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The antifungal activity of graphene oxide–silver nanocomposites

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ABSTRACT

Graphene oxide (GO)-based nanocomposites' antibacteria activity exhibits great potential in clinical application. Herein we reported for the first time the preparation and enhanced antifungal activity of carbon nanoscrolls (CNSs) filled with silver nanoparticles (AgNPs). The nanoscrolls filled with silver nanoparticles were prepared by sonication, TEM picture showed AgNPs filled and wrapped inside prepared nanocomposites, the antifungal test showed that CNSs-AgNPs exhibited ideal lengthened activities against *Candida albicans* and *Candida tropical* compared with the GO–AgNPs nanocomposites based on silver nanoparticles directly deposited on the surface of grapheme oxides, which is caused by CNS-AgNPs' controlled durative slow-releasing of silver ion. It is also observed that graphene oxides exhibited no antifungal activity. In conclusion, the carbon nanoscrolls composed of graphene oxides and silver nanoparticles own enhanced and lengthened antifungal activity, and have great potential in applications such as clinical nosocomial infections and local antifungal therapy.

1. Introduction

Silver has the inherent advantage of broad antimicrobial activities against bacteria [1,2], fungus [3] and possesses the minimal resistant development [4]. Silver-based compounds have been widely used clinically for centuries [4,5], for example, the treatment of burn wounds [5–8]. The antimicrobial ability of silver-based compounds comes from the dissolved silver cation (Ag^+) and soluble complexes [9]. The silver ion-based germicidal characterization mainly derives from the following pathways: (i) to generate sustained flux of Ag^+ from the silver compounds that deposited on special substrates or imbedded in colloidal or semisolid matrices. (ii) To transport active Ag^+ to sensitive targets on plasma membrane of microbes or enter the cells *via* endocytosis pathway [10]. So far, how to control the burst release of silver ions and keep a continual flux of Ag^+ have become our concerns. The bulk silver materials cannot realize the desired concentrations of Ag^+ due to its less surface area and higher cost. Whereas, silver nanoparticle (AgNP) is one kind of ideal material due to its larger surface area and tardo release properties [11]. However, the practical application of AgNPs is often hampered by the self-aggregation or precipitation, and lose of antibacterial activity [12]. Therefore, to develop a stable, dispersed AgNPs substrates and control the release of Ag^+ have become a critical challenge.

Graphene, a single-atom-thick of carbon atoms closely packed into honeycomb two-dimensional (2D) lattice, has attracted broad attention recently [13,14]. Its sp^2 hybrid carbon framework possesses unique thermal, mechanical, and electrical properties [15], are being actively explored for potential applications such as nanoelectronics, conductive thin films, supercapacitors, biosensors and nanomedicine [16–18]. GO owns specific high surface area, and has a great deal of oxygen bonds in its edges and defective sites, such as hydroxyl (C–OH), carboxylic (–COOH), carbonyl (C=O), epoxide groups (C–O–C) on both accessible sides [17,19]. Therefore, GO is strongly hydrophilic, and forms stable colloidal dispersions in water [20]. Such functional groups have been confirmed to own reducibility [21] and have been actively used to build new composites [22–25]. We have used GO and silver nanoparticles to prepare carbon nanoscrolls *via* several hours sonication in our previous work [26], which provides a fascinating suspension matrix for ion or metal nanoparticle intercalation.

The synthesis procedures of GO–AgNPs hybrids mainly include the utility of extra reducing agent such as sodium borohydride [27,28] and hydrazine monohydrate, or modified by PEI [28], the shortage of these method is very obvious: even the reducing agent had been scavenged by chemical or physical ways after the synthesis process, the residues of reducing agent may still possess cytotoxicity partially. Furthermore, the flammables, explosives properties and chemical poisonousness of hydrazine monohydrate

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and sodium borohydride will hinder their further application in biomedical engineering. Notwithstanding some biocompatible reducing agents were introduced into the processes of GO–AgNPs synthesis and the antibacterial activities were discussed extendedly [29,30], for a promising nanomaterial of GO–AgNPs, the morphological related release characteristic and controlled release kinetics are still stay in concealment, especially for fungus, the most common infection pathogen in clinical, the antifungal activity and fungicidal kinetics of GO–AgNPs hybrids are desiderating development.

With the aim of evaluating the antifungal efficiency of Ag nanoparticles deposited on different shape of GO matrix, as shown in Scheme 1, we used sonication method to make CNSs–AgNPs composites and further investigated the antifungal activity and the release kinetics of GO based AgNPs hybrids with different morphology. Moreover, in the interest of GO based AgNPs hybrids' histocompatibility and its application in biomedical engineering, the cytotoxicity experiments treated with these nanomaterials were also carried out.

2. Materials and methods

2.1. Synthesis and characterization of GO–AgNPs and CNSs–AgNPs hybrids

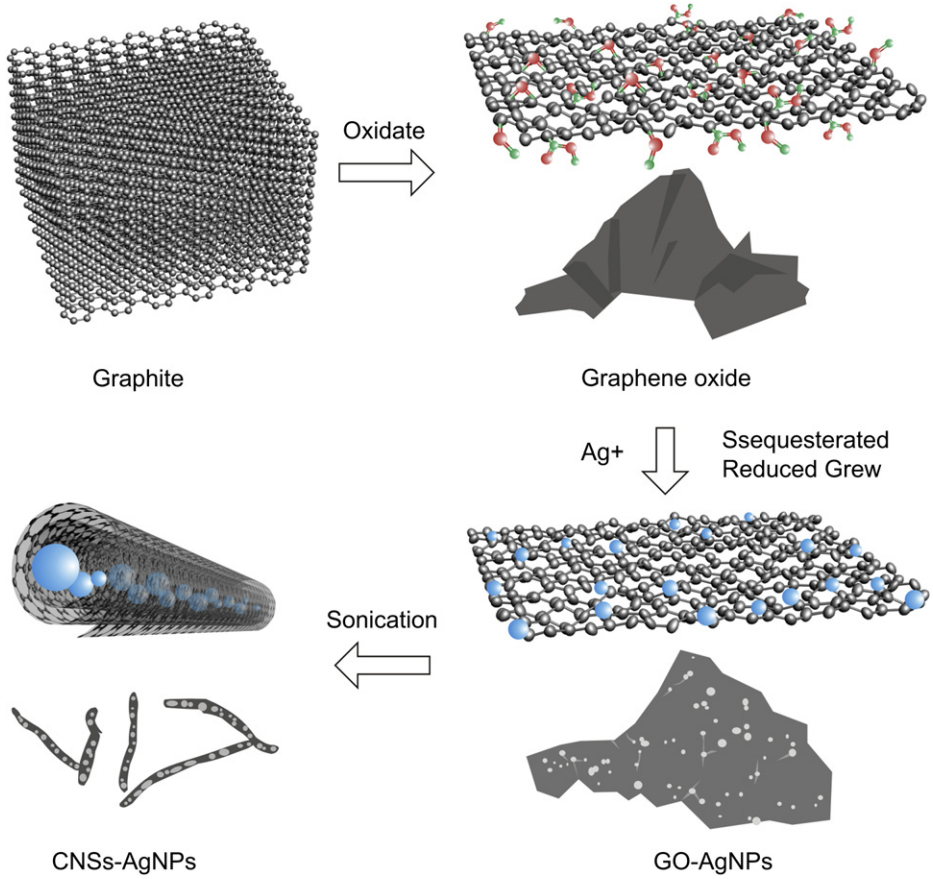
Briefly, the water soluble GO were prepared by oxidizing pristine graphite according to the modified Hummers method [31] and our previous reports [32–34] (Scheme 1 and S1, Support information). AgNPs were synthesized by in situ reducing silver nitrate solution on the surfaces of GO: the aqueous solution of $AgNO_3$ (20 mM) was gradually added to 5 mL of an aqueous solution of GO (0.5 mg mL^{-1}), by 1 mL per time for five times, while under vigorously stirring at room temperature for 48 h. The resulting mixture was then separated from the solution by centrifugation at 14,000 rpm for 10 min and further washed in ultrapure water twice to remove residual ionic silver. The dry GO–AgNPs obtained after the lyophilization and the dry composite was weighed and dissolved quantificationally in ultrapure water for next sonicate. After 6 h sonicate, most of the exfoliated GO curl into scroll and mainly part of AgNPs wrapped into carbon nanoscrolls. The size and characterization of the prepared nanoparticles were determined by UV absorption spectrum, Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) analysis (Fig. 1). Further characteristic of GO, GO–AgNPs, CNSs–AgNPs was determined with X-ray Diffraction (XRD), Raman spectra (Fig. 2) and Fourier Transform Infrared Spectroscopy (FTIR) (Fig. S4, Support information).

2.2. Antifungal activity

Silver nanoparticles own strong antibacterial [35,36], antifungal [37] and antivirus effects [38]. In order to explore the antifungal activities of synthesized GO and its AgNPs composites, the *Candida albicans* (ATCC 90029) and *Candida tropical* (isolated from a urethritis patient of our affiliated hospital) were introduced in our experiments.

At first, the antifungal activity of GO, GO–AgNPs and CNSs–AgNPs against *C. albicans* and *C. tropical* were evaluated by modified agar disk diffusion method that recommended by the Clinical and Laboratory Standards Institute (CLSI, 2011). Approximately 10^6 colony forming units (CFUs) of each fungus were inoculated on modified Sabouraud's agar plates (bio-KONT, Ltd. China), 20 μL of GO, GO–AgNPs and CNSs–AgNPs nanocomposite aqueous dispersions (100 μg/mL) were added to a 6.35 mm filter paper, and then placed the filter paper onto the seeded agar plate. After 8 h, 12 h, 20 h and 24 h incubation at 37 °C, the diameters of the inhibition zones were measured and optical images were documented by an ordinary camera at the same time.

Secondly, the MICs of GO, GO–AgNPs and CNSs–AgNPs against *C. albicans* and *C. tropical* were carried out by broth microdilution method (CLSI, 2011). *C. albicans* and *C. tropical* cells were dispensed in ultrapure water with final concentration of 10^5 CFU/mL, and then 50 μL of the dispersions were seeded to the microtiter plates (96 wells, flat bottom, Corning. USA) contained with 100 μL of double concentration nutrient broth (bio-KONT, Ltd. China) in each well. 50 μL diluted suspension of GO, GO–AgNPs and CNSs–AgNPs were added in. The final concentration of GO, GO–AgNPs and CNSs–AgNPs was 0.125, 0.25, 0.5, 1, 2, 4, and 8 μg/mL respectively. 200 μL pure broth without nanoparticle treatment and yeast cell inoculation were served as the negative control. After incubated in air condition (37 ± 1 °C) for 24 h, the optical densities (OD) were measured in a microtiter plate (ELISA) reader



Scheme 1. Schematic illustration of the fabricate processes of GO–AgNPs and CNSs–AgNPs hybrids.