Colloids and Surfaces B: Biointerfaces 149 (2017) 322–329

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Controlled release and long-term antibacterial activity of reduced graphene oxide/quaternary ammonium salt nanocomposites prepared by non-covalent modification

Xiaoli Ye[a,b], Jin Feng[a], Jingxian Zhang[b], Xiujiang Yang[a], Xiaoyan Liao[c], Qingshan Shi[a,*], Shaozao Tan[b,*]

[a] State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology, Guangzhou 510070, China
[b] Department of Chemistry, Jinan University, Guangzhou 510632, China
[c] Department of Light Chemical Engineering, Guangdong Polytechnic, Foshan, 528041, China

ARTICLE INFO

Article history:
Received 19 May 2016
Received in revised form
29 September 2016
Accepted 9 October 2016
Available online 14 October 2016

Keywords:
Reduced graphene oxide
Quaternary ammonium salts
π-π interactions
Long-term antibacterial property
Controlled release

ABSTRACT

In order to control the long-term antibacterial property of quaternary ammonium salts, dodecyl dimethyl benzyl ammonium chloride (rGO-1227) and rGO-bromohexadecyl pyridine (rGO-CPB) were self-assembled on surfaces of reduced graphene oxide (rGO) via π-π interactions. The obtained rGO-1227 and rGO-CPB nanocompounds were characterized by X-ray diffraction (XRD), fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), field emission scanning electron microscopy (FESEM), and transmission electron microscopy (TEM).The antibacterial activities were evaluated on Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*. Both rGO-CPB and rGO-1227 reduced the cytotoxicity of the pure antimicrobial agents and presented strong antimicrobial properties. Especially, CPB could be loaded efficiently on the surface of rGO via π-π conjugate effect, which resulted in a nanocomposite presenting a long-term antibacterial capability due to the more important quantity of free π electrons compared to that of 1227. When comparing the advantages of both prepared nanocomposites, rGO-CPB displayed a better specific-targeting capability and a longer-term antibacterial property.

1. Introduction

Microbial contamination is a challenge of paramount importance threatening human health notably because it concerns a large range of daily activities such as drinking water and water purification systems, aquaculture, food packaging, hospital furniture and surgical equipment, medical equipment, and textiles among others [1–7]. Antimicrobial agents destroy the pathogenic microorganisms and are applied in the formulations of water and soil disinfectants, antimicrobial drugs, or food preservatives [8]. However, the increasing antimicrobial resistance could jeopardize the effective prevention and treatment of microbial infections. Quaternary ammonium salts (QAS) are efficient antimicrobial agents that do not promote antimicrobial resistance [9,10]. Indeed, their bactericidal action is based on the strong electrostatic interactions with the positively charged bacteria, which leads to the destruction of the cell membrane of the bacteria, inducing therefore the leakage of intracellular components from the bacterial cell [11,12]. They are widely used in antibacterial treatment of daily activities and exhibit antibacterial activity against a wide range of bacteria, fungi, and virus [13]. However, pure quaternary ammonium salts present a non-controllable and slow release presenting only short-term antibacterial activity [14]. Moreover, the unnecessary release of biocides could directly cause unpredictable long-term pollution [15]. Therefore, the development of release carriers enabling a long-term sustainability is needed. As a matter of fact, a long-term antibacterial action enhances the antimicrobial efficiency and environmentally friendliness of the agent [16].

Graphene was herein considered as a potential carrier for ammonium quaternary salts. The material is characterized by two-dimensional carbon sheets of a single-atom thick and is used in many fields of application (nanoelectronics, conductive thin film, super capacitors, nanosensors, and drug-delivery) due to its high

* Corresponding authors.
E-mail addresses: shiqingshan@hotmail.com (Q. Shi), tanshaozao@163.com (S. Tan).

http://dx.doi.org/10.1016/j.colsurfb.2016.10.016
0927-7765/

conductivity, specific surface area, and chemical stability and good thermal, mechanical, and electrical performances [17–20]. Specifically, reduced graphene oxide sheets (rGO) are efficient carriers for materials exhibiting an electronically conjugated chemical structure. Recently, H. Kim et al. reported that some drugs of special structure could assemble on the surface of rGO via π-π interactions [21]. However, the irreversible agglomeration of rGO caused by the strong hydrophobicity of the rGO surface complicates the processing and challenges the use of rGO as a drug-delivery material. Fortunately,we found that quaternary ammonium salts could disperse homogeneously on graphene layers by acting as efficient surfactants.

In this paper, the two antimicrobial quaternary ammonium salts, dodecyl dimethyl benzyl ammonium chloride (1227) [22] characterized by a benzene ring structure and bromohexadecyl pyridine (CPB) presenting a pyridine ring structure (Fig. 1), could be assembled on the surfaces of rGO via π-π interactions. More specifically, the electronically conjugated structure of the quaternary ammonium salts could directly interact with the carboxyl groups on the rGO surface via electrostatic interactions [23]. We compared the rGO interaction affinity of the benzene ring with the pyridine ring of 1227 and CPB, respectively. Moreover, both compounds are known to improve the dispersion of graphene. In a previous work, we have shown that electronically conjugated structured antimicrobial drugs attached to rGO more efficiently than on GO [24] achieving long-term antibacterial properties and simultaneously reducing the toxicity of the antibacterial drug. To better understand the influence of rGO on the antibacterial activities of 1227 and CPB, we studied two types of graphene-based materials (rGO-1227 and rGO-CPB) by comparing their antibacterial and long-term antibacterial activities and cytotoxicities.

2. Materials and methods

2.1. Materials

Dodecyl dimethyl benzyl ammonium chloride (1227) and bromohexadecyl pyridine (CPB) were purchased from Jinhuada Chemical ReagentCo., Ltd (Guangzhou, China).Graphite powder (spectral pure), $KMnO_4$, P_2O_5, H_2O_2 and concentrated H_2SO_4were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hydrazine hydrate (80%) was purchased from FuyuChemical Reagent Co., Ltd (Tianjin, China). Gram-negative *E. coli* ATCC 25922 and Gram-positive *S. Aureus* ATCC 6538 were supplied by the Guangdong Institute of Microbiology (Guangzhou,China). Nutrient agar culture mediumwas provided by Huankai Microorganism Co., Ltd. (Guangzhou, China).All aqueous solutions were prepared with ultra-pure water (> 18 MU) from a Milli-Q Plus system (Millipore).

2.2. Preparation of rGO-1227 and rGO-CPB

The chemical precursor GO was prepared from natural graphite powder following the modified Hummers method [24–28]. Then, GO and 1227 were added in a flask and dispersed in H_2O (20 mL). After mixing for 4 h, hydrazine hydrate was added in the reaction mixture followed by stirring at 95 °C for 2 h. The obtained product was washed with deionized water and then freeze-dried. The composite was further vacuum dried at 60 °C for storage, resulting in the final product labeled rGO-1227. The product rGO-CPB was prepared following the same way.

2.3. Characterizations

X-ray diffraction (XRD) spectra were recorded on a RigakuD/max 2500 v/pc X-ray diffract meter with Cu Kαradiation(Kα = 0.15405 nm) at a generator voltage of 36 kV and electric current of 20 mA, measurements were conducted within a 2θ range of 5.0–40.0° at a scanning rate of 4.0°/min. Fourier transform infrared (FTIR) spectra between 400 and 4000 cm^{-1} were obtained on a Nicolet700 spectrometer. Thermogravimetric analysis (TGA) experiments were carried out by using an SDT Q600 V20.9 Build 20 thermogravimetric analyzer from room temperature to 800 °C at a heating rate of 10 °C/min in the nitrogen atmosphere. The morphologies ofsamples were observed on ZEISS field emission scanning electronmicroscope (FESEM); analysis the samples which should be wrapped with gold.Transmission electron microscopy (TEM) images were observed by using a JEOL JEM-2100F transmission electron microscope.

2.4. Antibacterial activity test

A specific concentration of rGO, rGO-1227, rGO-CPB, 1227, and CPB suspensions were separately introduced in a tube and then mixed with 10^5-10^6 cfu/mL of *E. coli* or *S. aureus* both containing 0.8% saline buffer. The control experiment did not involve Gram-negative *E. coli* ATCC 25922 nor Gram-positive *S. aureus* ATCC 6538 but only 0.8% saline buffer. All the samples were incubated at 37 °C in a shaker for 24 h. At the beginning of the experiment, a sample was taken and diluted in order to determine easily the initial concentration of bacteria suspensions. The suspensions at a gradient concentration were inoculated on nutrient agar culture medium and incubated at 37 °C for 24 h. The count of bacterial colonies was repeated three times.

2.5. Cytotoxicity assay

Cytotoxicity of sample was tested using the MTT assay based on the cellular uptake of MTT and its subsequent reduction in the mitochondria of living cells to dark blue MTT formazan crystals. NIH-3T3 cells were seeded on 96-well plates (1.5–2 × 104 cells/well) in corresponding medium. Then, the NIH-3T3 cells were treated with the samples for 24 h. After that, MTT (5 mg/mL in PBS) was added to each well and incubated for additional 4 h (37 °C, 5% CO_2). The cells were then lyzed in dimethyl sulfoxide (150 μL/well) and the plates were allowed to stay in the incubator (37 °C, 5% CO_2) to dissolve the purple formazan crystals. The color intensity reflecting cell viability was read at 490 nm using a Model-550 Enzyme-linked immunosorbent microplate (Bio-Rad, USA), and the morphologic changes of NIH-3T3 cells were photographed by an IX-70 inverted phase contrast microscope (Olympus, Japan). All the experiments were repeated four times and Statistical Product and Service Solutions software were used to assess statistical significance of the differences among treatment groups.

2.6. Release property

The drug release of rGO-1227 and rGO-CPB were evaluated by dialysis experiments [29]. The dialysis tubes (Spectra/Por Biotech, cellulose ester; MWCO 100 000) contained 5 mL of rGO-1227 or rGO-CPB dispersions and were dialyzed in 500 mL of ultra-pure water under and continuous stirring at 35 °C. The concentrations of the antibacterial agents were measured by ultraviolet-visible (UV–vis) spectroscopy.

3. Results and discussion

3.1. Morphologies of the prepared composites

Fig. 1. The rGO-1227 and rGO-CPB compounds were prepared by a three-step synthesis involving uniquely GO as suitable reagent (Fig. 1(A)). The quaternary ammonium salts 1227 and CPB could assemble on the surface of rGO via π-π interactions. We found