Synthesis and Cytotoxicity of Luminescent InP Quantum Dots

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ABSTRACT

As a potential biological imaging probe with a long-wavelength of emission, InP quantum dots were prepared via a high-temperature organic solution approach, and successfully transferred into an aqueous system through a ligand-exchange process using various functional surfactants. Photoluminescence and X-ray characterizations confirmed the desired properties of the InP quantum dots. The cytotoxicity of the water-soluble InP quantum dots against phaeochromocytoma PC12 cells as evaluated by the MTS cell viability assay was low relative to a positive control, poly(ethyleneimine). This study suggests a bright potential for this new type of InP quantum dots in bioimaging applications.

INTRODUCTION

Semiconductor quantum dots (QDs) attract great attention due to their unique size-dependent optical properties and many possible applications such as LED and lasers [1-5]. Recently, QDs are increasingly being used as a new class of luminescence probes for biological detection and labeling. For in vivo imaging applications, the QDs should ideally emit in the long wavelength region to minimize background interference. However, the frequently used II-VI CdSe and CdTe QDs often exhibit emission properties too close to the optimal biological window of transmission. In addition, toxicity derived from Cd diminishes the potential of these series of QDs in biological applications.

As a result, non-cadmium-based QDs have attracted interest from many research groups in recent years. Among III-V semiconductor QDs, InP has become the most extensively studied system [6-10] due to its relative ease of synthesis and appropriate emission region, offering comparable or even better optical properties than CdSe QDs. Moreover, bulk InP generally exhibits low toxicity, and the structural robustness of InP can also confer optical stability [11, 12]. However, toxicity information of InP QDs has been lacking. Another issue is the unavailability of aqueous solution-based synthesis of InP QDs. The latter would require the development of a robust processing by which the high emission efficiency, narrow size-
distribution and long-wavelength emission InP QDs [13] prepared from a high-temperature organic solution be successfully transferred into an aqueous system.

By adopting an established synthetic strategy [13], we prepared high-quality InP QDs from a high-temperature organic solution system, and successfully transferred these QDs into aqueous phase through a ligand-exchange process using various functional surfactants including Trichloro-s-triazine modified mPEG. Subsequently, we evaluated the cytotoxicity of these water-soluble InP QDs against phaeochromocytoma PC12 cells by the MTS cell viability assay. The promising results justify further development of these advanced structured III-V QDs for bioimaging applications.

EXPERIMENTS

Synthesis steps of InP QDs

In a typical synthesis of InP QDs, 0.20 mmol of indium acetate (In(Ac)₃) was mixed with 0.60 mmol of myristic acid (MA) in a three-neck flask equipped with a condenser and attached to a Schlenk line under an argon stream. 8.0 mL of 1-octadecene (ODE) was then injected to the mixture. The system was heated to 120 °C and vacuumed for 1 hour using a mechanical pump, resulting in a clear solution. After the mixture was further purged with argon three times, the temperature was raised to 300 °C with vigorous stirring under an argon flow. 1.0 mL of pre-prepared tris(trimethylsilyl)phosphine ((TMS)₃P) solution (0.1 M in ODE) was subsequently injected into the hot solution, causing a temperature dropping-down to 280 °C. Before the reaction was ceased by adding room-temperature ethanol into the flask, the temperature was kept at 280 °C for 60 min to allow a sufficient growth of the resultant InP QDs. The as-prepared InP QDs were collected by centrifugation and re-dispersed into an organic solvent such as chloroform for further use. No size selection post-treatment was conducted.

Conversion of InP QDs into aqueous phase

Myristic acid as a surface-capping agent is believed to bond on the as-prepared InP QDs, resulting in a high solubility of these colloids in a non-polar organic solvent. The conversion of InP QDs from hexane to water was achieved through a ligand-exchange process with trichloro-s-triazine (TsT) modified monomethoxy-poly(ethylene glycol) (mPEG) [14]. Briefly, 22 mg of TsT was mixed with 1.0 g of anhydrous sodium carbonate in 20 ml anhydrous benzene. 200 mg of mPEG 2000 was then introduced into the solution with vigorous stirring, and kept overnight at room temperature to allow a complete reaction. The TsT-modified mPEG (TsT-mPEG) was eventually precipitated out by adding 30 mL of petroleum ether and collected by centrifugation for further use. In a typical procedure, 6.0 mg of dopamine hydrochloride was dissolved in 2.0 mL of 1,4-dioxane in the presence of sodium carbonate (30 mg). 60 mg of as-prepared TsT-mPEG in 2.0 mL 1,4-dioxane was subsequently added dropwise within 5 minutes into the system with stirring at room temperature, and the reaction was then kept for additional 3 hours. The product was precipitated by adding petroleum ether and separated from the solution by centrifugation. The product was re-dissolved in chloroform and mixed with 1.0 mL of...
concentrated InP QD suspensions (~1.2 mg/mL). The mixture was vigorously stirred for overnight under argon atmosphere at room temperature. The resultant TsT-mPEG-Dopamine-coated InP QDs was then precipitated by centrifugation, washed with hexane, and dispersed in a deionized water or buffer (PBS).

**Cytotoxicity test of InP QDs**

Phaeochromocytoma PC12 cells were maintained in 75 cm² cell culture flasks coated with Bovine Collagen I in ATCC-formulated F-12K medium containing 2.5 % fetal bovine serum, 15 % horse serum, 100 U/mL penicillin and 100 g/mL streptomycin (complete growth medium). The cells were maintained at 37 °C, 5 % CO₂ and passaged at 70-80% confluency every 2 days. Cellular viability was determined by MTS, which measures the reduction of (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) to formazan by viable cells [15]. Briefly, 5 x 10⁴ cells were plated onto collagen coated 96 well plates. After incubation with the indicated dose of quantum dots for 48 h, the amount of formazan product was measured at 490nm. Included in the study was poly(ethyleneimine) (PEI), a polymeric gene carrier as a positive control and commercial quantum dots, QD605-NH₂ (Invitrogen). The mean absorbance of cells with no QD dosing was the reference value for calculating 100% cellular viability.

**Characterizations of InP QDs**

Photoluminescence spectra of all the InP samples were recorded on a VARIN Cary Eclipse fluorescence spectrophotometer at room temperature. UV/Vis absorption spectra of the samples were recorded using a VARIN Cary 50-Bio UV/Vis spectrophotometer. Hitachi 7000 and JEOL-2010 transmission electron microscope (TEM) were used for sample imaging in regular and high resolution, respectively. An X-ray diffractometer (PANalytical X’Pert system) equipped with CuKα radiation source (λ=0.15406 nm) was employed for determining the microstructure of these QDs.

**RESULTS AND DISCUSSION**

The as-prepared InP QDs (from organic solution) were characterized by both X-ray and TEM. Figure 1(a) depicts an X-ray diffraction (XRD) pattern of a typical sample grown at 290°C for 1 hour. All the detectable diffraction peaks in Figure 1(a) can be indexed to those of the cubic zinc blend structured InP (ICDD PDF card No. 73-1983). The broad nature of these peaks indicates an extremely small size of the nanoparticles. Figure 1(b) is a TEM micrograph of monodispersed InP QDs. The average size of dot-shaped particles is about (4.5 ± 0.5 nm) based on statistical sampling of this image. Inset in Figure 1(b) is a high-resolution TEM image, showing lattice structures of the individual InP QDs. These results clearly demonstrate that the InP QDs possess high crystallinity with a relatively narrow size-distribution.
The as-prepared InP QDs exhibit considerably good photoluminescence (PL) property. The emission peak can be easily tuned from 580 to 625 nm by varying the concentration of MA when an excitation wavelength of 366 nm was used. At emission peaks 580 and 625 nm as shown in Figure 2(a) and 2(b), the full widths at half maximum (FWHM) of the spectra are about 60 nm and 90 nm, respectively. The wider FWHM at 625 nm indicates that the size-distribution of larger InP QDs (which correspond to a long-wavelength of emission) is relatively broader. Using a standard method[16], the PL quantum yields for both the small sized (emission of 580 nm, using quinine sulfate as the standard dye) and large sized (emission of 625 nm, using rhodamine 101 as the standard dye) samples were determined as 3.2% and 5.1%, respectively. We realize that the PL efficiency could be greatly improved by coating ZnS over the InP QDs. Further investigation towards core/shell InP/ZnS study is in progress.

Figure 1. a) XRD spectrum and b) TEM image of an InP QD sample which possesses an emission at 625 nm (refer to Figure 2). Inset in b) is a high-resolution image of InP QDs.
As a first step towards evaluating the biological properties of the InP QDs, we determined their cytotoxicity against PC-12 cells. PEI, a widely used polymeric transfection agent, and QD605-NH₂ were used as controls. As shown in Figure 3, cells treated with either the as-synthesized InP QDs or the commercial QD605-NH₂ exhibited minimal decline in cell viability after exposure for 48h. The QD doses tested would be sufficient for many bioimaging applications. This low toxicity of the as-synthesized InP QDs is speculated to be a result of the structural stability of InP QD, which inhibits the release of indium ions. As such, these InP QDs could be highly promising probes for biological applications.

Figure 3. Viability of PC-12 cells after a treatment with different concentrations of InP QDs at 37°C in the humidified atmosphere with 5% CO₂ for 48 hour. PEI was included in this study as known positive control.
CONCLUSIONS

We have successfully prepared water-dispersible InP QDs with tunable and considerably long wavelength of photoluminescence emission through a two-step facile approach. No prolonged heating time and multi-injection operation were needed in the InP QD synthesis. The InP QDs could be readily transferred to deionized water through a ligand-exchange treatment. The cytotoxicity of InP QDs was examined by the cell viability (MTS) assay on PC-12 cells. With a relatively low cytotoxicity and reasonable optical properties, the results suggest that these InP QDs could be promising “core” materials for bioimaging application.

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REFERENCES