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Controllable synthesis and adjustable antineoplastic activity of bovine serum albumin-conjugated PbS/Ag_2S core/shell nano-composites

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ABSTRACT

Series of mono-dispersed bovine serum albumin (BSA)-conjugated PbS/Ag_2S core/shell nano-composites with different Pb/Ag ratios had been successfully synthesized by an ion-exchange method under the gentle conditions using BSA-conjugated PbS nano-crystals as precursors, which were prepared by a biomimetic method. Fourier transform infrared spectra analysis and transmission electron microscopy (TEM) observation demonstrated that BSA was a key factor to control the morphology and size of final products. Additionally, the real-time TEM observation, X-ray powder diffraction and atomic absorption spectroscopy analysis were applied to monitor the synthesis process. The results indicated that the shell thickness and ratio of Pb to Ag could be controlled by adjusting the ion-exchange time. Both metabolic and morphological methods revealed that the proliferation of rat pheochromocytoma (PC 12) cells could be inhibited by BSA-conjugated PbS/Ag_2S core/shell nano-composites, and the antineoplastic activity was Pb/Ag ratio-dependent. It might be explained by a Trojan horse-type mechanism. Summarily, the present study would be helpful to find a new core/shell nano-composite with higher and controllable antineoplastic activity due to the synergistic reaction of different metal ions.

1. Introduction

Keeping in view of the fact that tumor is one of the most frequently occurring diseases and still a therapeutic challenge to the human, the successful applications of nano-technology in tumor cure, such as tumor cells tracking and targeted delivery, give hope to tumor patients for a cure [1–8]. For example, Chen et al. [9] prepared CdSe/ZnS quantum dots linked to alpha-fetoprotein monoclonal antibody. The results in vitro and in vivo indicated that the resulting quantum dots had good stability, specificity and biocompatibility for ultrasensitive fluorescence imaging of molecular targets in the liver cancer model system. We also fabricated bovine serum albumin (BSA)-conjugated Ag_2S nano-particles, nano-rods and nano-wires in the previous work by a biomimetic method and found that the antineoplastic activity of different nano-materials on C6 glioma cells was dose-, size- and cell type-dependent [10,11].

In fact, the antineoplastic activity of nano-materials containing metal ions can directly kill the tumor cells through different ways [12,13]. For example, Nam et al. [12] prepared "smart" gold nano-particles with size of 10 nm in diameter, which could switch its surface charge-switch trigger by the environmental pH, form aggregates in cells by electrostatic attractions and further efficiently destroy cancerous cells in response to external illumination. Lee et al. [14] investigated the bioactivity of water-soluble CdSe/ZnS core/shell quantum dots. The in vitro and in vivo results demonstrated that quantum dots could invoke the toxicity by inducing robust activation of tumor necrosis factor-α (TNF-α) and CXC-chemokine ligand (CXCL) 8 expression through reactive oxygen species (ROS)- and mitogen-activated protein kinases (MAPKs)-dependent pathways. Consequently, is it possible to control the antineoplastic activity of nano-materials containing metal ions by an appropriate combination of metal ions?

Based on the fact that nano-materials killing tumor cells can be attributed to the leaching of heavy metal ions, we hypothesized that the synthesis of nano-composites with controllable antineoplastic activity might be accomplished by adjusting the metal ion types and ratios in nano-materials. Moreover, we further fabricated the BSA-conjugated PbS/Ag_2S (BPbS/Ag_2S) core/shell nano-composites by an ion-exchange technique using BSA-conjugated PbS (BPbS) nano-particles as the precursor. The formation mechanism was well investigated by monitoring the preparation process with a real-time transmission electron microscopy (TEM) observation, X-ray powder diffraction (XRD) analysis and atomic absorption spectroscopy (AAS) test. The relationship between the Pb/Ag ratio and antineoplastic activity was verified by using rat pheochromocytoma PC 12 cells as the model.

2. Experimental

2.1. Materials

Silver nitrate (≧99.8%, Mw = 169.87 AR), plumbum nitrate (≧99%, Mw = 331.21 A.R.) and thioacetamide (TAA) (≧99.0%, Mw = 75.13

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AR) were purchased from Tianjin Chemical Reagent Factory (Tianjin, China). BSA (≧98%, Mw = 68,000) was purchased from Xiamen Sanland Chemicals Company Limited (Xiamen, China).

2.2. Preparation of BSA-conjugated PbS nano-particles

Protein-conjugated PbS nano-particles were prepared by the aqueous chemistry method according to our previous work [15]. Briefly, 50 mL of 13.25 mg/mL plumbum nitrate aqueous solution was mixed with 150 mL of 1.3 mg/mL BSA aqueous solution. The mixture was kept static for 12 h at room temperature in the dark. Then 40 mL of 3.75 mg/mL TAA was added under stirring and the mixed reaction solution was incubated at room temperature for 3 days in the dark. Finally, the as-prepared samples were separated by centrifugation at 10,000 rpm for 10 min. The collected solid-state product was washed with double distilled water and dried in a vacuum freeze-drying for 8 h.

2.3. Preparation of BPbS/Ag_2S core/shell nano-composites

The BPbS/Ag_2S core/shell nano-composites were fabricated by the ion-exchange method using BSA-conjugated PbS nano-particles as the precursor. Typically, 12.5 mg BSA-conjugated PbS nano-particles were dispersed in 20 mL of distil water and then 5 mL of 2 mg/mL silver nitrate solution was quickly added under stirring. 10 min later, the mixed solution was transferred to a 25 mL Teflon-lined autoclave and maintained at 80 °C for 4 h. The final products were rinsed three times with distilled water and dried in a vacuum freeze-drying for 8 h.

2.4. Characterization

High-resolution transmission electron microscopy (HRTEM) observations were carried out in a JEM-2010 electron microscope working at 200 kV for measuring the lattice spacing of core/shell nano-composites.

Fourier transform infrared spectra (FTIR) were recorded on a Bio-Rad FTS-40 Fourier transform infrared spectrophotometer in the wavenumber range of 4000–400 cm^{-1}. The spectra were collected at 2 cm^{-1} resolution with 128 scans by preparing KBr pellets with a 3:100 "sample-to-KBr" ratio.

Thermogravimetry–differential scanning calorimetry (TG/DSC) measurement was carried out on an EXSTAR TG/DTA 6300 instrument (Seiko, Japan) to measure the content of BSA in BPbS/Ag_2S core/shell nano-composites.

2.5. Real time analysis

The real time analysis was performed on the morphology, crystalline phase and Pb/Ag ratio of samples obtained with the different ion-exchange time by TEM (FEI-Philips Tecnai G2, Philips, Holland), XRD (D & Advance, Bruker, Germany) and AAS (Z-5000, Hitachi, Japan). Briefly, as for the samples obtained by 0, 2, 4, 6 and 8 h of ion-exchange, a 5 μL droplet of dilute alcohol solution of samples was dripped onto a holey carbon-coated formvar support for the observation of TEM.

To determine the crystalline phase, XRD measurements were performed on a Bruker D & Advance X-ray powder diffractometer with graphite monochromatized Cu/Kα (γ = 0.15406 nm). A scanning rate of 0.05 °/s was applied to record the pattern in the 2θ range of 10–90°.

At the same time, the samples were also digested by nitric acid and the Pb and Ag concentrations were detected by AAS test.

2.6. Exposure of cells to nano-composites

2.6.1. Cell seeding

PC 12 cells were routinely cultured in tissue culture flasks with high glucose-Dulbecco modified Eagle medium (H-DMEM), containing 10% fetal bovine serum and incubated at 37 °C in a humidified atmosphere with 95% air and 5% CO_2. The culture medium was refreshed every two days. When the cells became almost confluent after 5 days, they were released by treatment with 0.25% trypsin. Then the cells were counted to 10^4 cells/cm^2 and 200 μL of the cell suspension was pipetted into 96-well tissue culture plate. After 12 h of culture, the medium was replaced with the fresh H-DMEM medium containing 0.8–500 μg/mL of nano-materials. In addition, the normal group without any nano-materials was chosen as the control group.

2.6.2. Cell viability

The metabolic analysis was performed by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay (MTT) based on succinic dehydrogenase activity at OD490 nm (n = 3); 630 nm was chosen as the reference wavelength [16]. Briefly, after 48 h of cell culture, the wells were carefully washed with phosphate buffered solution (PBS, pH 7.2, 0.1 M). 20 μL of MTT was added in 180 μL of culture medium, and cell culture was continued for another 4 h. Then the solution was removed and the wells were washed twice with PBS. 200 μL of dimethyl sulfoxide was pipetted into each well and optical density (OD) values were read on a microplate reader (Multiskan MK3, Thermo Labsystems, USA). The inhibition rate of heavy metal sulfides on cells was calculated as follows:

$$\text{Inhibition rate} = (\text{ODc} - \text{ODt})/\text{ODc} \times 100\%$$

where ODc and ODt represent the OD490 nm values of the control group and the treatment group, respectively.

2.6.3. Cell proliferation

The number and distribution of attached cells on plate treated with nano-materials were measured by the cell counting method. Briefly, after 48 h of cell culture, all substrates were clearly rinsed in PBS (pH 7.2, 0.1 M) and then the cells were counted on the microscope (Axioskop 40, ZEISS, Germany).

2.7. Statistical analysis

The number of independent replica was listed individually for each experiment. Where applicable, all data were mean ± SD. The analysis of data for cell proliferation was performed by one-way factorial analysis of variances (ANOVA) and multiple comparisons (Fisher's method as Post-Hoc test, $p < 0.05$).

3. Results and discussion

In order to present the data clearly, BSA-conjugated PbS/Ag_2S nano-composites obtained by ion exchange for 0, 1, 2, 4, 6 and 8 h were abbreviated as BPbS/$Ag_2S^{@0h}$, BPbS/$Ag_2S^{@1h}$, BPbS/$Ag_2S^{@2h}$, BPbS/$Ag_2S^{@4h}$, BPbS/$Ag_2S^{@6h}$ and BPbS/$Ag_2S^{@8h}$, respectively.

3.1. Preparation of BPbS/Ag_2S core/shell nano-composites

BPbS/Ag_2S core/shell nano-composites were prepared under the gentle conditions by combination of the biomimetic method and the ion-exchange method. Here, BSA was expected to act as the template to control the morphology and size of nano-composites. Firstly, TEM observation was applied to confirm the template effect of BSA. Fig. 1 shows the TEM images and corresponding size distribution of bulk PbS control, BPbS/$Ag_2S^{@0h}$ and BPbS/$Ag_2S^{@4h}$ nano-composites. PbS