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Antimicrobial behavior comparison and antimicrobial mechanism of silver coated carbon nanocomposites

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ABSTRACT

Nanomaterials have been intensively used as antibacterial agents, due to their efficient disinfection without harmful disinfection byproducts. Silver nanoparticles have attracted considerable attention. However, silver nanoparticles are likely to aggregate. In this work, we used a simple and facile one-step approach for the preparation of carbon nanotubes-silver (including single-walled carbon nanotubes-silver (SWCNTs-Ag) and multi-walled carbon nanotubes-silver (MWCNTs-Ag)) and graphene oxide-silver (GO-Ag) nanoparticles. The synthesized carbon-silver nanocomposites were characterized by XPS, TEM, EDS and Zeta-sizer. We compared the disinfection activity of six materials (i.e. GO, SWCNTs, MWCNTs, GO-Ag, SWCNTs-Ag and MWCNTs-Ag) toward two strains including Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Staphylococcus aureus* (*S. aureus*). Under similar concentration and incubation conditions, GO-Ag showed the highest disinfection activity. Antioxidant enzyme activities and lipid peroxidation assays induced by GO-Ag proved that GO-Ag was capable of inducing $O_2^{\cdot-}$ oxidative stress on bacterial. Subsequently affected the cell membrane integrity and thus resulted in cell death. GO-Ag with excellent disinfection efficiency against *E. coli* and *S. aureus* highlighted the potential application of GO-Ag in water disinfection.

1. Introduction

The provision of clean drinking water involves not only the chemical risks but also the microbial risks (Benabbou et al., 2007). Pathogenic bacteria are responsible for water borne diseases, but effective removal of them is quite difficult (Gao et al., 2013). Although conventional drinking water disinfection processes such as chlorination, ozonization and advanced filtration technology are effective for eliminating most of pathogenic microorganisms, while these processes still have several drawbacks such as formation of potentially mutagenic and carcinogenic disinfection by-products (DBPs), high costs or low efficiencies (Wang and Lim, 2013). Therefore, it is necessary to find new disinfecting agents which have good antimicrobial properties and continuous antimicrobial effects, and harmless to human health.

Recent advances of nanomaterials have brought about a new opportunity for the resistance to pathogenic bacteria. Nanomaterials often show unique physical, chemical and biological properties compared to their macro scaled counterparts (Sharma et al., 2009). A number of nanomaterials have been proposed to inactivate microorganisms in drinking water, wastewater, surface water and other sources, such as photocatalytic titanium dioxide (Dimitroula et al., 2012),

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magnesium oxide (Ganguly et al., 2011), zinc oxide (Gordon et al., 2011), nano-silver (Kaegi et al., 2011) and zero valent iron (Crane and Scott, 2012).

Nano-silver is the most widely used material due to its low toxicity and microbial inactivation in water (Amin et al., 2014). The superior antibacterial performance of Ag nanoparticles (NPs) partially originates from their large specific surface area owing to their small sizes (Zahed and Hosseini-Monfared, 2015). However, Ag NPs are likely to aggregate due to the extremely high surface energy (Yuan et al., 2014). The general problem can be overcome by using a framework to support the NPs. Following this strategy, carbon-based nanomaterials such as carbon nano-tubes (CNTs) (Chen et al., 2015b; Han et al., 2015), graphene oxide (GO) (Yuan et al., 2012) and carbon nanofibers (Numnuam et al., 2014) have been used as supports for Ag NPs.

Graphene oxide (GO) is chemically oxidized graphene, GO has a single-layer sheet of sp^2-hybridized carbon atoms with high surface area and superior mechanical and electrical properties, provides an extraordinary platform for preparing composite nanomaterials (Schwegmann et al., 2013). CNTs are built of pure carbon and their fundamental structure is a hexagonal graphene network in which the carbon atoms are in sp^2 hybridisation (Goscianska et al., 2014). CNTs have become attractive over the past years due to their fast electron transfer rate, high chemical stability, strong adsorptive ability and excellent biocompatibility (Lin et al., 2014). In addition, the large surface area and high electric conductivity of CNT endow it excellent property to load metal NPs (Lin et al., 2014). CNTs can be classified as single-walled carbon nanotubes (SWCNTs), which are hollow tubes of carbon capped at either end with a hemi-fullerene, and multi-walled carbon nanotubes (MWCNTs) consisting of concentric layers of graphene sheets, where smaller diameter tubes are encased in larger diameter tubes (Chen et al., 2015a). Although, carbon-coated silver composites have been proven to enhance the disinfection ability, there was no report about the comparison of their disinfection properties under the same experimental conditions. In addition, the disinfection mechanism of silver coated carbon nanocomposites is not very clear.

In the present study, to better understand the different carbon nanomaterials' dispersion properties of silver ions, we used the same chemical synthesis method synthesized three carbon-silver nanocomposite materials (including graphene oxide-silver (GO-Ag), single-walled carbon nanotubes-silver (SWCNTs-Ag) and multi-walled carbon nanotubes-silver (MWCNTs-Ag) and the disinfection properties of three kinds of materials were compared. *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) typical Gram-negative bacterium and Gram-positive bacterium, were employed as models due to their well-known pathogen commonly involved in water contamination and widely used in reference tests to measure bactericidal properties. The various characterization methods were used to explain the different antibacterial properties. We selected the best antibacterial material from GO-Ag, SWCNTs-Ag and MWCNTs-Ag nanocomposite materials. The underlying antibacterial mechanism of the GO-Ag was investigated through a series of enzyme activity tests.

2. Materials and methods

2.1. Strains and raw materials

The bacteria strains *E. coli* ATCC 25900 and *S. aureus* ATCC 6538 were purchased from China Center for Type Culture Collection (Beijing, China). Graphite powders were supplied from Jim Shan Ting New Chemical Factory (Shanghai, China). SWCNTs (outer diameter <2 nm; length 5–30 μm) and MWCNTs (outer diameter 40–60 nm; length 5–15 μm) were obtained from Chengdu Organic Chemicals Co (Chinese Academy of Sciences, China). Catalase (CAT) kit, Total superoxide dismutase (T-SOD) kit and Malonaldehyde (MDA) kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Sodium nitrate ($NaNO_3$), concentrated sulfuric acid (H_2SO_4, 98%), potassium permanganate ($KMnO_4$), hydrogen peroxide (H_2O_2, 30%), absolute ethanol (CH_3CH_2OH), silver nitrate ($AgNO_3$), ammonium formate ($HCOONH_4$), sodium chloride (NaCl), yeast extract, tryptone and glutaraldehyde were all purchased from Sinopharm Chemical Reagent Co.(Shanghai, China). Mannitol Salt agar medium and Eosin-methylene blue agar medium were obtained from Haling Biological Technology Co (Shanghai, China). All chemicals were analytical grade.

2.2. Preparation of GO

GO was produced via a modified Hummers' method (Deng et al., 2014; Pavagadhi et al., 2013). Graphite powders (1.0 g) were stirred in an ice bath. $NaNO_3$ (0.5 g) and H_2SO_4 (23 mL) were added into a conical flask. $KMnO_4$ (3.0 g) was then added slowly with stirring. Once the mixture was homogeneous, the solution was transferred to an oil bath maintained at 35 °C and stirred for 30 min. A thick paste was formed and deionized (DI) water (46 mL) was then added. The reaction was continued at the temperature of 98 °C for 30 min and H_2O_2 (2.5 mL) was added to reduce the residual permanganate and manganese dioxide. The colour of the suspension changed from brown to yellow. The resulting yellow suspension was centrifuged and washed with DI water and absolute ethanol sequentially until the pH of the supernatant was 7. The GO samples were recovered finally and then dried in a vacuum desiccator.

2.3. Synthesis of GO-Ag, SWCNTs-Ag and MWCNTs-Ag composites

The GO-Ag, SWCNTs-Ag and MWCNTs-Ag were prepared in one step reaction. In a typical procedure, GO (250 mg), SWCNTs (250 mg) and MWCNTs (250 mg) dispersed in DI water (100 mL) with ultra-sonication 1 h to form suspension, respectively. In the above three kinds of suspension, $AgNO_3$ (300 mg) was added under stirring, respectively. The ammonium formate (25 mL, 0.5 mol/L) was gradually added to the mixtures with magnetic stirring for 30 min. Subsequently, three kinds of compounds were transferred to an oil bath and kept at 30 °C for 1 h under constant stirring. The final compounds were left undisturbed at room temperature for 24 h. The slurry-like products were centrifuged and washed with DI water repeatedly to remove excess reagents. The samples were kept in the vacuum drier overnight.

2.4. Characterization

The morphologies of the GO, SWCNTs, MWCNTs, GO-Ag, SWCNTs-Ag and MWCNTs-Ag were evaluated by a transmission electron microscopy (TEM) (JEM-3010). Energy dispersive X-ray (EDS) was used to illustrate the Ag content of GO-Ag, SWCNTs-Ag and MWCNTs-Ag. EDS measurements were conducted using the EDAX system attached to the same transmission electron microscopy (JEM-3010). The structure and crystal phase of synthesized samples were examined by X-ray