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Preparation of highly fluorescent magnetic nanoparticles for analytes-enrichment and subsequent biodetection

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ABSTRACT

Bifunctional nanoparticles with highly fluorescence and decent magnetic properties have been widely used in biomedical application. In this study, highly fluorescent magnetic nanoparticles (FMNPs) with uniform size of ca. 40 nm are prepared by encapsulation of both magnetic nanoparticles (MNPs) and shell/core quantum dots (QDs) with well-designed shell structure/compositions into silica matrix via a one-pot reverse microemulsion approach. The spectral analysis shows that the FMNPs hold high fluorescent quantum yield (QY). The QYs and saturation magnetization of the FMNPs can be regulated by varying the ratio of the encapsulated QDs to MNPs. Moreover, the surface of the FMNPs can be modified to offer chemical groups for antibody conjugation for following use in target-enrichment and subsequent fluorescent detection. The *in vitro* immunofluorescence assay and flow cytometric analysis indicate that the bifunctional FMNPs-antibody bioconjugates are capable of target-enrichment, magnetic separation and can also be used as alternative fluorescent probes on flow cytometry for biodetection.

1. Introduction

Multifunctional nanoparticles are attracting more and more attentions of material scientists, chemists and biologists, since such multifunctional particles combine multiple properties and have various applications [1–5]. Especially, considerable efforts have been devoted to design fluorescent magnetic nanoparticles (FMNPs) with both fluorescence and magnetism functionalities for potential application as dual-modality imaging probes [6–11]. In addition to the use as imaging probes, FMNPs have potential applications in bio-analysis [12,13]. FMNPs can be employed to separate and enrich the analytes from complicated samples, and then the enriched analytes can be detected with the fluorescent signal generated by FMNPs. The preparations of FMNPs have been extensively reported these years [14–18]. However, one of the common problems during the preparation of FMNPs is fluorescent quenching, resulting in the low QY of FMNPs [15,19]. The fluorescent quenching is mainly owing to two factors. The first one is the photo/chemical stability of the used fluorophores. FMNPs are usually prepared and used in the complicated chemical solution, and many chemicals could be the quencher of fluorophores [20–24]. The second factor is the influence of MNPs because of the strong absorption cross section of the magnetic nanoparticles [15] and the possible energy transfer between fluorophores and MNPs [25]. The second quenching effect could be weakened by adjusting the mol ratio of embedded fluorophores to MNPs [17]. However, the saturation magnetization of the FMNPs will be insufficient if the ratio of MNPs to fluorophores is too low. Thus, the most promising solution to solve fluorescent quenching is the selection of proper fluorophores.

Semiconductor nanocrystals called QDs [26] have high photostability, high emission quantum yield, narrow emission peaks, size-dependent wavelength tunability in comparison with organic dyes and fluorescent proteins, which make them more interesting for potential biomedical application [27]. Most of QDs are hydrophobic, since they are capped by organic ligands. And they are photo stable in organic solution. However, hydrophobic QDs should be transferred into hydrophile for biomedical application. One of the most widely used modification methods is silanization. However, the fluorescence of QDs will be quenched in various degrees when they are transferred into water phase [28,29]. Our group has done some work about the changes of optical property of QDs before and after silica-coating. And study data indicated that QDs with appropriate structure and composition of shells can retain the initial QY more efficiently after silanization [30]. Based on the results from our past work, in this study, the well-designed seven layered shell/core QDs ($CdSe/CdS/CdS/Cd_{0.75}Zn_{0.25}S/Cd_{0.5}Zn_{0.5}S/Cd_{0.25}Zn_{0.75}S/ZnS/ZnS$) were selected to prepare highly florescent FMNPs with the classical silica-coating via the reverse microemulsion approach [31]. After the successful preparation of FMNPs, the prepared samples were chemically-activated by amino groups for antibody-labeling and the following target-enrichment and subsequent fluorescent detection on a flow cytometry.

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2. Materials and methods

2.1. Materials

Selenium powder (100 mesh, 99.99%, Aldrich), cadmium oxide (CdO, 99.5%, Aldrich), zinc oxide (ZnO, 99.9%, Sigma), $Fe(acac)_3$ (Alfa Aesar), 1,2-hexadecanediol (Alfa Aesar), benzyl ether (Alfa Aesar), sulphur (99.98%, Aldrich), tri-n-butylphosphine (TBP, 90%, TCI, Japan), tri-*n*-octylphosphine oxide (TOPO, 90%,Aldrich), octadecylamino (ODA, 90%, ACROS), 1-octadecene (ODE, 90%, ACROS), oleic acid (OA, 90%, Aldrich), Carboxyl-polystyrene (PS) microspheres (Tianjin BaseLine ChromTech Research Center); Bovine Serum Albumin (BSA), human IgG: purified total IgG from normal human serum, in which heavy chain is 50,000 Da, light chain is 25,000 Da. Goat anti-human IgG: pure human total IgG immune against goat until 1:100,000 (ELISA), above supplied by BEIJING DINGGUO BIOTECHNOLOGY CO. Ltd. 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC, GL Biochem (Shanghai) Ltd.), 3-aminopropyltriethoxysilane (APTES, Alfa Aesar), NP-40 (Fluka), TEOS, aqueous ammonia solution (25 wt.%), cyclohexane, glutaraldehyde, acetone, and argon (oxygen free) were obtained from local suppliers. All chemicals were used without further purification.

2.2. Synthesis of TOPO-capped bare/core CdSe QDs and shell/core QDs

Bare/core QDs were synthesised according to a previously reported protocols with minor modifications [32,33]. Briefly, 0.3 mmol of CdO, 0.4 mL of OA, 4.0 mL of ODE were loaded into a 50 mL flask. First, the mixture was heated to 300 °C under an Ar flow, and CdO was dissolved to generate a colorless homogeneous solution. Next, the solution was cooled to room temperature (RT), and then 2.50 g ODA and 0.50 g TOPO were added into the flask. Then the system was heated again to 280 °C under an Ar flow. After that, a selenium solution (1.8 mmol of Se powder dissolved in 2 mL of TBP) was injected quickly. Following the injection of selenium, nanocrystals were grown at 260 °C for different amounts of time depending on the desired sizes, and the solution underwent color changes from colorless, to green, to yellow and finally to red, which is the indication of QDs formation. Next, the solution was injected into chloroform. The TOPO-capped QDs were precipitated by adding dry ethanol, collected by centrifugation, washed with methanol several times, and vacuum dried for use. A typical synthesis of shell/core QDs was performed as follows: CdSe nanocrystals dissolved in 10 mL of hexane were mixed with 1.5 g of ODA and 5.0 g of ODE in a 25 mL three-neck flask. Then, the flask was switched to Ar flow to replace the air for 30 min, and then heated to 100 °C for another 5–10 min to remove hexane from the system. Subsequently, the reaction mixture was further heated to 240 °C for the injections. The procedures of Cd, Zn and S resource injections were according to Ref. [33]. The final product was diluted by hexane that was followed by a methanol extraction, or acetone precipitation of the nanocrystals. Excess aminos were further removed by dissolving the nanocrystals in chloroform and precipitating them with acetone. In this paper, seven layered shell/core QDs were prepared. The structure and compositions were CdSe/CdS/CdS/$Cd_{0.75}Zn_{0.25}S$/$Cd_{0.5}Zn_{0.5}S$/$Cd_{0.25}Zn_{0.75}S$/ZnS/ZnS (7Layers) with QY of 47.8%. For comparison, other shell/core QDs were prepared. And the shell structure and compositions were CdSe/CdS/CdS/CdS (QY, 43.5%) and CdSe/ZnS/ZnS/ZnS (QY, 50.8%).

2.3. Synthesis of MNPs

Fe_3O_4 MNPs were synthesised with minor modifications according to a previously published procedure [34]. Fe $(acac)_3$ (1 mmol), 1,2-hexadecanediol (6 mmol), oleic acid (4 mmol), oleylamino (3 mmol), and benzyl ether (12 mL) were mixed and magnetically stirred under a flow of nitrogen. The mixture was heated to 200 °C for 2 h and then heated to reflux (300 °C) for 1 h. The black-colored mixture was cooled to RT by removing the heat source. Under ambient conditions, ethanol (20 mL) was added to the mixture, and a black material was precipitated and separated via centrifugation.

2.4. Synthesis of FMNPs

FMNPs were synthesised according to a previously published procedure with minor modifications [31]. Typically, 10 mL of cyclohexane, 1.3 mL of NP-40, 200 μL of QDs (3.3×10^{-6} M) stock solution in chloroform, 100 μL of MNPs in chloroform (5 mg/mL), and 120 μL of TEOS were added into a flask under vigorous stirring. Thirty minutes after the microemulsion system was formed, 100 μL of ammonia aqueous solution (25 wt.%) was introduced to initiate the polymerization process. The silica growth was completed after 24 h of stirring at RT. Chemically-actived silica-coated nanoparticles were prepared by adding 20 μL APTES for the introduction of NH_2 group into above reaction system after 24 h polymerization. The resulting nanoparticles were isolated from the microemulsion using acetone and ethanol four times to remove any surfactant and unreacted molecules.

2.5. Covalent immobilization of antibody and antigen onto FMNPs surface and carboxylated PS microspheres respectively

The goat anti-human IgG antibody was directly immobilized onto the FMNPs with well-established glutaraldehyde method shown below. (1) Fluorescent FMNPs 10 mg was dispersed into the 1.0 mL phosphate buffered saline (PBS) buffer containing 5% glutaraldehyde for about 2 h at RT. (2) The nanoparticles were separated by centrifugation and washed with PBS three times. After the nanoparticles were redispersed in PBS, they were further incubated with IgG antibody (10.0 mg) for 3 h at RT with gentle shaking. (3) The antibody-labeled FMNPs were washed with PBS several times to remove excess IgG antibody and kept at 4 °C in PBS (0.01 M, pH 7.2, 0.5% BSA). Highly carboxylated PS microspheres were coated with human IgG (as positive control) and BSA (as negative control) via the covalent bonds between human IgG molecules and microspheres. The solutions of IgG or BSA (5.0 mg), EDC (10.0 mg) and microspheres (10.0 mg) were gently mixed in the PBS buffer for 2 h at RT using an orbital shaker followed by storage at 4 °C.

2.6. In vitro target-enrichment, magnetic separation and flow cytometric analysis with antibody-labeled FMNPs

The immunofluorescence assay for the positive and negative control experiments were both carried out in the same manner. The IgG-coated PS microspheres (2.0 mg/mL, 1.0 mL) were firstly blocked for 30 min at RT in the PBS-BSA buffer (0.01 M, pH7.2, 0.5% BSA), then gently mixed with anti-human IgG labeled FMNPs bioconjugates (20 nmol/L, 0.1 mL) using an orbital shaker at RT. After 30 min, external magnetic field was applied to enrich and following separate microsphere-FMNPs bioconjugates. Finally the captured microspheres were first examined under an OLYMPUS.BX51 fluorescence microscope equipped with an OLYMPUS MICRO DP 70 camera and a broad band light source (ultraviolet 330–385 nm). The illumination light was from an O-LH100HG 100 W mercury lamp with automatic exposure control. And then the positive and negative microspheres treated with anti-human IgG labeled FMNPs were analyzed on the flow cytometry.