Advanced Powder Technology xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Advanced Powder Technology

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

Original Research Paper

Enhanced antibacterial activity and mechanism studies of Ag/Bi_2O_3 nanocomposites

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ARTICLE INFO

Article history:
Received 10 April 2018
Received in revised form 9 May 2018
Accepted 11 May 2018
Available online xxxx

Keywords:
Ag/Bi_2O_3
Nanocomposites
Drug-resistance
Antibacterial activity
Mechanism

ABSTRACT

In this work, sphere-like Ag/Bi_2O_3 nanocomposites with the average size of ca. 170 nm were successfully synthesized by simple deposition-precipitation method. The antibacterial activities of as-prepared Ag/Bi_2O_3 nanocomposites were evaluated by minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and colony counting methods. It was found that Ag/Bi_2O_3 nanocomposites displayed greatly improved antibacterial ability against common pathogenic Gram-positive and Gram-negative bacteria in comparison with single-component Bi_2O_3 nanospheres. More importantly, Ag/Bi_2O_3 nanocomposites exhibited remarkably outstanding antibacterial activities against clinical drug-resistant bacteria. The antibacterial activity of Ag/Bi_2O_3 nanocomposite increased with the increase of Ag content and 15 wt% Ag/Bi_2O_3 nanocomposites showed the highest antibacterial activity. Furthermore, a plausible antibacterial mechanism of Ag/Bi_2O_3 nanocomposite was proposed. It was believed that the enhanced generation of H_2O_2 could lead to the membrane leakage of cytosol and the inactivation of respiratory chain dehydrogenaes, which was possibly responsible for the enhanced antibacterial activities of nanocomposites.

1. Introduction

The inappropriate use of antibiotics often makes strains of bacteria to gain resistance for many common antibiotics, leading to numerous negative effects upon medicine and society [1,2]. Drug-resistant bacterial infections could result in higher dosages of drugs, addition of treatments with higher toxicity, longer hospital stays, and increased mortality [3,4]. It impelled researchers to develop new agents that can inhibit bacterial growth without showing cytotoxic effects on humans and other species. Among them, nanosized inorganic antibacterial agents have gained increased interest over the past decade due to their increased surface area, smaller particle size and enhanced antibacterial activities [5,6]. More importantly, unlike commonly antibiotics, these nanomaterials could affect bacteria growth through multiple action pathways, which made microbes difficult to acquire resistance toward these nanoparticles [6,7].

It is well known that Ag nanoparticles (AgNPs) exhibited excellent antibacterial activity towards various microorganisms [8–11]. However, the property of easy aggregation and high cytotoxicity greatly limited its practiced application [12–15]. Therefore, a series of Ag/semiconductor nanocomposites, including Ag/TiO_2, Ag/SiO_2 and Ag/ZnO have been developed to overcome these drawbacks [16–18]. It was found that the loading of AgNPs on semiconductors could facilitate the separation of charge with holes on the semiconductors surface and generate more reactive oxygen species (ROS), thus resulting in the enhancement of photocatalytic and antibacterial activity [19,20]. In recent years, bismuth-related nanomaterial was found to present efficient bacteria growth inhibition performance [20–23]. Particularly, bismuth oxide (Bi_2O_3) display remarkable antibacterial activity and has low cytotoxicity [24–26]. Furthermore, as an important p-type semiconductor, Bi_2O_3 has been extensively applied in photovoltaic cells, optical coating, gas sensing, fuel cells and photocatalysis [27,28]. It was believed that bismuth oxide could act as an ideal candidate to composited with Ag nanoparticles to improve the antibacterial activity of nanocomposites, as well as lower its cytotoxicity. In this work, spherical Ag/Bi_2O_3 nanocomposites with different Ag content were prepared, and their antibacterial activities against commonly pathogenic bacteria and clinical drug-resistant bacteria were evaluated for the first time. Moreover, to understand the antibacterial behavior of the nanocomposites, the changes of bacterial morphology, leakage of outer membrane, activity of respiratory chain dehydrogenase and lipid peroxidation of the bacteria after being treated with Ag/Bi_2O_3 nanocomposites were investigated. The in vitro

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https://doi.org/10.1016/j.apt.2018.05.015

cytotoxicity of Ag/Bi_2O_3 nanocomposites towards HEK293 cell was also evaluated.

2. Materials and methods

2.1. Materials

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 2-thiobarbituric acid (TBA), iodonitrotetrazolium chloride (INT) and trichloroacetic acid (TCA) were purchased from Aladdin (China). 3, 5-dinitrosalicylic acid, glutaraldehyde and coomassie blue were obtained from Sinopharm Chemical Reagent Co. (China). Silver nitrate, amoxicillin and piperacillin were purchased from Sigma-Aldrich Trading Co., Ltd (Germany). All the reagents were analytical grade and used directly without further purification.

2.2. 2Bacteria and cell strains

The strains of *S. aureus* (ATCC 9118) and *S. typhimurium* (CCTCC AB 94010) were supplied by Hua zhong Normal University. The strains of *B. subtilis* (CCTCC AB 90008), *E. coli* (CCTCC AB 93154), *P. aeruginosa* (CCTCC AB 93066) and *P. vulgaris* (CCTCC AB 91103) were supplied by China Center for Type Culture Collection. The clinical strains of methicillin-resistant *S. aureus* (MRSA), drug-resistant *P. aeruginosa* (DRPA) and drug-resistant *E. coli* (DREC) were supplied by Wuhan Third Hospital. Luria-Bertani (LB) medium was used in growing and maintaining all the bacteria. The human embryonic kidney cell line (HEK 293) was supplied by

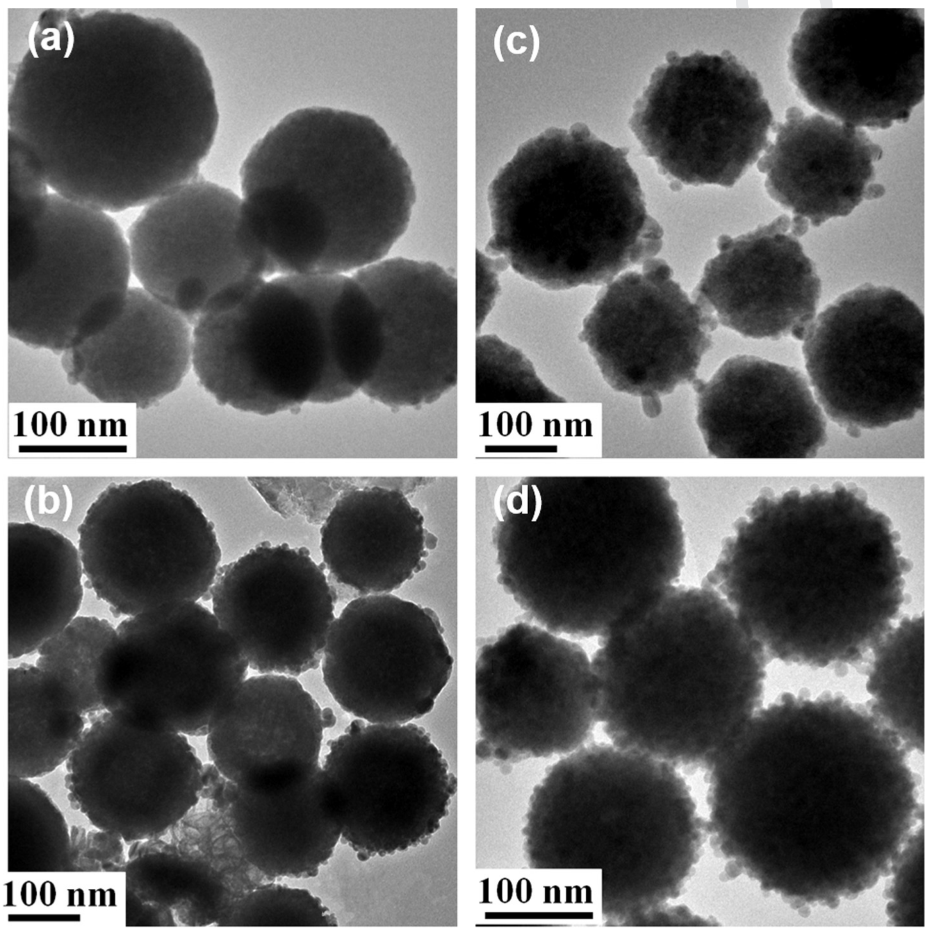

Fig. 1. TEM images of the as-synthesized ABNPs products: (a) ABNP-2.5, (b) ABNP-5, (c) ABNP-10 and (d) ABNP-15.

Table 1
The MIC and MBC values of BNPs, ABNPs and AgNPs against the bacteria.

	MIC (μg/mL)						MBC (μg/mL)					
	BNPs	ABNP −2.5	ABNP −5	ABNP −10	ABNP −15	ANPs	BNPs	ABNP −2.5	ABNP −5	ABNP −10	ABNP −15	ANPs
S. aureus	4	4	4	2	4	8	8	8	8	8	8	16
B. subtilis	>1000	220	180	140	80	4	>1000	260	220	160	100	8
E. coli	>1000	320	140	80	60	16	>1000	400	180	100	60	16
S. typhimurium	>1000	360	160	80	60	16	>1000	512	256	128	70	16
P. aeruginosa	>1000	550	250	150	70	8	>1000	600	350	200	90	16
P. vulgaris	>1000	200	128	64	20	8	>1000	250	160	100	40	16

Please cite this article in press as: Q. Liu et al., Enhanced antibacterial activity and mechanism studies of Ag/Bi_2O_3 nanocomposites, Advanced Powder Technology (2018), https://doi.org/10.1016/j.apt.2018.05.015