidal mechanism of AgNPs is not fully understood, three possible mechanisms are usually proposed: (i) gradual release of silver ions, which can affect on DNA replication and ATP production, (ii) direct damage to cellular membranes by AgNPs, or (iii) generation of reactive oxygen species (ROS) from AgNPs and Ag^+ [13]. It is believed that good dispersion of AgNPs is required for effective antibacterial activities [14]. However, colloidal AgNPs have a tendency to aggregate in practical applications due to colloidal instability, leading to deterioration of antibacterial performance [14]. Therefore, development of stable, dispersed AgNPs substrates is a critical challenge of AgNP-based antimicrobial materials because of the poorly colloidal stability of AgNPs [15–17]. To address this problem, loading AgNPs on supporting matrix is one of the most efficient strategies [17].

Graphene is a one-atom thick and two-dimensional carbon allotrope made up of a conjugated system of sp^2 carbons arranged in a honeycomb structure. It has attracted great attention and shown great potential applications for fabricating electronic devices, nanocomposites, sensors and nanocarriers [18]. Graphene oxide (GO) is strongly hydrophilic material, and easily forms stable colloidal dispersions in water because it has a great deal of oxygen bonds in its edges and defect sites, such as hydroxyl (C3OH), carboxylic (–COOH), carbonyl (C=O), epoxide groups (C3O3C) on both accessible sides [19]. The functional groups have been confirmed to be reducibility and have been actively used to build new composites [20–22]. For instance, we have synthesized a kind of thionin-bridged graphene–gold NP nanohybrids with efficient photothermal therapy ability through formation of amide bonds [23]. Cui and coauthors have used GO and AgNPs to prepare carbon nanoscrolls via several hours ultrasonic vibration, which exhibited enhanced antifungal activities [24].

In situ synthesis of GO–AgNPs with good antibacterial activity has been extensively reported [25,26]. For example, Tang and co-workers have in situ synthesized of a kind of Ag–polydopamine–graphene nanosheets as antibacterial agents [27]. However, the reduction of GO usually has a side effect on the dispersity and stability of GO–AgNPs [28] and the negative surface charge would

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weaken the contact between bacterial cells and the GO–Ag nanocomposites [25]. Self-assembly is another effective method to fabricate GO–NPs nanohybrids, in this approach NPs can be prepared as requirement and GO could be used as a supporting matrix without reduction [29].

Herein, a facile method to assemble AgNPs and GO sheets is demonstrated. In this process, poly(diallyldimethylammonium chloride) (PDDA) was employed as a linker for conjugating AgNPs to GO sheets. AgNPs with different densities, sizes and shapes can be loaded onto GO sheets easily in this way. The antibacterial activities of GO–AgNPs have been investigated using Gram-negative (G-) bacteria *Escherichia coli* (*E. coli*) and Gram-positive (G+) bacteria *Bacillus subtilis* (*B. subtilis*) as model bacteria. The effects of various AgNPs sizes, mass ratio of Ag to GO–PDDA and dosages on antibacterial activity of GO–AgNPs have been examined.

2. Experimental section

2.1. Materials

Graphite was purchased from Alfa Aesar co., Ltd. (USA). Poly (diallyldimethylammonium chloride) (PDDA, 35 wt% in water) was obtained purchased from Sigma-Aldrich co., Ltd. (USA). *E. coli* (ATCC BL21) bacterial strain and *B. subtilis* (ATCC 31785) were purchased from Dingguo co., Ltd. (Beijing, China). Tryptone and yeast extract were obtained from Oxoid Co., Ltd. (England). Baird-Parker agar base was purchased from Qingdao Hope Bio-Technology Co., Ltd. (Qingdao, China). Ascorbic acid, trisodium citrate, silver nitrate ($AgNO_3$) and other agents were purchased from Beijing Chemical Reagents Co., Ltd. (Beijing, China). All reagents were analytical grade and used as received without further purification. Milli-Q water (18.2 MΩ cm) was used in all experiments.

2.2. Apparatus

UV–vis–NIR spectra were obtained by TU-1901 UV–vis–NIR spectrophotometer (Purkinje General Instrument Co., Ltd. China). FTIR characterization was carried out on Vertex70 Fourier transform infrared spectroscope (Bruker Co., Ltd. Germany). X-ray photoelectron spectroscopy (XPS) measurements were carried out on EscA-LABMK II X-ray photoelectron spectroscope (VG Scientific Co., Ltd., UK). Raman spectra were obtained by a Renishaw 2000 Raman spectrophotometer (Gloucestershire, UK) equipped with an Ar^+ ion laser (514.5 nm wavelength) and a CCD detector. Transmission electron microscopy (TEM) measurements were made on H600 Transmission electron microscope (Hitachi Co,. Ltd. Japan) with an accelerating voltage of 100 kV. The composition of the sample was determined by iCAP 6300 inductively coupled plasma-optical emission spectrometer (ICP-OES, Thermo., USA). Zeta potential measurements were made on ZEN3600 Zetasizer (Malvern Co., Ltd. UK). Thermo-gravimetric analysis (TGA) of sample was performed on a Pyris Diamond TG/DTA thermogravimetric analyzer (Perkin-Elmer Co,. Ltd. USA). In TGA experiment, the samples were heated from room temperature to 900 °C at 10 °C min^{-1} under an air atmosphere [27,30].

2.3. Synthesis of silver nanoparticle–graphene oxide

GO was prepared from natural graphite powder by the modified Hummers method [31]. Silver nanoparticles (AgNPs) with ca. 14 nm were prepared according to previous report with modification [32]. In a typical synthesis, 48 mL water was mixed with 5 mg of ascorbic acid and 40 mg of sodium citrate, and the pH of solution was adjusted to 11 with 0.2 M NaOH. 2 mL of $AgNO_3$ solution (10 mg) was added dropwise on ice-water bath. After stirred for 30 min, the solution was transferred into a 100 °C water bath for 1 h. About 46 nm AgNPs were prepared using citrate reduction method of Lee and Meisel [33]. Typically, 50 μL of 0.2 mM ascorbic acid was added into 50 mL of boiling water. 10 mg of sodium citrate and 2.5 mg of $AgNO_3$ were mixed in 3 mL of water under stirring at room temperature. After 5 min incubation at room temperature, the mixture solution was injected into the ascorbic acid solution. The solution was further boiled and stirred for 1 h and cooled down to room temperature. Ag nanoplates were prepared according to previous report [34]. Generally, 1 mg of $AgNO_3$, 26 mg of sodium citrate and 60 μL of H_2O_2 (30 wt%) were added into 50 mL of water under stirring. After stirred for 1 min, 250 μL of $NaBH_4$ (1.4 mg) was injected into the solution rapidly, and the blue colored solution indicated formation of Ag nanoplates. All of the products were stored at 4 °C for further use.

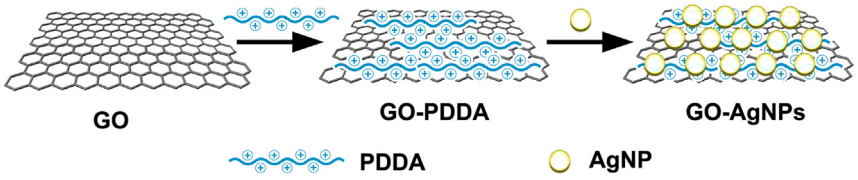
2 mL of as-prepared GO (3.0 mg mL^{-1}) was mixed with 1.6 mL of PDDA (10 mg mL^{-1}). The pH value of mixture was adjusted from 1.0 to 6.5 by 1.0 M NaOH solution under ultrasonic vibration. After ultrasonic vibration for 20 min, the mixture was centrifuged (13000 rpm, 10 min) for 3 times to remove excess PDDA. The obtained product, named as GO–PDDA, was dispersed in 10 mL water and stored at 4 °C. 100 μL of GO–PDDA was mixed with various volumes (12 mL, 6 mL, 2 mL, 670 μL or 200 μL) of AgNPs (120 μg/mL) (or 8 mL of as-prepared large AgNPs (30 μg/mL) or 15 mL of Ag nanoplates (12 μg/mL)) under ultrasonic vibration, respectively. The mixture was centrifuged (5000 rpm, 10 min) for 3 times after ultrasonic vibration for 1 min, and then the precipitate was re-dispersed in water, named as GO–AgNPs. For antibacterial test, the amounts of Ag in AgNPs and GO–AgNPs solutions were determined by ICP analysis.

2.4. Zeta potential measurement

For zeta potential measurements, 800 μL of samples dispersed in 20 mM HEPES (pH=7.4) were injected into the measuring cell. After equilibration at 25 °C for 120 s, the potential data were collected for 3 times.

2.5. Bacteria culturing

All bacterial strains were cultured in Luria-Bertani (LB) culture medium (10 g•L^{-1} tryptone, 5 g L^{-1} yeast extract, 5 g L^{-1} NaCl). The bacteria strains were incubated in LB medium with shaking at 37 °C. The optical density at 600 nm (OD_{600}) was measured to monitor bacterial growth. When the cultures reached an OD_{600} of 0.3 (the beginning of logarithmic phase) [35], the log phased strains were centrifuged at 5000 g for 8 min, and resuspended in



Scheme 1. Procedure for the self-assembly of AgNPs and PDDA onto GO nanosheets.