Nanostructures for Biolabeling

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Carboxylic Acid Enriched Nanospheres of Semiconductor Nanorods for Cell Imaging**

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During the past decades, nanomaterials such as noble-metal,[1,2] lanthanide-doped,[3–6] magnetic,[7,8] luminescent SiO_2,[9,10] and semiconductor[11–13] nanomaterials have been extensively investigated and used in the development of sensitive and selective detection and imaging in biological and medical fields. Owing to their novel optical properties, semiconductor nanocrystals are of great interest to many fields such as field-effect transistors,[14] waveguides,[15] nano-lasers,[16,17] photovoltaics,[18] electronics,[19,20] and biolabels.[11–13,21] Specific applications, for example, biolabels, require these nanocrystals to be not only highly luminescent but also water-soluble.[11–13,21] Up to now, lots of facile methods have been developed for the preparation of semiconductor nanoparticles and nanorods;[22–27] however, most of them are mainly based on the decomposition of organometallic precursors in hydrophobic solvents.[24,26–28] Also, these as-prepared nanocrystals are hydrophobic, which is inconvenient for bioapplications. It is still a challenge to synthesize monodisperse semiconductor nanorods by using common inorganic salts such as nitrates as precursors. Furthermore, the surface modification of hydrophobic nanocrystals is another challenge.

In this work, we have developed a facile strategy for the preparation of CdS and ZnS:Mn nanorods with nitrates as precursors. By simply modifying the solvothermal conditions, the sizes of the as-prepared nanorods were desirably tuned. Then, the hydrophobic short nanorods were encapsulated to form amphiphilic polymer coated nanospheres with a facile ultrasonication strategy. With an abundance of carboxylic acid groups in the polymer coating on the outer face, these colloidal nanospheres are water-soluble and bioconjugatable. These luminescent nanospheres were also used as biolabels in cell fluorescence imaging, as reported herein.

The synthesis was conducted in oleylamine with nitrates as precursors. At 160 °C, colloidal CdS and ZnS nanorods were grown in an oleylamine solution of nitrates (1 mmol) and thioacetamide (1.2 mmol). Figure 1 shows the TEM images of the semiconductor nanorods prepared in oleylamine by solvothermal processing for 12 h (or 24 h) at 160 °C.

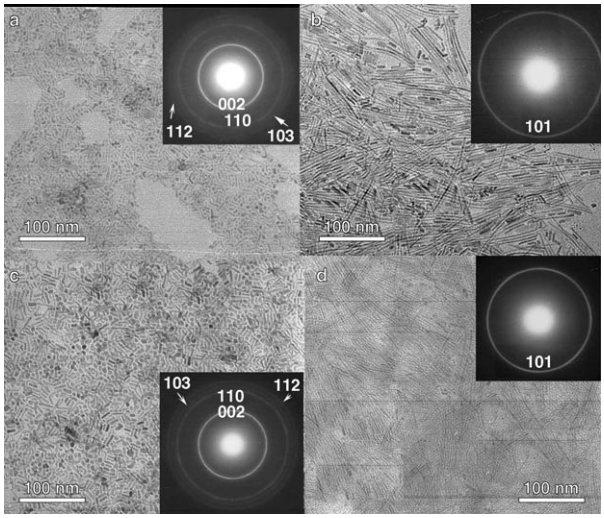

Figure 1. TEM images of the CdS (a,c) and ZnS (b,d) nanorods prepared in oleylamine; heated during synthesis at 160 °C for 12 h (a,b) or 24 h (c,d; see the Experimental Section). Electron diffraction patterns are shown in the insets.

It was found from the TEM observations that most of the CdS nanorods possessed a length of less than about 25 nm when the reaction time was 12 h (Figure 1 a). However, by prolonging the reaction time to 24 h (Figure 1 c), the length of the nanorods increased to about 40 nm. Under the same conditions, ZnS preferentially forms long nanorods. When the solvothermal time is 12 h (Figure 1 b) and 24 h (Figure 1 d), the respective lengths of the ZnS:Mn nanorods are about 110 nm and 180 nm. Although the nanorods grew longer with the increase in reaction time, its average diameter had no obvious change and was less than about 7 nm. Electron diffraction patterns, taken from large-area nanorods, are shown in the insets of Figure 1. The electron diffraction patterns of the CdS nanorods (Figure 1 a,c) can be indexed to hexagonal wurtzite CdS (JCPDS, 41-1049), and the electron diffraction patterns of ZnS nanorods (Figure 1 b,d) can be indexed to hexagonal wurtzite ZnS (JCPDS, 36-1450). As shown in Figure S1 of the Supporting Information, the XRD patterns of the nanocrystals further indicated their wurtzite structures. The position and relative intensities of all diffraction peaks matched well with those from the JCPDS card for CdS and ZnS, respectively. The as-prepared nanorods were further characterized with high-resolution TEM (HRTEM)

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images (Supporting Information). The results indicate that the nanorods are single-crystal hexagonal structures and grow along the (001) direction as depicted with the arrow in Figure S2 of the Supporting Information.

As far as we know, the hexagonal structure is preferable to epitaxial growth along the *c* axis, and the formation of nanorods can be induced by maintaining high chemical potential.[24,27] In a previous report on the formation mechanism of CdSe nanorods, Peng et al.[27] reported that excess cadmium precursor was essential to maintain high chemical potential for the preferential formation of nanorods. Interestingly, in the current case, excess thioacetamide resulted in CdS and ZnS nanorods. Otherwise, nanoparticles instead of nanorods are obtained (Supporting Information). These results imply that the less reactive precursor should be used in excess to maintain high chemical potential and, consequently, to obtain nanorods. In our work, because thioacetamide is less reactive than Cd^{2+} and Zn^{2+}, excess thioacetamide was essential to maintain high chemical potential. Besides the precursor, the appropriate choice of surfactants was also very important to the successful synthesis of nanorods.[28] In this work, when oleic acid was chosen in the synthesis process, neither CdS nor ZnS nanorods were generated (Supporting Information), probably because the carboxylic acid group binds too strongly to the cadmium and zinc ions and inhibits the epitaxial growth. The alkylamine is an intermediate-binding surfactant and has been identified as the appropriate choice for the preparation of CdS and ZnS nanorods.

The as-prepared nanorods not only have uniform shape and size, but also possess multicolor optical properties. As shown in Figure 2, under excitation with 365-nm light, the CdS nanocrystals emit strong blue fluorescence, and the most

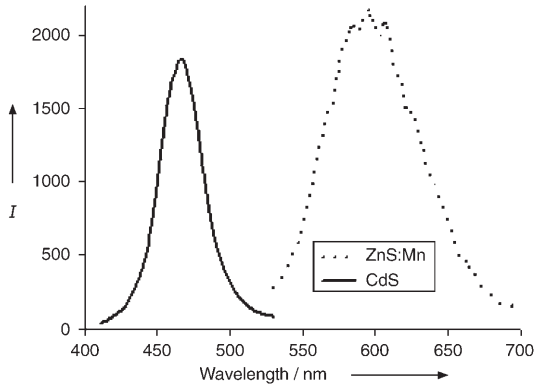

Figure 2. Fluorescence spectra of the as-prepared nanocrystals.

intense blue emission line is located at 470 nm. However, if we prolong the reaction time and increase the temperature, the obtained CdS nanocrystals emit strong yellow fluorescence for the increased particle sizes (see the luminescence photo in Figure 4). However, under the same excitation, the 5% Mn^{2+}-doped ZnS nanocrystals show strong red emission centered at 591 nm.

Although these nanocrystals are highly luminescent, they are hydrophobic, which greatly inhibits their application in biological and medical fields. To use the nanocrystals as luminescent biolabels, they should be transferred into aqueous solution. Recently, we have reported a facile strategy to fabricate hydrophilic colloid nanospheres with the hydrophobic nanocrystals as precursors.[29] Although this method is a general strategy for the preparation of hydrophilic nanospheres and can be used for various kinds of nanocrystals, the as-prepared nanospheres can not be used as biolabels because their surface has no bioconjugatable groups such as amine and carboxylic acid groups. The encapsulation procedure reported here is a modified version of the one used before.[29] In this work, we use an amphiphilic polymer, that is, a copolymer of styrene and methacrylic acid, to replace the sodium dodecylsulfate (SDS) surfactant used in the reported method. The carboxylic acid enriched nanospheres were successfully prepared and characterized by TEM. As shown in Figure 3, with the reported method,[29] the short CdS nanorods have been assembled into 100-nm nanospheres (Figure 3a). A

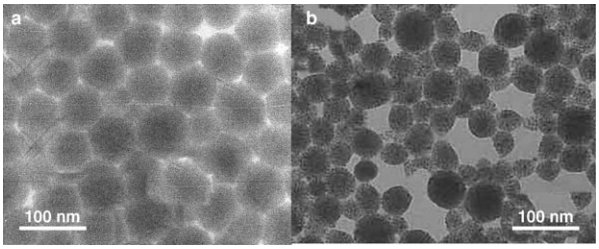

Figure 3. TEM images of the CdS colloid nanospheres prepared with the reported (a) and the modified (b) method; see the text for details.

TEM image of the polymer-coated nanospheres prepared, using the modified encapsulation strategy, is also shown in Figure 3b. And the average size of the polymer-coated nanospheres is less than 100 nm.

With this novel polymer-coating strategy, different luminescent nanocrystals were encapsulated into hydrophilic nanospheres. With an abundance of carboxyl groups on the surface, these as-synthesized nanospheres present outstanding stability in aqueous solution. The multicolor luminescence of the nanomaterials before and after encapsulation is demonstrated in Figure 4. From these photos, it can be seen that the CdS nanocrystals can emit fluorescence of different colors with different particle sizes. These nanocrystals are dispersible in hexane before encapsulation; however, they can be stably dispersed into water and maintain good optical properties after encapsulation.

For the coated polymer, these nanospheres can be easily dispersed in aqueous solution and remain stable for at least three months, which is preferable for bioapplications. To identify the existence of the polymer, these nanospheres were characterized by FTIR analysis. Figure S4 in the Supporting Information exhibits the FTIR spectrum of the polymer-encapsulated nanospheres (Figure S4a) and the nanocrystals prepared in oleylamine (Figure S4b).

The carboxylic acid groups on the nanospheres facilitate the conjugation of biomolecules to the nanospheres and, consequently, their bioapplication as luminescent labels. To investigate their ability as biolabels, the carboxylic acid enriched CdS nanospheres were applied in cell fluorescence

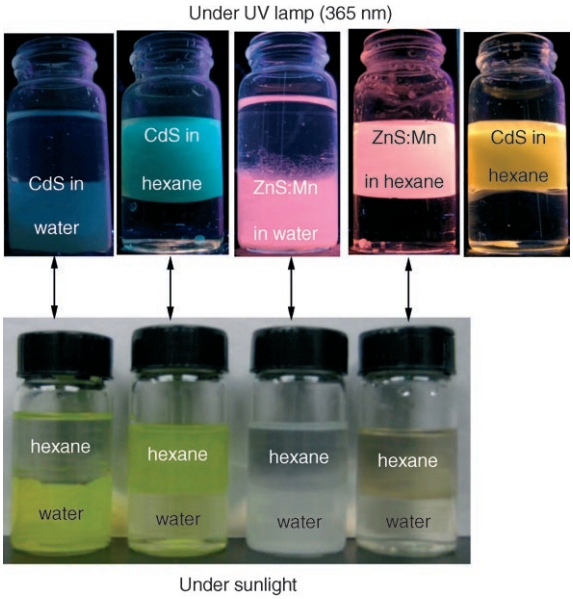

Figure 4. Luminescence photos of the hydrophobic nanocrystals (dispersed in hexane) and the hydrophilic polymer-encapsulated nanospheres (dispersed in water). The upper-right photograph shows dispersed CdS nanocrystals that were synthesized with a longer reaction time (24 h) and at a higher temperature (180 °C) and thus exhibit yellow luminescence instead of blue.

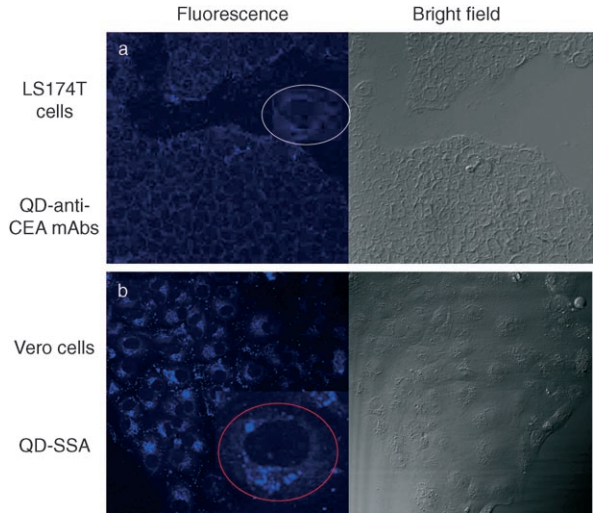

Figure 5. Fluorescence microscopy image of LS174T cancer cells (a) and Vero cells (b) labeled with polymer-encapsulated CdS nanospheres. Interaction mode: a) specific protein interaction between antigens on the cell and antibodies on the nanospheres; b) endocytosis of SSA-coated nanospheres. Areas marked in the images with ovals are higher magnifications of single cells. QD = quantum dot.

imaging. We demonstrate their potential as markers for biological imaging by labeling the carcinoembryonic antigen (CEA) of LS174T cancer cells. Anti-CEA monoclonal antibodies (mAbs) and their cell surface antigens are known to form a tight complex. Anti-CEA mAbs were covalently bound to the surface of the CdS nanospheres. Figure 5 a shows typical labeling of LS174T cancer cells with antibody-modified CdS nanospheres. One can see that the blue fluorescence of the CdS nanospheres is present on the surface of the cells. Figure 5 b presents an example of a fluorescent image of Vero cells labeled with CdS nanospheres under a fluorescent microscope. As shown in Figure 5 b, the sheep serum albumin coated (SSA-coated) CdS nanospheres are endocytosed by the Vero cells. The detailed cell labeling and fluorescence imaging of ZnS:Mn nanospheres are currently in progress. These preliminary results indicate that the as-prepared semiconductor nanospheres can be used as biolabels for cell fluorescence imaging.

Herein, with a facile solvothermal strategy, semiconductor nanorods were easily prepared with common nitrates as precursors. By controlling the amount of thioacetamide and choosing an appropriate surfactant, both monodisperse nanoparticles and nanorods were prepared. The as-prepared hydrophobic nanocrystals were also encapsulated with an amphiphilic polymer to form hydrophilic nanospheres. Then, the carboxylic acid enriched CdS nanospheres were conjugated with biological molecules and applied in cell imaging as fluorescent biolabels. The results indicate that this method is a facile and successful technology for the preparation of CdS and ZnS nanorods and may be extended to other chalcogenides. Cell imaging studies indicate that the reported polymer encapsulation method is a novel strategy for the preparation of bioconjugatable nanolabels from hydrophobic nanocrystals.

Experimental Section

Preparation of nanorods: $Cd(NO_3)_2$ (1.0 mmol) or $Zn(NO_3)_2$ (0.95 mmol) with $Mn(NO_3)_2$ (0.05 mmol) were added to oleylamine (25 mL) and vortexed for 10 min. Then, thioacetamide (1.2 mmol) was added, and the mixture was stirred for another 15 min. The mixture was then transferred into a 50-mL teflon-lined autoclave and heated at 160 °C for 12 h or 24 h. The final product was collected by centrifuging and washing of the powder with ethanol and hexane.

Preparation of carboxylic acid enriched nanospheres: The nanocrystal solution (10 mg mL^{-1}) and polymer solution (50 mg mL^{-1}) were prepared by dissolving the respective precursors into chloroform. Then the nanocrystal solution (800 μL) and polymer solution (200 μL) were mixed and injected into deionized water (10 mL) under ultrasonication and magnetic stirring, which were maintained for 5 min. The polymer-coated nanospheres were obtained after removing the chloroform by heating the solution at 70 °C for 2 h.

Bioconjugation and cell imaging: For LS174T cancer cell labeling, the monoclonal anti-CEA antibody was covalently conjugated to the CdS nanosphere. 1-Ethyl-3-(3-dimethly-aminopropyl) carbodiimide (EDC, 50 μL, 50 μg mL^{-1}), *N*-hydroxysuccinimide (NHS, 50 μL, 50 μg mL^{-1}), and deionized water (900 μL) were added into a vial and vortexed. Then the CdS nanospheres (ca. 1.5 mg) were dissolved into the mixing solution and incubated at room temperature for 30 min. The nanospheres were collected by centrifugation and washed twice with deionized water. The functionalized nanospheres were added into the mixture solution of monoclonal anti-CEA antibody (100 μL, 1 mg mL^{-1}) and NaOAc buffer solution (900 μL, pH 6.0, 0.2 mol L^{-1}) and vortexed, and the mixture was incubated at 4 °C for 2 h. The antibody-labeled CdS nanospheres were centrifuged and washed twice with phosphate buffer solution (PBS, pH 7.0) and incubated in Tris solution (pH 7.5, 50 mmol L^{-1}) at room temperature for 30 min. Then, the antibody-labeled CdS nanospheres were purified and dispersed into a PBS solution (1 mL). For the cell

labeling, the antibody-labeled nanospheres were incubated with the LS174T cancer cells at 37 °C for 1 h and purified. The photographs were taken with an Olympus FV1000 and clearly showed the typical blue fluorescence of the CdS nanocrystals.

Prior to conducting the endocytosis tests with Vero cells, the polymer-encapsulated CdS nanospheres were coated with sheep serum albumin (SSA) by suspending the CdS nanospheres (ca. 5 mg) in 5% SSA solution at room temperature for 20 min. After the incubation, the SSA-coated CdS nanospheres were diluted in DMEM (Dulbecco's Modified Eagle Medium, 1.8 mL) to a concentration of 0.25 mg mL^{-1}. To examine cellular uptake of the quantum dot nanospheres, Vero cells were incubated with the nanosphere–SSA complexes at 37 °C for 2 h. After removal of the culture medium, the cells were washed twice with PBS and incubated in DMEM culture for 4 h.

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