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Preparation and Characterization of Aqueous Bifunctional Fe_3O_4/CdSe/CdS Nanocomposites and Its Application in Immunolabelling of Cancer Cells

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A novel method to prepare aqueous magnetic and luminescent Fe_3O_4/CdSe/CdS nanocomposites was demonstrated. Fe_3O_4 magnetic nanoparticles were first synthesized by a chemical coprecipitation method with PEG4000 as stabilizer. After being modified by citric acid, the Fe_3O_4-COOH magnetic nanoparticles were coated with silica shells by Stober method, followed by modification with thiol groups through the sol-gel method. The surface-modified Fe_3O_4 nanoparticles were lastly linked to CdSe/CdS quantum dots, which were synthesized in aqueous solution with thioglycolic acid as stabilizer, to form Fe_3O_4/CdSe/CdS bifunctional nanocomposites through coordination. Their optical and structural properties were characterized by photoluminescence, vibrating sample magnetometer and X-ray diffraction studies. It was indicted that the water soluble nanocomposites possess both the magnetic and photoluminescence properties. It was used as fluorescent label in detection of carcinoembryonic antigen, a cancer marker expressed on the surface of HeLa cells. The present study demonstrates the practicability of the prepared nanocomposites as an attractive type of fluorescent labels for biological applications.

Keywords: Fe_3O_4/CdSe/CdS nanocomposites, Quantum dots, Magnetic nanoparticles, Cell imaging.

INTRODUCTION

Compared with traditional organic dyes, quantum dots have broad excitation spectra, narrow emission bandwidths and tunable size-dependent emission[1]. Recently, quantum dots play a crucial role in active bio-analytical fields[2], which have attracted much attention of analysts. Particularly in terms of biological imaging[3,4], quantum dots exhibite so many advantages compared with organic dyes. In cells or tumors labeling, quantum dots are always linked with antibody or biomarker[5]. Although those methods could achieve specific imaging or detection, they lead to the existence of redundant reagent in testing solution except the residual synthetic reactant. So, these redundant reagents should be separated. Among all kinds of methods, magnetic separation is one of the good techniques.

Magnetic (Fe_3O_4) nanoparticles, in various iron oxide nanoparticles, are a prominent class due to the low cost and the simply synthesizing method. One of the properties of Fe_3O_4 nanoparticles was their relative simply linked with some molecules such as luminescence probes[6], medicine[7]. They have been applied in cell separation and drug targeting due to the unique magnetic properties[8]. More and more researches pay their attention to the combining quantum dots with magnetic nanoparticles for achieving multifunctional nanocomposites[9].

Sun *et al.*[10] have reported the preparation of water soluble Fe_3O_4/CdTe nanocomposites using several reagent modifications and the nanocomposites exhibit magnetic and fluorescent properties favorable for their applications in magnetic separation and guiding as well as fluorescent imaging. In this study, CdSe/CdS quantum dots were synthesized in a water soluble system. Fe_3O_4 nanoparticles were prepared by a chemical coprecipitation method. Fe_3O_4 nanoparticles were sequentially modified with citric acid, SiO_2 and 3-mercaptopropyltrimethoxysilane. The surface-modified Fe_3O_4 nanoparticles were then linked to CdSe/CdS quantum dots to form the Fe_3O_4/CdSe/CdS bifunctional nanocomposites through coordination. They could be used in magnetic separation and luminescence detection.

EXPERIMENTAL

Ferric chloride, polyethylene glycol (PEG 4000), tetraethyl orthosilicate (TEOS) and 3-mercaptopropyltrimethoxysilane (MPS) were obtained from Shanghai Chemical Reagent Co. Ltd. Selenium powder, sulfur powder, cadmium chloride, thioglycolic acid and sodium borohydride were purchased from Beijing Chemical Reagent Co. Ltd. Water used in all experiments was prepared using a double-distilling system.

N-hydroxysuccinimide (NHS) was obtained from Dingguo Bio. The human cancer marker HeLa cells were obtained from IBCB; CAS and cell media were from Gibco (USA). Primary antibody (Ab) rabbit anti-CEA8 and bovine serum albumin (BSA) were all purchased from Beijing Biosynthesis Biotechnology Co. Ltd.

Synthesis of Fe_3O_4/CdSe/CdS nanocomposites

Synthesis of Fe_3O_4 nanoparticles: Fe_3O_4 nanoparticles were synthesized by chemical coprecipitation method[11] and then PEG 4000 was added for their better dispersibility in pH 9.8 solution. After washing with water, the Fe_3O_4-PEG nanoparticles were firstly modified with citric acid under ultrasonic for 2 h and then the upper solution was washed with acetone. Dried at 40 °C for 2 h and grinded, the Fe_3O_4 nanoparticles were achieved.

Synthesis of Fe_3O_4/SiO_2 nanocomposites: For making Fe_3O_4 nanoparticles be stably dispersed in water and achieving the linking with CdSe/CdS quantum dots, SiO_2 layer and thiol modifier were used on their surface. SiO_2 layer were synthesized by Stober method[12]. Briefly, some Fe_3O_4 nanoparticles prepared above were firstly dispersed in water with stirring and ultrasound for 0.5 h and then some ethanol and ammonia were added. Lastly, some TEOS were slowly dropping in above solution through a constant pressure dropping funnel. After reacting at 35 °C for 10 h, Fe_3O_4/SiO_2 solution was obtianed. 3-Mercaptopropyltrimethoxysilane solved in ethanol was then dropped in Fe_3O_4/SiO_2 solution and reacted at 35 °C for 14 h, thus thiol capping reagent was modified on their surface.

Synthesis of Fe_3O_4/CdSe/CdS nanocomposites: CdSe/CdS quantum dots capped with thioglycolic acid were prepared in aqueous solution according to the procedure described elsewhere[13].

Thiol capped Fe_3O_4/SiO_2 nanoparticles were firstly mixed with CdSe/CdS quantum dots at the ratio of 1:1.4 and then the pH value was adjusted to 11 with 2 mol/L NaOH solution. The Fe_3O_4/CdSe/CdS nanocomposites were achieved after reacting at 35 °C for 6 h.

Preparation of conjugates of nanocomposites-carcinoembryonic antigen: The water-soluble Fe_3O_4/CdSe/CdS nanocomposites were first conjugated with the primary antibody (rabbit anti-CEA8), using N-hydroxy succinimide as a cross-linker. Typically, 50 µL 0.1 mg/mL rabbit anti-CEA8 solution in 10 mmol/L phosphate buffered solution (PBS, pH 7.4), was mixed with 50 µL 20 mg/mL nanocomposites solution in the same buffer and reacted for 60 min at 37 °C in a reciprocating oscillator after being activated by 100 µL 0.1 mg/mL N-hydroxy succinimide for 10 min at room temperature. Then the solution was separated under a magnetic field for removing unbound antibody. At last, the nanocomposites-carcinoembryonic antigen was redispersed in 400 µL PBS and HeLa cells were incubated with quantum dots-antibody conjugates at 4 °C for 1 h.

Characterization: The photoluminescence spectra were measured using a Hitachi F-4600 luminescence spectrometer equipped with a xenon lamp as a light source. The powder X-ray diffraction (pXRD) patterns of particles were obtained on a PW3040/60 X' Pert Pro MDP (Holland Panalytical B.V.). Hysteresis loops were measured on a vibrating sample magnetometer 7407 vibrating sample magnetometer (LakeShore, USA). The cancer marker detections were performed using a Leica DMIL inverted fluorescence microscope (with a × 40/0.5 objective) and the fluorescence microscopic images were captured using blue excitation (488 nm).

Cell culture, fixation and fluorescence microscopic imaging: In a 96 well plate, HeLa cells were cultured (at 37 °C, 5 % CO_2) in RPMI 1640 medium containing 10 % fetal bovine serum and 1 % penicillin/streptomycin overnight in a culture box (Heraeus BB16UV). To label the fixed cells, the cells were gently washed three times with PBS and fixed with 4 % formaldehyde for 10 min at room temperature and then they were blocked in PBS containing 1 % BSA for 20 min at 4 °C. HeLa cells were incubated in the conjugates solution of NCs-CEA at 37 °C for 0.5 h to achieve cells labeling. After being labeling, the cancer cells detections were performed using a Leica DMIL inverted fluorescence microscope. Prior to imaging, the cells were washed thoroughly with PBS to remove any unbound reagents.

RESULTS AND DISCUSSION

As shown in Fig. 1, Fe_3O_4/CdSe/CdS nanocomposites were achieved through the reaction performed between the thiol group on the surface of Fe_3O_4/SiO_2 nanoparticles and Cd^{2+} on the surface of CdSe/CdS quantum dots. For achieving the nice dispersibility and bio-functionalization, three strategies were carried out in this work. (1) Citric acid and PEG4000 could enhance the dispersibility of Fe_3O_4 nanoparticles. Three carboxyl groups in citric acid can be linked with hydroxyl through hydrogen bonding and electrostatic interactions. (2) SiO_2 was coated on the surface of Fe_3O_4 nanoparticles to improve their bio-functionalization because SiO_2 has good biocompatibility and chemical stability. (3) Thiol group was successively formed on the surface of Fe_3O_4/SiO_2 nanocomposites by the hydrolyzation of 3-mercaptopropyltrimethoxysilane. There are strong force between -SH and Cd^{2+}, which ensured that the formation of Fe_3O_4/CdSe/CdS nanocomposites.

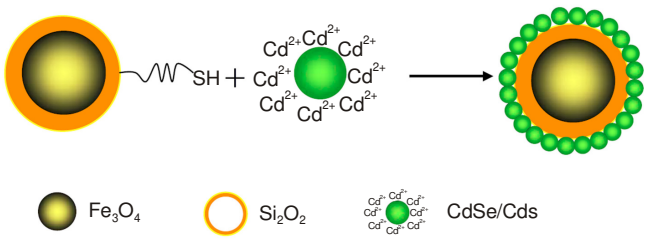

Fig. 1. Schematic illustration of formation of Fe_3O_4/CdSe/CdS nanocomposites

Optical properties of CdSe/CdS quantum dots and Fe_3O_4/CdSe/CdS nanocomposites: The emission (λ_{ex} = 320 nm) and absorption spectra of the as-prepared CdSe/CdS quantum dots dissolved in water (Ex slit 5 nm, Em slit 5 nm and scan speed 1200 nm/min) were shown in Fig. 2a and b, respectively. Typical FWHM of those as-synthesized nanocomposites were in the range of 25-50 nm; even the biggest FWHM was less than 55 nm. It indicated that these nanocomposites had good optical characteristics, desirable dispersibility and uniformity. The maximum emission peak of Fe_3O_4/CdSe/CdS

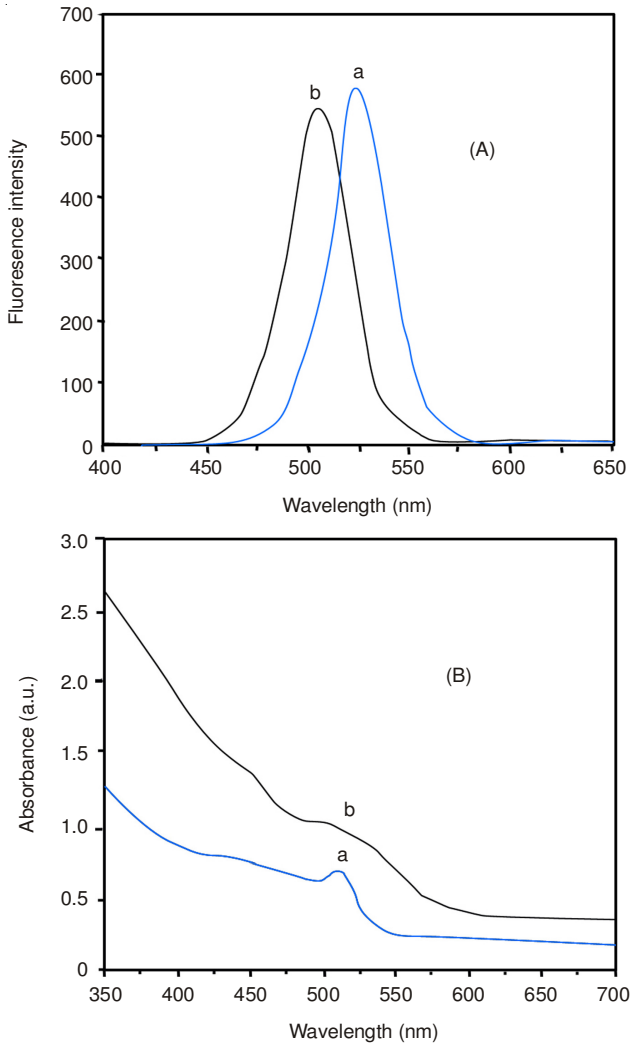

Fig. 2. Fluorescence spectra (A) and UV-visible absorption spectra (B); (a) CdSe/CdS quantum dots; (b) Fe_3O_4/CdSe/CdS nanocomposites

nanocomposites exhibited some blue-shift (about 8 nm) compared with CdSe/CdS (QD525) quantum dots. The blue-shift maybe results from the different solvent environment between the two nanoparticles. The relative lower polarity around Fe_3O_4/CdSe/CdS nanocomposites than that of CdSe/CdS quantum dots reduces stokes shift. The absorption spectrum was continuous and broad in the range 200-480 nm with a characteristic peak for CdSe/CdS quantum dots. Comparing with CdSe/CdS quantum dots, the absorption spectrum of Fe_3O_4/CdSe/CdS nanocomposites changed flat, which resulted from the absorption of Fe_3O_4 in the visible range.

Magnetization curves of Fe_3O_4 and Fe_3O_4/CdSe/CdS nanoparticles: The magnetic properties of Fe_3O_4 and Fe_3O_4/CdSe/CdS nanoparticles were measured with a vibrating sample magnetometer at room temperature. As shown in Fig. 3, the saturation magnetization of Fe_3O_4 nanoparticles was 49.30 emu/g, while that of Fe_3O_4/CdSe/CdS nanocomposites was only 2.16 emu/g. The variation of magnetization was due to the presence of SiO_2 and CdSe/CdS layers, which could make the magnetism lower. Under a magnetic field, Fe_3O_4/CdSe/CdS nanoparticles can be gathered to the side of magnet. With the luminescence and magnetic property, the Fe_3O_4/CdSe/CdS nanoparticles can be used as a biological probe.

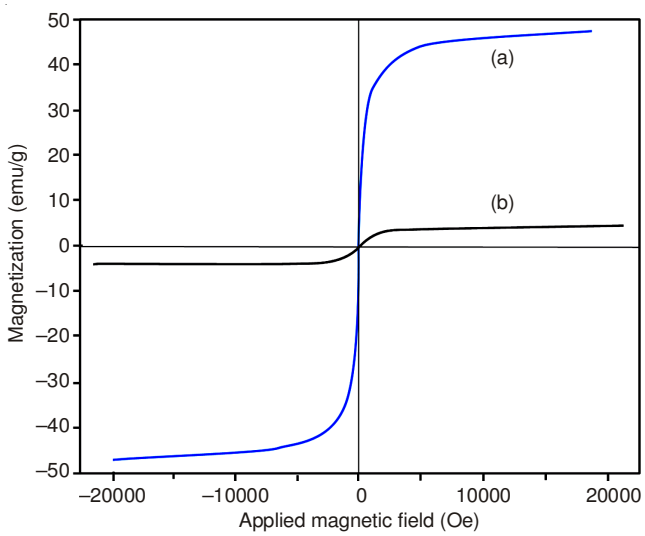

Fig. 3. Magnetization curves obtained by vibrating sample magnetometer (a) Fe_3O_4 nanoparticles; (b) Fe_3O_4/CdSe/CdS nanocomposites

Structure characteristic of Fe_3O_4/CdSe/CdS nanocomposites: As shown in Fig. 4, the structure and composition of Fe_3O_4, CdSe/CdS quantum dots and Fe_3O_4/CdSe/CdS nanocomposites were characterized with XRD measurement. The peaks of CdSe/CdS were 26.03, 43.37 and 51.22 and the corresponding positions of Fe_3O_4 nanoparticles were 18.23, 30.21, 35.31, 43.18, 57.22, 62.81. Fe_3O_4/CdSe/CdS nanocomposites exhibited the peaks in both nanoparticles, which confirmed the formation of nanocomposites. The sizes of CdSe/CdS quantum dots and Fe_3O_4/CdSe/CdS nanocomposites could be calculated approximately from Debye-Scherrer equation ($D = K\lambda/B_{1/2}\cos\theta$), where λ was the wavelength of X-ray radiation (CuKα, 1.5406 nm), θ is the Bragg diffraction angle, K was normally equal to 0.89 and $B_{1/2}$ is FWHM of diffraction peak. By this calculation, the size of bare CdSe/CdS quantum dots was about 3.9 nm and that of Fe_3O_4/CdSe/CdS nanocomposites was about 28 nm.

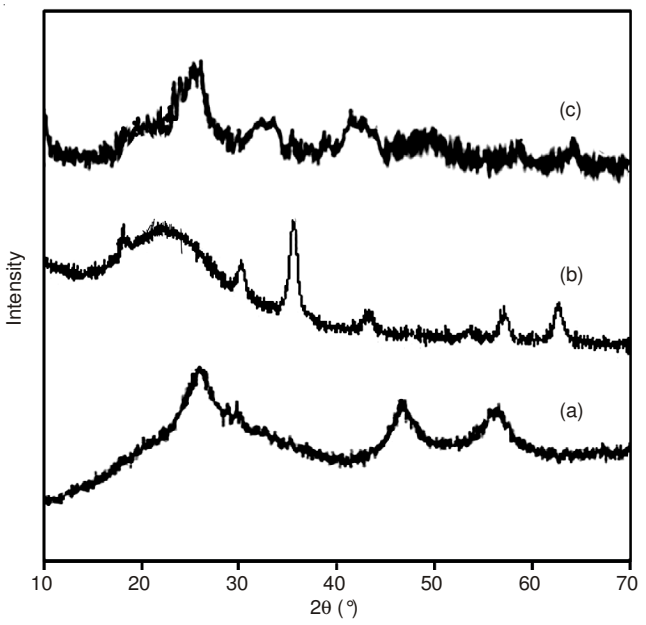

Fig. 4. X-ray powder diffraction pattern; (a) CdSe/CdS quantum dots; (b) Fe_3O_4 nanoparticles; (c) Fe_3O_4/CdSe/CdS nanocomposites

Immunolabelling of HeLa Cells: In this work, HeLa cells were labeled by the direct method *via* specific identification and combination between antigen and antibody. As shown in Fig. 5, the prepared Fe_3O_4/CdSe/CdS nanocomposites were first linked with carcinoembryonic antigen and then the conjugates were incubated with BSA-blocked live and fixed cells, respectively. Through the immunoreaction between antigens and carcinoembryonic antigen expressed on HeLa cells membrane, quantum dots recognized the targets and exhibited bright fluorescence over the cells.

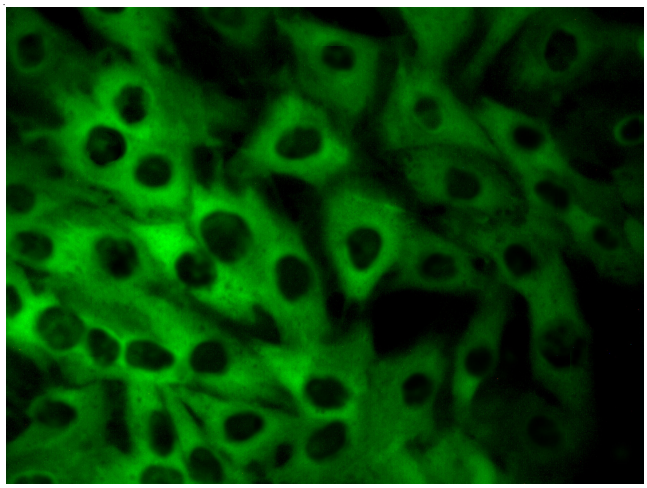

Fig. 5. Images of fixed cell membranes directly labeled by Fe_3O_4/CdSe/CdS nanocomposites

Conclusions

In this study, double functional Fe_3O_4/CdSe/CdS nanocomposites were prepared in a water soluble system, where unreacted or redundant reagents could be removed from reaction system result from the magnetic properties. Moreover, the CdSe/CdS quantum dots could supply bright fluorescent for subsequent using in cell imaging. With the immunization between antibody and antigen, Fe_3O_4/CdSe/CdS nanocomposites were successfully used to directly label fixed HeLa cells, with excellent specificity.

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