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Silica coating of luminescent quantum dots prepared in aqueous media for cellular labeling

Yunfei Ma, Yan Li *, Xinhua Zhong *

Shanghai Key Laboratory of Functional Materials Chemistry, Institute of Applied Chemistry, East China University of Science and Technology, Shanghai 200237, China

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ABSTRACT

Silica coating is an effective approach for rendering luminescent quantum dots (QDs) with water dispersibility and biocompatibility. However, it is still challenging to prepare silica-coated QDs (QD@SiO_2) with high emission efficiency, small size and great stability in favor for bioapplication. Herein, we reported a modified Stöber method for silica coating of aqueously-prepared CdTe/CdS QDs. With the coexistence of Cd^{2+} and thioglycolic acid (TGA), a thin silica shell was formed around QDs by the hydrolysis of tetraethyl orthosilicate (TEOS). The resultant QD@SiO_2 with a small size (~5 nm in diameter) exhibits significantly higher emission efficiencies than that of the initial QDs. Also, QD@SiO_2 has extraordinary photo and colloidal stability (pH range of 5–13, 4.0 M NaCl solution). Protected by the silica shell, the cytotoxicity of QDs could be reduced. Moreover, the QD@SiO_2 conjugated with folic acid (FA) presents high specific binding toward receptor-positive HeLa cells over receptor-negative A549 cells.

1. Introduction

Due to the unique optoelectronic properties determined by the quantum size effect, colloidal quantum dots (QDs) have attracted increasing attention in both fundamental research and industrial applications in recent years [1–4]. In light of the high quantum yields, narrow emission, broad absorption spectra and surface modification properties, QDs become promising candidates in biosensors and biological imaging [5–10]. However, the photo and colloidal instability of QDs remains questionable under harsh environments to realize their applications in biosystems. It is therefore imperative to develop facile methods for the preparation of high-quality QDs with photo and colloidal stability, high photoluminescence (PL) emission efficiency and compatibility with biological manipulations. The effective handling of the above problems is to encapsulate QDs with biocompatible silica, which can not only protect QDs from deteriorating but also enable further functionalization on the surface of silica with diverse groups, thus enabling the coupling and labeling of biotargets with selectivity and specificity [11–14].

The preparation of silica-coated QDs (QD@SiO_2) may follow two approaches: reverse microemulsion method [15–24] and Stöber method [25–30]. The former method is performed in a water-in-oil (W/O) reverse microemulsion, in which the hydrolysis and condensation of silica precursors occur at the W/O interface or in the water phase. Usually each silica particle only contains a single QD in most resultant QD@SiO_2 nanoparticles by the microemulsion method, which is beneficial for maintaining its spectra purity. Nevertheless, the resultant QD@SiO_2 particles commonly with a size distribution of 30–150 nm are too large to the efficient subcellular labeling [23]. The Stöber method begins with the preparation of hydrophilic QDs, followed by the growth of the silica shell on the QDs in a mixture of ethanol and water. QDs can be encapsulated in the silica sphere with a board thickness from a few to hundreds of nanometers. However, the specific number of QDs in per silica sphere is difficult to be controlled [31]. What is more, QDs with high luminescent efficiency used in the Stöber method must be alcohol-dispersible, seriously restricting the application of this method. Compared with the original QDs, the QD@SiO_2 particles prepared by both methods always present a drastically reduced PL efficiency due to the coating of the silica shell [16–21,27–29].

Recent efforts are focusing mainly on retaining the luminescent efficiency of QDs after silica coating. Gao and co-workers incubated CdTe QDs in an alkaline solution prior to the reverse micro-emulsion process. This leads to the preservation of high fluorescent brightness of the initial CdTe QDs during the silica coating process [32]. Xu and co-workers prepared sandwich-like SiO_2@CdTe@SiO_2 nanoparticles 60–80 nm in diameter with high fluorescence QYs [33]. However, their application in cell labeling is limited by the

* Corresponding authors. Fax: +86 21 6425 0281.
E-mail addresses: yli@ecust.edu.cn (Y. Li), zhongxh@ecust.edu.cn (X. Zhong).

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large size of the sandwich structure. Beginning with the aqueously prepared QDs, Murase et al. successfully prepared small-sized QD@SiO_2 with the coexistence of Cd^{2+} and sulfur precursors. Interestingly, under refluxing condition the PL efficiency of QD@SiO_2 increases obviously, together with a significant red shift. The authors attributed these phenomena to the formation of CdS-like clusters in the vicinity of the QDs in the silica shell [34–36]. Although some promising results have been reported, still the current method for silica coating of QDs cannot satisfy the comprehensive requirements, such as high luminescence brightness, small size, good photo and colloidal stability, and low cytotoxicity, for practical applications.

Herein, we present a simple route based on the modified Stöber method for the preparation of silica-coated QDs with small size in aqueous solutions. As briefly outlined in Fig. 1, an aqueously synthesized CdTe/CdS QDs solution containing Cd^{2+} and thioglycolic acid (TGA) was directly employed to form the thin silica shell by the hydrolysis/condensation of tetraethyl orthosilicate (TEOS) in the water media. The resultant QD@SiO_2 exhibits great dispersibility in aqueous solution, along with a small size of ~5 nm in diameter. In addition, the PL efficiency was significantly increased from 36–54% to 45–81% after silica coating. It was found the prepared QD@SiO_2 presents extraordinary photo and colloidal stability. It was also demonstrated that the thin silica shell could efficiently eliminate the cytotoxicity and also provide the access of bioconjugation. The QDs@SiO_2 conjugated with folic acid (FA) as a versatile luminescent probe for evaluating the tumor targeting in cells was also examined.

2. Experimental

2.1. Chemicals

Tellurium powder (−200 mesh, 99.8%), cadmium chloride hemi (pentahydrate) ($CdCl_2 \cdot 2.5H_2O$, 99+%), thiourea (97%), 3-mercaptopropionic acid (MPA, 99%), sodium borohydride ($NaBH_4$, 99%), chromic acetate, thioglycolic acid (TGA), fluorescamine, aminopropyltriethoxysilane (APTES), folic acid (FA), 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoliumbromide (MTT), *N*-hydroxysuccinimide (NHS), and dicyclohexylcarbodiimide (DCC) were received from Aldrich. Tetraethylorthosilicate (TEOS), $NH_3 \cdot H_2O$ (25–28%) and dimethyl sulphoxide (DMSO) were ordered from Shanghai Lingfeng Chemical Reagent Co., Ltd. DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% fetal bovine serum, and sodium dodecyl sulfate (SDS) were purchased from Shanghai Futurebio Technologies Co., Ltd. Deionized (DI) water was used throughout.

2.2. Silica coating of CdTe/CdS QDs

The initial CdTe/CdS QDs were synthesized in aqueous media and the detailed procedure was available in the Supporting information (SI). Typically, the as-prepared CdTe/CdS colloidal solution was purified by acetone and re-dispersed in 5 mL of DI water in a 50 mL plastic centrifuge tube (absorbance is 0.4 at the first absorption peak position), followed by addition of 25 μL of Cd $(OAc)_2$ aqueous solution (0.8 mol/L) and 25 μL of TGA aqueous solution (1.0 mol/L) subsequently. The pH value was adjusted to 7.5 to obtain clear colloidal solution. Then 60 μL of ammonia and 60 μL of TEOS were added to the above solution with vigorous shaking for 12 h to form a thin silica layer containing Cd^{2+} and TGA around QDs. The obtained colloidal solution was treated by a rapid rotary evaporation at 40 °C for 10 min to facilitate the purification of obtained QD@SiO_2 by acetone. Finally, the QD@SiO_2 re-dispersed in 2 mL of DI water for further characterization.

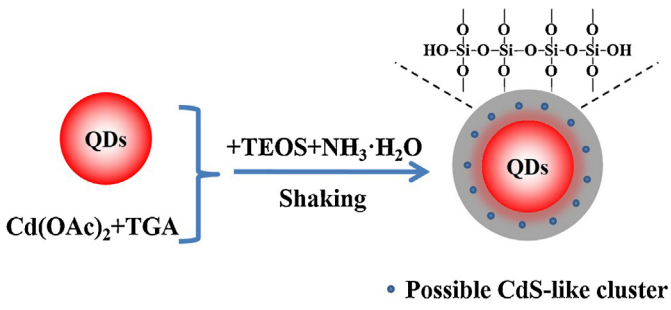

Fig. 1. Schematic illustration for the synthesis of silica-coated QDs.

2.3. Conjugation of amino-functionalized QD@SiO_2 with folic acid

Amino-functionalized QD@SiO_2 was prepared by using APTES as the silane coupling agent. Briefly, 2 mL of aqueous solution of as-synthesized QD@SiO_2 were dispersed in 20 mL of ethanol in a 50 mL plastic centrifuge tube. Then 50 μL of APTES solution (1:100 by volume in ethanol) was added under vigorous shaking for 2 h. The precipitate was obtained by centrifuge and washed with 2-propanol and acetone several times to remove the excess APTES. The resultant amino-functionalized QD@SiO_2 composite were dispersed in 5 mL of 0.01 mol/L PBS buffer. Then DCC chemical strategy was used to combine amino-functionalized QD@SiO_2 with FA. Typically, 25 μL of FA (0.05 M solution in DMSO) were mixed with the 5 mL PBS buffer of amino-functionalized QD@SiO_2 in a 50 mL plastic centrifuge tube. 25 μL of DCC (0.05 M solution in DMSO) and an equal amount of NHS (0.05 M solution in DMSO) were then added to the above solution. With shaking overnight, the QD@SiO_2–FA was obtained and re-dispersed in 2 mL of DI water after being purified using acetone.

2.4. Cytotoxicity assay by MTT method

Cytotoxicity was estimated by performing MTT assays in the HeLa cells. HeLa cells were seeded at 5×10^4 per cell into a 96-well cell culture plate in DMEM with 10% fetal bovine serum at 37 °C for 24 h under the humidified atmosphere (5% CO_2). The cells were then incubated with different concentrations of CdTe/CdS QDs and QD@SiO_2 for 24 h, respectively. Thereafter, MTT (10 μL, 5 mg/mL) was added to each well, and the plate was incubated for another 4 h at 37 °C. The HeLa cells were lysed with acidulated SDS, and their absorbance was measured at 570 nm by using an automatic ELISA analyzer. Each data point was collected by averaging that of six wells and the untreated cells were used as controls.

2.5. Cell labeling and fluorescent imaging

To study the uptake and imaging of the conjugated FA, HeLa and A549 cells were cultured on glass chamber slides in a 6-well plate with 2 mL of DMEM medium with 10% (v/v) calf serum at 37 °C (5% CO_2), respectively. After 24 h of incubation, the cells were carefully rinsed with PBS solution, into which 2 mL of the corresponding fresh media and 20 μL of QD@SiO_2–FA was added sequently. With the incubation for 2 h, the labeled cells were carefully rinsed with PBS to remove the unbonded QD@SiO_2–FA, and then incubated for another 10 min with fresh serum-free medium to preserve the cellular viability. Finally, HeLa and A549 cells incubated with QD@SiO_2–FA were directly imaged with a confocal microscope. The fluorescence images of the cells were obtained with an OLYMPUS ZX81 laser scanning microscopy with 488 nm argon laser excitation.