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In-situ reduction of monodisperse nanosilver on hierarchical wrinkled mesoporous silica with radial pore channels and its antibacterial performance

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ABSTRACT

Monodisperse silver nanoparticles (Ag NPs) were facilely loaded on the inner and outer surface of hierarchical wrinkled mesoporous silica (WMSs) via an in situ chemical reduction, and the antibacterial capacity of the obtained nanocomposite was investigated in detail. Typical sulfydryl-functionalized wrinkled mesoporous silica nanoparticle with radical pore channels was firstly prepared through sol-gel technique with cetyltrimethylammonium bromide (CTAB) as the templating surfactant. After sulfonation of the as-prepared WMSs, Ag^+ ions were then densely locked up on the inner and outer surface of WMSs via electrostatic interactions. Well distributed Ag NPs (ca. 3–5 nm) on WMSs without any agglomeration were finally obtained via a simple in situ reduction reaction with sodium borohydride. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) test indicated that the obtained products can achieve durable and much better antibacterial performance both against Gram-negative bacterium *Escherichia coli* (*E. coli*) and Gram-positive bacterium *Staphylococcus aureus* (*S. aureus*) comparing to pure colloidal silver nanoparticles, which rendered them as favorable candidate for the development of effective antibacterial agents.

1. Introduction

Bacterial contamination is of great concern in food sanitation, environmental safety and public health [1], and the exploration of broad-spectrum and effective bactericide has been a research focus during the past decades. Ag nanoparticle (Ag NP) possesses diverse advantageous such as good adsorbability, high surface energy and strong activity, and is wildly utilized as antibacterial agent in medical treatment and public health management during the past decades [2,3]. However, nano-scale Ag particles, in the form of colloids, often encounter practical problems such as poor dispersion, quick release and inferior storage stability, which strictly limited its practical applications [4]. Considering the above-mentioned situation, attaching Ag NPs to organic/inorganic materials such as silica [5–9], titanium dioxide [10–13], phosphate [14,15], carbon materials [16,17], assembled polymers [18–20] or other composite materials [21,22] with excellent stability may successfully solve the problem, which may also prevent Ag NPs from aggregation and sustain the steady release of Ag ions during the antibacterial performance.

Mesoporous silica has been widely accepted as an ideal candidate for carrier material owing to its high specific surface area, large pore volume, good biocompatibility, high chemical stability and easy surface modification [23], which has been extensively used in numerous fundamental research and practical applications (catalysis [24,25], separation [26,27], chemical sensing [28,29], and controlled release [30,31]). The development of mesoporous silica-supported Ag NPs has also arouse tremendous research interest [32–39]. Generally, two strategies have been employed to construct Ag/MS nanocomposite. One relies on the development of core-shell structure via direct sol-gel coating on Ag NPs, which can achieve effective dispersion and immobilization of nanosilver cores with mesoporous silica shells [40–42]. Another one focuses on the adsorption or in situ reduction of Ag NPs on the as-prepared mesoporous silica materials [43–45]. In order to guarantee the dispersibility of Ag NPs, the amount of Ag loaded in the core-shell structure is relatively low in a majority of cases. Moreover, the Ag NPs are totally embedded in the MS framework, and these two factors may greatly decrease their antibacterial capacity. As for the second strategy, a majority of Ag NPs generated on the MS matrix was far greater than their regular tiny mesopores, thus most of the loaded Ag NPs are merely dispersed on the surface of the MS nanoparticles. Accordingly, the burst release of Ag^+ ions and rapid consumption of Ag NPs without rational

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protection of mesoporous matrix will consequently contribute to a rapid loss of antibacterial capacity in a short period of time.

Recently, great deals of effort have been made towards the development of Ag/MS nanocomposite with appropriate structure regulation and enhanced antibacterial capacity. Shen [46] proposed a two-step method which can accurately load Ag NPs onto the interior wall of a hollow mesoporous silica shell. This unique structure can protect the inner Ag NPs from aggregation and increase the durability of the antibacterial agent. However, multiple preparation process including the synthesis, modification and polymeric template removing process, accompanied with the sol-gel formation of mesoporous silica shell made this elaborate design hard to be utilized for popularization and application. Quite recently, Jiang and his cooperators [47] dispersed Ag NPs in the frame work of MCM-41 type mesoporous silica nanoparticles via a facile one-pot co-condensation process, and the obtained nanocomposite exhibited long-term antibacterial activity on Gram-negative and Gram-positive bacteria at a low concentration.

Recently, wrinkled mesoporous silica materials (WMSs) have aroused great research interest due to the presence of hierarchical pore structures and center-radial open pores [48–50], which endowed this material with additional advantages in the cargo loading process than traditional MCMs or SBAs with ordered and uniform mesopores [51]. Herein we attempt to load Ag NPs onto the inside and outside surface of wrinkled mesoporous silica with typical radial pore channels (WMSs). First, center-radial open pores have more accessibility for various sized molecules to enter into the internal surface of the particles, which is in favor of the loading and the high dispersion stability of Ag NPs. Meanwhile, spherical SiO_2 shell will protect Ag NPs embedded in pores from deterioration, and reduce their consumption rate, which can extend its life cycle. Finally, the synergistic combination of multiple-scale pores will harmonize the diffusion of Ag^+ through the porous matrices just as mesoporous silica-based drug carriers [30,31], resulting in an enhanced antibacterial performance.

2. Experimental

2.1. Materials

Cetyltrimethylammonium bromide (CTAB, AR, 99.0%), tetraethoxysilane (TEOS, AR, 28%), (3-mercaptopropyl) trimethoxysilane (MPS) and all the solvents were purchased from Sinopharm Chemical Reagent Company. Poly(*N*-vinylpyrrolidone) (PVP-K30, Wt 40,000) was obtained from Sigma-Aldrich. Sodium borohydride ($NaBH_4$, AR, 98.0%) and silver nitrate ($AgNO_3$, AR, 99.8%) were purchased from Aladdin Industrial Corporation and used without further purification.

2.2. Synthesis of hierarchical wrinkled mesoporous silica nanoparticles (WMSs)

CTAB (0.50 g) was dissolved in 70 mL water, followed by the addition of 15 mL diethyl ether, 5 mL ethyl alcohol and 0.80 mL ammonium hydroxide under magnetic stirring. TEOS (2.50 mL) and MPS (0.20 mL) were then added dropwise to the reaction mixture by a dropping funnel at room temperature. After vigorous stirring for 4 h, the reaction mixture was centrifuged and washed with ethyl alcohol and water thrice. The obtained sample was subsequently refluxed in the mixture of 120 mL ethyl alcohol and 15 mL concentrated HCl overnight. The hierarchical wrinkled mesoporous silica nanoparticles were finally obtained by repeated centrifugation and washing procedure with water.

2.3. Synthesis of sulfoacid-modified WMSs (WMSs-SO_3^-)

WMSs (1 g) were dispersed in 100 mL methylbenzene under sonication. MPS (2 mL) was then added and the obtained reaction mixture was kept stirring for 18 h at 80 °C. After centrifugation and washing procedure thrice, the obtained sulfydryl-modified WMSs (WMSs-SH) were re-dispersed in 50 mL H_2O_2 for sulfonation reaction at room temperature. After vigorous stirring for another 48 h, the reaction mixture was centrifuged and washed with ethyl alcohol and water thrice. WMSs-SO_3^- was finally obtained by vacuum drying at 60 °C.

2.4. In-situ reduction of Ag NPs on sulfoacid-modified WMSs (AgNPs@WMSs-SO_3^-)

WMSs-SO_3^- (20 mg) precursor was dispersed in 10 mL ethyl alcohol under sonication, followed by the addition of $AgNO_3$ aqueous solution (3.6 mg $AgNO_3$ in 10 mL water). The obtained reaction mixture was stirred at room temperature under dark for 80 min. After the electrostatic interaction, PVP-K30 (2 mg) in 5 mL water was then added into the above reaction mixture. Finally, $NaBH_4$ (1 mg) in 5 mL water was added dropwise. After vigorous stirring for another 15 min, the reaction mixture was centrifuged and washed with ethyl alcohol and water thrice, then freeze-dried for 15 h.

2.5. Ag^+ ions release from the AgNPs@WMSs-SO_3^- sample

The Ag^+ ions release from the AgNPs@WMSs-SO_3^- sample was determined quantitatively by the ICP spectrometry [52,53]. The sample (0.1 g) was placed into 80 mL of distilled water at 37 ± 0.1 °C under gentle stirring for certain length of time (1, 2, 3, 4 and 6 days). Parallel experiments were conducted simultaneously. At the end of the experiment, the solution was centrifuged and the ICP was employed to determine the concentration of Ag^+ ions in the separated supernatant.

2.6. Evaluation of the antibacterial properties of AgNPs@WMSs-SO_3^- sample

2.6.1. Minimal inhibition concentration (MIC) test

Fresh bacteria were first inoculated in liquid medium and grown to an approximate OD_{600} of 0.5 (i.e. the initial concentration is approximately 1×10^8 CFU/mL). The bacterial suspension used in experiment was then prepared by diluting the above suspension to 1×10^6 CFU/mL and then mixed with various concentrations of AgNPs@WMSs-SO_3^- samples. MIC was defined as the minimal concentration of AgNPs@WMSs-SO_3^- at which the mixed suspension was clear and without any change of OD_{600} after cultivating for 24 h by an orbital shaker at 37 °C.

2.6.2. Minimal bactericidal concentration (MBC) test

After cultivating for 24 h, 0.1 mL of the above sterile suspension at proper dilution degree was extracted and quickly spread on solid medium and cultured at 37 °C for another 24 h, and the average number of discrete colonies was counted as the number of the remaining bacteria by repeating the above procedure three times with error bars. MBC was defined as the minimal concentration of AgNPs@WMSs-SO_3^- at which the growth rate of bacteria reduced >99%.

2.7. Characterization

High resolution transmission electron microscopic (HRTEM) and scanning electron microscopic (SEM) images of the obtained samples were taken on a JEOL-3000F electron microscope and a Hitachi-S4800FESEM. X-ray photoelectron spectroscopy (XPS) measurements were carried out in an X-ray photoelectron spectrometer (Kratos AXIS Ultra DLD, Shimadzu Co., Japan) using 300 W Al Kα radiation. Powder X-ray diffraction (XRD) patterns are recorded on an X-ray diffractometer (D8 ADVANCE SS, Germany) with mono-chromated Cu-Kα radiation (λ = 1.54060 Å) at a scanning rate of 5.0°/min. The Brunauer-Emmett-Teller (BET) surface area is determined by nitrogen adsorption and desorption using a specific surface area analyzer (Micromeritics ASAP 2020, USA). Using the Barrett-Joyner-Halenda (BJH) model, the pore volumes and pore size distributions are derived from the desorption