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The application of $CdTe@SiO_2$ particles in immunoassay

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ABSTRACT

$CdTe@SiO_2$ fluorescent particles were synthesized via hydrolysis and condensation of tetraethyl orthosilicate (TEOS) in water-in-oil (W/O) emulsion. Uniform luminophore-doped silica nanoparticles with 100 nm in diameter were obtained using microemulsion method and characterized by SEM. Antibody proteins were successfully conjugated to the fluorescent particles by the reaction of avidin and biotin, which were confirmed by fluorescence spectra. $CdTe@SiO_2$ fluorescent particles were potentially useful for the applications in biolabeling and imaging.

1. Introduction

Semiconductor nanoparticles made a significant impact on biotechnological and bioanalytical research [1–3], due to its advantages of desirable fluorescent properties, such as tunable emission spectra, high photostability, resistance to photobleaching, controllable surface characteristics, etc. [4–6].

The first milestone application of Quantum dots (QDs) as luminescence labels in bioimaging was reported by Alivisatos' group, and the multicolor labeling of fixed mouse 3T3 fibroblasts was demonstrated [7]. To be used in bioconjugation, a silica shell was intentionally coated onto the nanocrystals (NCs), followed by coupling of ligands to the silica. Biotinylated QDs with red photoluminescence selectively stained cytoskeletal filaments modified with streptavidin. Green-emitting QDs with trimethoxysilylpropyl urea and acetate groups showed high affinity to the cell nucleus. Constant excitation of bioactive QDs over 4 h with an Ar^+ laser resulted in constant emission with little decay. Almost at the same time, Chan and Nie prepared water soluble CdSe QDs by surface exchange of the organic ligands for mercaptoacetic acid, which could offer pendant carboxylic acid groups for further coupling [8]. CdSe QDs that were labeled with the protein transferrin underwent receptor-mediated endocytosis in cultured HeLa cells, and those QDs that were labeled with immunomolecules recognized specific antibodies or antigens. These two papers reported in 1998 laid the foundation of QDs as biolabels and opened the door to biological applications of semiconductor nanoparticles (NPs).

Quantum dots, however, have characteristics that limit their effectiveness for such applications. These limitations include particle growth, photoinduced decomposition, and conjugate aggregation [7]. Especially, they are rapidly photobleached in the presence of oxygen. It is a major problem in their use of bioanalytical application, such as biosensor and real-time imaging applications. Amorphous silica shells show some prime advantages necessary for bioconjugation of QDs. The silica shell prevents flocculation of particles and species from adsorbing onto the surface, and help to maintain the photoluminescence. In previous work [9,10], the development of organic-dye-doped silica NPs have been reported, but the fluorescence intensity of organic-dye-doped silica NPs was limited due to the relative low quantum yield of organic dyes, these NPs were unsuitable for bioanalysis because of their high hydrophobicity and poor dispersibility in water [11,12].

In order to solve this problem, coating inorganic nanoparticles with silica has been widely investigated. Using the well-known StÖber method, Liz-Marzán and his co-workers succeeded in coating aqueous CdS NCs stabilized by sodium citrate and obtained $CdS@SiO_2$ particles with core-shell structure [13]. Nann and Mulvaney further demonstrated that the silica coating on organic-soluble NCs by the StÖber process could also lead to well-defined structures [14]. Under optimized conditions, they successfully obtained silica spheres with 30–120 nm in diameter and single-quantum-dot cores. Murase and his co-workers reported the preparation of fluorescent CdTe-silica particles by the reverse microemulsion method. It emerged that the CdTe NCs were only

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situated on the surface of the resultant silica particles [15]. Kirchner et al. reported high cell viability of various cell lines including MDA-MB-435S breast cancer and NRK cell lines when ingested and adsorbed with silica-coated CdSe/ZnS nanoparticles, silica coated QDs showed there were no cytotoxic effects in the cell lines investigated with concentrations as high as 30 μM [16].

In this work, we developed a method for producing "biofriendly," spherical, stable, easily preserved, and monodisperse silica nanoparticles. Silica was used as a matrix in this work because it has been one of the most commonly used matrices for making nanoparticles for in vivo applications, and it has several superior properties. Silicate glass is a porous, high-purity, optically transparent and homogeneous material [17], so these make it an ideal choice as a matrix for the encapsulation of optically active reagents. Also, the preparation of silica particles is technically simple, and tailoring their physicochemical properties, i.e., pore size or innersurface hydrophobicity, could be achieved easily by varying the processing conditions and the concentration or type of reactants used [18]. Furthermore, the silica matrix made it possible to retain to a much larger extent compared to organic polymers, the specificity and reactivity of biologic molecules (i.e., proteins) in the solid state and provides morphologic and structural control, but that is not available when the biologic molecules are simply dissolved in aqueous media [19].

As it is well known that CdTe nanoparticles lose their highly fluorescent properties in the presence of common buffers such as phosphate-buffered saline (PBS), the growth of a silica shell has been found to significantly enhance the photoluminescence stability of CdTe QDs in buffer solution, provided a versatile platform for decoration with any number of functional groups and for successful conjugation to IgG proteins, which confirmed both bioactivity and binding specificity. Also it is common knowledge that Cd is highly toxic, and this could be a major concern for its vivo applications. The surface oxidation of the core QDs can be reduced by using silica based shell layers, which could create a barrier for oxygen diffusion. Remarkably, Kirchner et al. [16] reported that silanized CdSe did not elicit genes related to exposure to heavy metals. Silanization of various metal and semiconductor nanoparticle systems have shown great success in protecting their surface characteristics. This indicates that silica is an outstanding biocompatible protective layer and may hold the best promise for non-cytotoxic bioimaging and site-directed surgeries.

To further expand the usefulness of silica nanoparticles, a variety of surface modification and immobilization procedures are utilized and can be developed to couple the nanoparticles to various biomolecule targets. For example, oligonucleotides, enzymes, antibodies, and other proteins have been used for bioanalytical detection after the nanoparticles are linked to probe biomolecules. The silica surface can be modified to contain avidin, sulfide, amine, or carboxylate groups [20]. Avidin can be passively adsorbed on the silica surface followed by glutaraldehyde cross-linking. The resulting avidin-coated nanoparticles can then be conjugated with biotinylated molecules based on the strong avidin–biotin affinity. The capacity of silica to bind with avidin will allow the use of the nanoparticles to assays requiring commonly used and widely available biotinylated compounds.

In this paper, the CdTe@SiO_2 fluorescent particles were synthesized via hydrolysis and condensation of tetraethyl orthosilicate (TEOS) in water-in-oil (W/O) emulsions. CdTe nanocrystals were stabilized by 3-mercaptopropyl acid (MPA). Under optimized conditions, highly fluorescent SiO_2 spherical particles with CdTe nanocrystal cores were obtained, and terminated with an outermost layer of avidin. The optical investigation showed that the avidin-bound CdTe–silica particles retain bioactivity and had specific biorecognition ability, a fluoroimmunoassay employing bioconjugation of antibody and antigens was carried out in this experiment. The avidin-bound CdTe-silica particles were treated in biotinylated goat-anti-rabbit IgG solution to form new outermost layer through the strong affinity between avidin and biotin, the antibody (biotinylated goat-anti-rabbit IgG) could combine to biospecific antigen (FITC-rabbit-anti-goat IgG) to form a typical immunoreaction on the surface of CdTe-silica particles. The inclusion of nanoparticles into the silica matrix was demonstrated and discussed briefly. Also given were specific protein-binding studies that relate to the biocompatibility of the silica nanoparticles.

2. Materials and methods

2.1. Materials and reagents

All chemicals used in this experiment were of analytical reagent grade without further purification. Triton X-100, 3-mercaptopropyl acid (99+%), tellurium powder (~200 mesh, 99.8%), $CdCl_2$ (99+%), $NaBH_4$ (99%) were purchased from Aldrich Chemical Co. The following biochemicals were used: avidin (10 mg, Rockland), biotinylated goat-anti-rabbit IgG (1.0 mg/mL, Beijing Dingguo Biotechnology Ltd. China), FITC-rabbit-anti-goat IgG (1.5 mg/mL, Beijing Dingguo Biotechnology Ltd. China); BSA (5 g, Beijing Dingguo Biotechnology Ltd. China). BSA and avidin powder were dissolved in a 2 mmol/L phosphate buffered saline solution (PBS, pH 7.4) to obtain 1 mg/mL solution and all the solutions were stored at 0–4 °C, diluted only prior to immediate use. Water used throughout was doubly distilled water (>18 MΩ cm).

2.2. Instrument and spectrometry

Fluorescence spectra were recorded by a Shimadzu RF-5301 PC spectrofluorophotometer. UV–vis absorption spectra were obtained using a Varian GBC Cintra 10e UV–vis spectrometer. In both experiments, a 1-cm path-length quartz cuvette was used to measure the absorption or fluorescence spectrum. All pH values were measured with a PHS-3C pH meter (Hangzhou, China). A bath ultrasonic cleaner (Autoscience AS 3120, Tianjin, China) was used to disperse the microspheres. All optical measurements were carried out at room temperature under ambient conditions.

2.3. Synthesis of aqueous-compatible CdTe quantum dots

Stable water-compatible CdTe quantum dots were prepared by derivatizing nanocrystal surfaces with MPA as described in previous papers [21,22]. In brief, freshly prepared oxygen-free NaHTe solution was added to nitrogen-saturated 1.25×10^{-3} M $CdCl_2$ aqueous solution at pH 11.4 in the presence of MPA as a stabilizing agent. NaHTe was produced in an aqueous solution by reaction of $NaBH_4$ with tellurium powder at a molar ratio of 2:1. The molar ratio of Cd^{2+}:MPA:HTe^- was fixed at 1:2.4:0.5. The resulting mixture was then subjected to refluxing to control the size of the CdTe nanocrystals. Finally, QDs with different sizes were synthesized under different refluxing conditions respectively. The final clean and stable aqueous QDs were well-dispersed in basic solutions (pH > 6), and the luminescence quantum yield of 25% was obtained for the CdTe nanoparticles at room temperature compared with the fluorescent emission of Rhodaminutese 6G [23]. Stable water-compatible MPA-capped CdTe QDs with emission maximum at 610 nm were used in this study.

2.4. Preparation of biofunctional CdTe@SiO_2 particles

CdTe@SiO_2 particles were prepared as follows: typically, 7.5 mL cyclohexane, 1.77 mL Triton X-100, 1.8 mL *n*-hexanol, 48 μL aque-