The Preparation and Property Research of Bismuth Oxide Nanospheres

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***Abstract*—Nanomaterials are a new option to successfully treat the multiresistant microorganisms. The as-prepared bismuth oxide with the optimal concentration exhibits stronger antibacterial abilities than others. Meanwhile, it exhibits well biocompatibility. In one word, the present approach can shed new light on designing of antibacterial material like bismuth oxide with promising applications in biological sciences.**

Keywords-bismuth oxide (Bi_2O_3); preparation; antibacterial; cytocompatibility

I. INTRODUCTION

Recently, Nanostructured materials have attracted considerable research interest because of their great potential in diverse fields of magnetic separation, biomedicine, catalysis and biological sciences [1]. Nanomaterials are a new option to successfully treat the multiresistant microorganisms. The metal ions of antibacterial products contact with bacteria can cause microbial inherent component damage or dysfunction. It is well-known that zinc oxide nanoparticles (nZnO) possesses antibacterial activity and currently is used in many cosmetic materials [2]. The nano-silica silver nanocomposite (NSAgNC) is as antibacterial effect on gram-negative bacteria viz. Pseudomonas aeruginosa and Escherichia coil has been investigated, however, the studies further demonstrated the down regulation of protein and fragmentations of DNA expression in NSAgNC treated cells leading to the cell death [3]. Recently, it was demonstrated that the bactericidal activity of zero-valent bismuth colloidal nanoparticles inhibited the growth of Streptococcus mutans. Typically, bismuth is found as bismite (bismuth oxide [Bi_2O_3]), bismuthite (bismuth carbonate) and bismuthinite (bismuth sulfide), in medicine, bismuth, has been employed as an antidiarrheal to treat vomiting, stomach, and pain nauseas. Bi2O3 shows a distinctive polymorphism, including the following solid state phases: α-Bi_2O_3, β-Bi_2O_3, γ-Bi_2O_3, δ-Bi_2O_3, and the recently characterized ε-Bi_2O_3. The α-Bi_2O_3 is the most thermodynamically stable phase at room temperature and pressure [4]. So, under standard reaction conditions in aqueous solutions, the α-Bi_2O_3 is formed, a poorly water-soluble specie that carries surface hydroxyl groups, α-Bi_2O_3 is a basic oxide and its Bi-O bonds are predominantly ionic; it is a p-type semiconductor material [5]. Bi_2O_3 is a derivative of great technological importance, and it is used in the manufacture of glass and ceramic products, and also, as catalyst in the oxidation of hydrocarbons. It is widely used in applications, such as microelectronics, and sensor and optical technology [6-8]. Recently, the Bi_2O_3 porous nanospheres demonstrated outstanding performance in visible-light-driven photocatalysis for Cr (VI) and organic dye removal, inactivation of Gram-negative and Gram-positive bacteria, as well as template-synthesis for fabrication of bismuth related hollow nanostructures [9]. It is important to emphasize that we did not find any report concerning cytotoxicity of common bismuth nanostructured derivatives. And it is necessary to determine the possible toxicity of Bi_2O_3 NPs in human fibroblasts cultures and analyze their potential use in humans.

This paper reports the synthesis of Bi_2O_3 nanospheres and antibacterial test and cytocompatibility evaluation of as obtained Bi_2O_3 nanospheres.

II. EXPERIMENTS

A. Materials

$Bi(NO_3)_3 \cdot 5H_2O$ was purchased from Tianjin Chemical Reagent Co., Ltd. HNO_3 was purchased from Xilong Chemical Co., Ltd. PEG-4000 were purchased from Sinopharm Chemical Reagent Co., Ltd. NaOH was obtained from Tianjin Chemical Reagent Co., Ltd. ethyl alcohol was obtained from Tianjin Chemical Reagent Co., Ltd. Dulbecco's modified Eagle's medium (DMEM) and Dimethyl sulfoxide (DMSO) were purchased from Gibco. Trypsin-EDTA solution and MTT, guaranteed reagent were supplied by Sigma. Fibroblastic cells (L929) were provided by the Fourth Military Medical University. Ultrapure water was used for rinsing and as the solvent as well.

B. The Preparation of Bi_2O_3 Nanospheres

All chemicals used in the experiment were analytical reagents and were used as received. In a typical procedure, 24.25 g $Bi(NO_3)_3 \cdot 5H_2O$ was dissolved by 30mL0.05 mol L^{-1} HNO_3 to obtain transparent aqueous solution, then 20mL polyethylene glycol (PEG-4000) was added as dispersant. Then 50mL 4 mol L^{-1} NaOH aqueous solution was quickly poured into the prepared solution under vigorous stirring, which immediately resulted in the formation of yellowish precipitates in the beaker. The as-produced precipitates were filtered after kept at 90°C for 2 h under stirring, then washed with alcohol and ultrapure water for several times and dried at 60°C in a vacuum drier to obtain the yellow Bi_2O_3 samples. The as-produced Bi_2O_3 powders were milled by ball mill with the 400 rotary velocities for 400min to obtain final powders.

Bi_2O_3 powder was dissolved in distilled water and stirred until complete dissolution, then different concentrations of suspension liquid (0.25, 0.5, 1, 1.5 mg mL^{-1}, respectively) were obtained.

C. Characteristics

The morphology of as-synthesized samples was observed with a scanning electron microscope (SEM, JEOL JSM–6701F) at 5.0 kV and a transmission electron microscope (TEM, JEOLJEM –2010). The structure and phase composition of as-synthesized samples were characterized by X-ray diffraction (XRD, Rigaku D/Max–2400 diffractometer, CuKa ¼ λ =1.54056 Å).

D. Antibacterial Activity Measurement

The bacteria (S. aureus) used were cultured in the Luria Bertani (LB) liquid medium at 37°C for 24 h. Before the antibacterial test, all the samples and materials in the experiments were sterilized at 121°C for 20 min. The antibacterial activities of Bi_2O_3 samples towards S. aureus were evaluated by colony counting methods. The colony counting test was performed by mixed 2 mL 10^6 CFU mL^{-1} diluted bacteria and 2mL Bi_2O_3 samples (0.25, 0.5, 1, 1.5 mg mL^{-1}) into tubes, then the mixtures were incubated for 4 h at 37°C with shaking at 300 rpm. Then, 0.1 mL of the mixtures were diluted into 10^{-6}, 10^{-7}, 10^{-8}, and then plated onto LB agar plates and the number of the colonies was counted after incubation for 24 h at 37°C.

E. Fibroblastic Cells Culture

In this research we used L929 cells to analyze the cytotoxic effect of Bi2O3 NPs. The fibroblastic cells were grown in DMEM containing 10% fetal bovine serum, 4.5 g/L^{-1}glucose, 2mM L-glutamine, and 1% antibiotic/antimycotic solution. The cells were kept under sterile conditions at 37°C and 5%CO_2. The media were refreshed every day until the cells reached confluence.

F. Cellular Viability by MTT Assay

Cell viability was determined by the MTT assay, which is based on the reductive cleavage of MTT to formazan by mitochondrial dehydrogenase of living cells [10]. All the samples and materials in the experiments were sterilized at 121°C for 20 min, and then placed into a 24-well plate and seeding density was 2×10^4 cells mL^{-1} each well. At a set period of time (1, 2, 3 days), each well was infused with 100μL MTT solution, then cultured for 4h. The upper solvent was removed, and the blue formazan reaction product was dissolved by adding DMSO (200μL) after culturing. The ultimate dissolvable solution was transferred into a 96-well plate, and then its absorbance of 490nm was recorded with a microplate reader. The data of three parallel experiments were averaged. The cellular survival rate were calibrated by blank and control groups. To this end, culture media were added only into the blank group, while the control group

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was designed to cells and culture media without samples. The optical density (OD) values of the blank, control, and experimental groups are enciphered as OD_{bla}, OD_{con}, and OD_{exp}; and the cellular survival rates are calculated as

$$\text{Survival Rate} = (OD_{exp} - OD_{bla})/(OD_{con} - OD_{bla}) * 100\% \quad (1)$$

Results are expressed as mean ± standard deviation and are analyzed with the Student's t-test.

III. Results and Discussion

A. Characterization of Bi_2O_3

Figure 1A shows the morphology of Bi_2O_3 without milling, the SEM image shows stick crystals with an average length of 15μm. Figure 1B shows typical XRD patterns of the as-synthesized Bi_2O_3 without milling, all the diffraction peaks are well indexed to Bi_2O_3 (JCPDS 76-2478), indicative of the high purity of Bi_2O_3. It indicates well-crystallized Bi_2O_3 can be successfully produced via the solution-based approach without having resort to the high-temperature calcination process as have to be taken by the traditional methods. Figure 1C shows the morphology of milled Bi_2O_3, the SEM image illustrates that final Bi_2O_3 powders are composed of large-scale uniform solid Bi_2O_3. TEM image (Figure 1D) further demonstrates the Bi_2O_3 nanospheres with an average diameter of 350 nm.

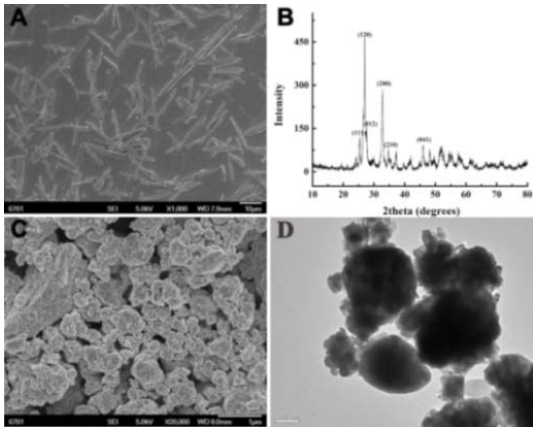

Figure 1. The Characterization of Bi_2O_3

B. Antibacterial Performance

To explore the possible antimicrobial activity of Bi_2O_3 NPs, their effect on S. aureus growth is determined. Figure 2 shows the images of antimicrobial activity of Bi_2O_3 NPs, and Table I shows the activity of the colony. The results show that 1.5 mg mL^{-1} Bi_2O_3 NPs reduce the number of S. aureus by 45%, in comparison with control without Bi_2O_3. This result is very important because it demonstrates the effectiveness of the nanostructured material. The mechanism of the antimicrobial activity of inorganic NPs is not completely understood, and their precise mechanism of action against bacteria remains to be fully elucidated. It has been shown that positive charges on the metal ion are critical for the antimicrobial activity, allowing for the electrostatic attraction between a negatively charged cell membrane and the positively charged NPs.

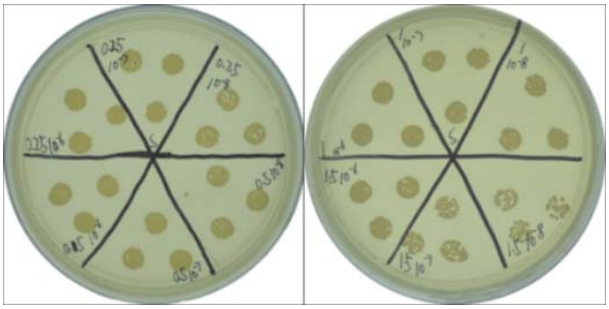

Figure 2. Photographs showing the antibacterial activity of Bi2O3 NPs (S. aureus)

TABLE I. The Activity of The S. Aureus of Bi_2O_3 NPs

Bi_2O_3 (mg mL^{-1})	Activity of the colony (%)
0.25	88.93
0.5	88.14
1	86.27
1.5	54.88

C. MTT Assay

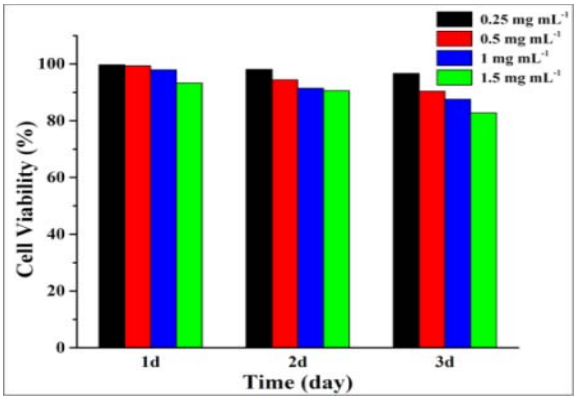

Figure 3. Cellular proliferation analysis, the cells were respectively

incubated for 1, 2, and 3 days with Bi_2O_3 NPs.

MTT assay is one of the methods commonly used to assess biocompatibility of materials. L929 fibroblasts were seeded directly in 96-well plates, and incubated for 1, 2, 3 days, respectively, and their viability were assessed by MTT assay. As shown in Figure 3, the Bi2O3 NPs do not promote cytotoxic effects in the L929 cells at 24 hours of exposure compared with the cells without NP. The cells looked very similar in the presence or absence of Bi2O3 NPs. These results suggest the absence of cytotoxicity by Bi2O3 NPs, under our experimental conditions.

▪ IV. Conclusions

In conclusion, Bi_2O_3 NPs of 350 nm average size have an antimicrobial activity inhibiting the growth of S. aureus. Additionally, our results suggest that the Bi_2O_3 NPs, under the experimental tested conditions and concentrations, do not exhibit cytotoxicity. The present approach can shed new light on designing of antibacterial material like bismuth oxide with promising applications in biological sciences.

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