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Preparation and Characterization of Various Surface-modified Semiconductor Nanocrystals

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Fluorescent semiconductor quantum dots (QDs) are a novel class of fluorescent probe whose unique optical properties suggest that they are superior to conventional organic dyes for many biological applications. Semiconducting quantum dot have been shown to possess several photophysical properties that are superior to those of organic fluorophores: high-absorption cross sections, excellent photostability, broad excitation spectra, narrow symmetric, materials- and size-dependent emission spectra, and narrow emission spectra.[1] Because of these characteristics, QDs are an interesting alternative to organic fluorophores in some biotechnological and biomedical applications.[2] These attractive fluorescence properties are particularly appealing for the visualization of cellular processes, as they can potentially facilitate long-term and multicolor labeling of both fixed and living cells.[3]

Recently, many research groups in the world have researched QDs including II-VI or III-V compounds[4] and have focused upon generating high quantum efficiency through changes of the raw materials of QDs or *via* synthesis techniques.[5] Also, industrial scale QDs have been synthesized using simple methods that do not involve injection techniques.[6] These methods often result in a more uniform particle size and improved optical properties. Recent improvements in synthesis methods and protective coatings for water solubility make QDs promising fluorescent labels for certain life sciences research. Different QD solubilization strategies have been devised over the past few years, including (i) ligand exchange with simple thiol-containing molecules; (ii) encapsulation by a layer of amphilic diblock or triblock copolymers or in silica shell; (iii) combinations of layers of different molecules conferring the required colloidal stability to QDs.[7] Indeed, QDs have been used successfully in a variety of biological experiments, such as long-term multicolor imaging, single-particle tracking in live cells, fluorescence in situ hybridization in human chromosomes, Xenopus embryo development imaging, multiphoton imaging in live mice, cancer targeting and metastasis studies *in vivo*, FRET-based biosensors, and multiplexed biocoding.

In this context, CdS-coated CdTe nanocrystal QDs was synthesized. CdTe/CdS, the core/shell structure, was synthesized with a coordinating organic solvent to improve the quan-

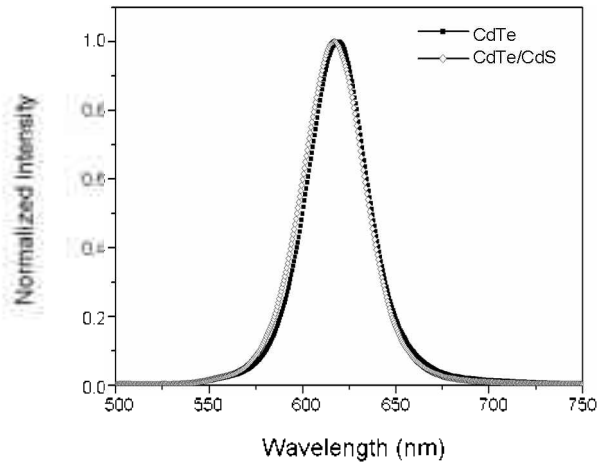

Figure 1. Emission spectra of CdTe and CdTe/CdS QDs.

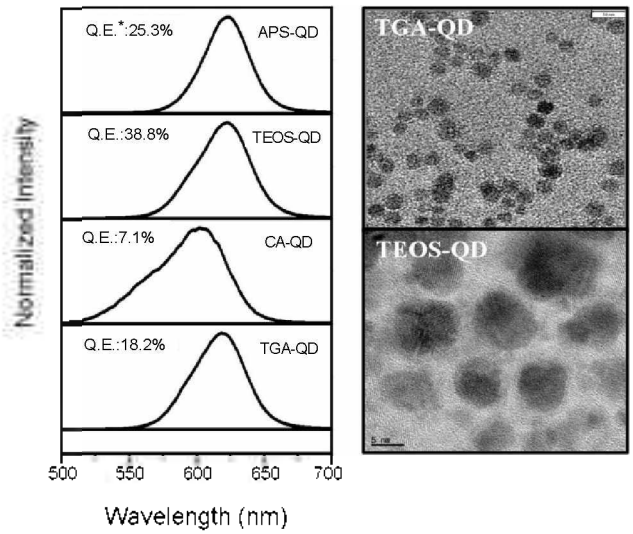

Figure 2. Optical Characteristics of QDs. **Left**: emission spectra of various surface-modified QDs; **Right**: TEM images of TGA-QD and TEOS-QD. The particle diameters of both QDs were approximately < 4 nm and < 6 nm, respectively. *Quantum efficiency.

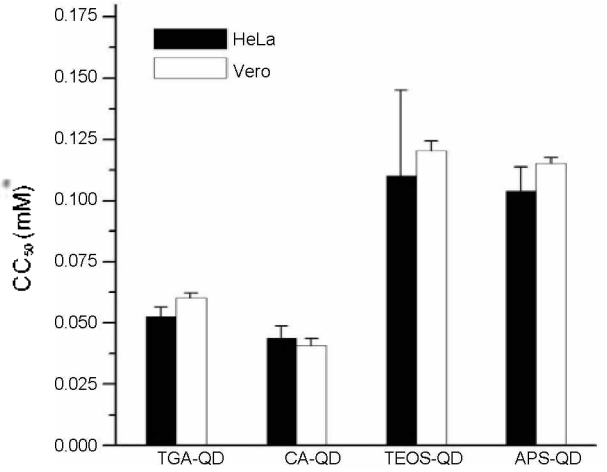

Figure 3. Cell cytotoxicity of the four surface-modified QDs (sample size n = 6). *Cytotoxicity activity.

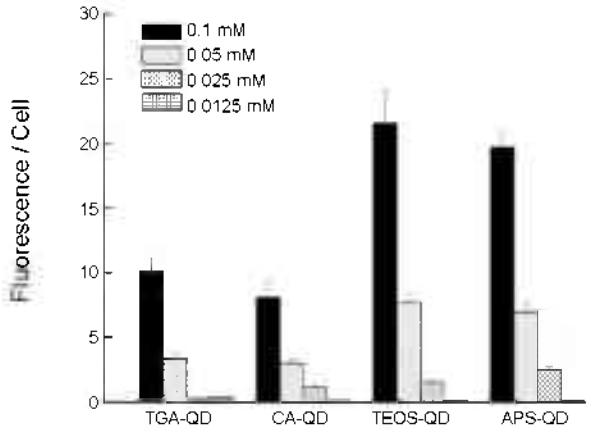

Figure 4. Intracellular uptake of various surface-coated CdTe/CdS QDs (sample size n = 6).

tum yield and stability. The synthesis and application of modifiable QDs is conducted. We examined the fluorescence emission of these two types (CdTe and CdTe/CdS) using a fluorescence spectrometer (Figure 1). The emission of CdTe and CdTe/CdS were detected red light at 619 nm and 617 nm and the full-width-at-half-maximum (FWHM) values are 37 nm and 39.5 nm, respectively. From the prepared CdTe/CdS material, we synthesized the four surface-modified QDs: 1) thioglycolic acid (TGA), 2) cysteamine (CA), 3) tetraethyl orthosilicate (TEOS), 4) aminopropyl-triethoxysilane (APS). It was observed that the optical properties of various surface-coated QDs are affected by the reagents and the used methods (Figure 2). TGA-QD has very small shift of emission spectrum compared with only CdTe/CdS. TEOS-QD and APS-QD show a shift of nearly 8 nm. However, CA-QD shows a large shift to a short wavelength of about 15 nm with a shoulder at 557 nm. The FWHM of the emission spectrum of CA-QD was widely broadened compared to the others, as the emission spectra have a shoulder that measures approximately 557 nm. Figure 2 shows TEM images of TGA-QD and TEOS-QD. The sizes of these samples are < 4 nm and < 6 nm, respectively. The core/

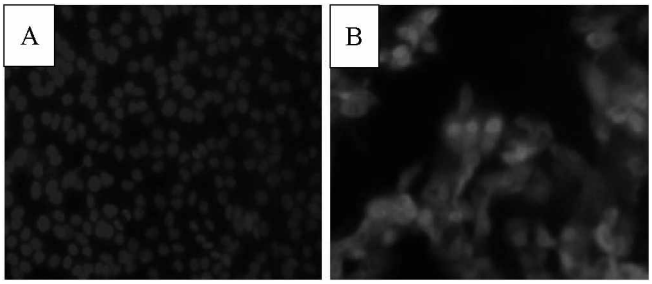

Figure 5. Luminescence images of cultured mock-infected (A) and virus-infected (B) HeLa cells that were incubated with antibody conjugated TGA-QD. The QDs (red) and the DAPI-stained nuclei (blue) were recorded with fluorescence microscopy.

shell structure of TGA-QD is not observable due to the very thin CdS layers on the CdTe core. However, the silica coating layer is depicted by the blurry image on the CdTe/CdS spheres. In the image of TEOS-QD, CdTe/CdS shows distinct lattices with a spherical shape. This is a synthesized silica layer with a thickness less than 1 nm.

We characterized the cytotoxicity of various surface-coated QDs based on the extracellular nanoparticle exposure levels in cells. A cell cytotoxicity assay was conducted to confirm whether the QDs damage the cells or not. To determine the cytotoxicity of different surface-modified QDs was investigated with HeLa and Vero cells. A 50% cytotoxic concentration (CC_{50}) was defined as a concentration of a compound that reduced the absorbance of the control samples by 50% (Figure 3). The silica coating QDs (TEOS-QD and APS-QD) improved the optical properties and reduced the cytotoxicity, as the silica, located in the outmost shell of QDs, protects from contamination by toxic Cd^{2-} ions. Also, the quantum yield of quantum dots was 140 - 210% increase in the TEOS or APS surface-coated QD than in the TGA surface-coated QD. Therefore, a significantly increased quantum yield and low level of cytotoxicity of CdTe QDs caused by the silica coating, which was composed of a very thin silica layer and required a short processing time. Improvements of the quantum efficacy resulted that the silanization process removed the shallow trap states from the QD surface. The low cytotoxicity resulted from the silica shell, which prevents the leakage of toxic Cd^{2+}.[8]

To demonstrate the effect of various surface-coating on the cellular uptake of QDs, these levels were measured the intensity of all surface-modified QDs using a fluorometric imaging plate reader (FlexStation, Molecular Devices, Sunnyvale, CA), obtained by incubation at appropriate concentrations in HeLa cells (Figure 4). The results showed that QD uptake in cells was influenced significantly by surface-coating modifications. As shown in Figure 4, CA-QD showed little QD uptake. The uptake levels of silane or silicate coating QD were higher than those of CA capping QD.

One of the most fascinating capabilities of many biological molecules is that of molecular recognition. Certain biological molecules can recognize and bind to other molecules with extremely high selectivity and specificity. Concerning molecular recognition, two classes of biological molecules are of special interest: antibodies and oligonucleotides. We used a

bioconjugated QD probes for protein targeting and imaging. Fluorescence has long been used to visualize cell biology at many levels, from molecules to complete organisms. Fluorescence is mainly observed from small organic dyes attached by means of antibodies to the specific proteins. For biological application, the intracellular localization of protein (HSV-1 strain F Thymidine Kinase (TK)) with antibody (Ab) conjugated QD was measured *via* fluorescence microscopy (Figure 5). We used to crosslink free carboxylic acid groups with amine-containing antibodies. Briefly, the TGA-coated QDs (TGA-QD) were activated with EDC. The activated QDs were reacted with the antibody bearing a primary amine group. The mock-infected HeLa cells showed little fluorescence signals with QD-Ab clusters. However, the fluorescence of the QD clusters was visible in virus-infected HeLa cells throughout the cytoplasm and nucleus.

In conclusion, QD-surface modification does play a significant role in cell cytotoxicity. In addition, increasing cytotoxicity with higher QD concentration for all surface-modified QDs was observed, with CA-coated QDs exhibiting the highest cell toxicity. By demonstrating how nanoparticle surface coatings can influence cell toxicity and intracellular uptake of QD, these results serve to suggest an additional factor to be considered in the design of biocompatible nanomaterials for future biological applications. Also, this study demonstrates the successful application of these nanoparticles to the detection of intracellular proteins. Therefore, the prepared QDs are attractive for various biological applications due to feasible cytotoxicity and photostability characteristics.

Experimental

Synthesis of Quantum Dot. The CdTe/CdS, core/shell structure were modified using synthetic methods outlined in earlier reports. All surface-modified samples were based on indicated CdTe/CdS materials. It was synthesized using 0.4535 g $Cd(NO_3)_2{\cdot}4H_2O$, which was dissolved in a mixed solution, dodecylamine and trioctylphosphine oxide. This solution was heated to 160 °C and a trioctylphosphine (TOP)-Te solution prepared using 0.16 g Te was dissolved in 5 g TOP at a high temperature and was then added to the original mixture. This was heated for 1 h at a constant temperature. Residual solution was removed by washing and centrifuging with chloroform and methanol several times. The CdS shell was synthesized onto the CdTe core. The core was dispersed into 20 g of dodecylamine and was heated to 130 °C. Dissolved solutions composed of 0.907 g $Cd(NO_3)_2{\cdot}4H_2O$ and 0.198 g S into 10 g TOP were put onto the dispersed core solution and heated for 1 h. The core-shell was obtained by washing and centrifuging. The surface of this powder were treated for dispersal in water using appropriate amounts of pH-controlled TGA (pH > 13) with KOH. Water was added this solution and separated in two parts: water that contained QDs and chloroform. Only the water part was decoupled from this solution.

The CA-QD sample was prepared by the follow process: The core/shell structure was conducted *via* the method of TGA-QD. CA-QDs were prepared using a CA. The pH of CA solution was controlled with KOH to be over 9. The other process is same that of TGA-QD.

The TEOS-QD and APS-QD samples were prepared by the follow process: The core, shell and thiol treatments were carried through the method of TGA-QD. TEOS-QDs were treated with TGA-QD. It was prepared using TEOS and ammonia solutions at 50 °C for 1 h. APS-QDs were treated with TGA- QD. It was prepared using tetraethyl silicate and APS at 50 °C for 30 min.

The surface coating was cross-linked further by EDC (1-ethyl-3-(3-dimethylamino propyl)carbodiimide)-mediated coupling to antibodies. EDC reacts with a carboxyl group first and forms an amine-reactive *O*-acylisourea intermediate that quickly reacts with an amino group to form an amide bond and release of an isourea by-product. The antibody bearing a primary amine group was attached to the surface-modified QD (TGA-QD). These QDs were activated by adding 10 mM EDC solution. EDC solution was added to QDs before the primary amine group containing antibodies. The mixture solution was incubated at RT for 1 h with continuous gentle mixing. To quench the reaction, add 2-mercaptoethanol to a final concentration of 10 mM. The excess amount of compound was removed by a desalt column. Purify the conjugated by size-exclusion with superdex200 resin.

To evaluate the characteristics of the prepared samples, a TEM image was taken *via* FE-TEM from JEOL and TEM (TECNAI G^2 from FEI) at 200 kV. The emission and absorption spectra were obtained using a Perkin-Elmer LS-50B and UV-vis spectrophotometer (UV-2501PC from Shimadzu).

Cell Cytotoxicity Test. A cell cytotoxicity test was assessed by a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Confluent cells grown in 96-well tissue culture microplates were added with 100 μL of QDs diluted with DMEM/2% FBS. After incubation for 24 h, the residual QDs were removed and a MTT solution at a concentration of 2.5 mg/mL in phosphate buffered saline (PBS(-)) was added to each well. The wells were then incubated in a humidified CO_2 incubator at 37 °C for 1.5 h. 100 μL of acidified isopropanol/10% Triton X-100 solution was then added and the plates were shaken to dissolve the formazan products. The absorbance levels at dual wavelength (540 nm as the main and 690 nm as the reference) were measured with a microplate reader (Thermomix, Molecular Devices, Sunny Vale, CA). The cell survival rate in the control wells without QD solutions was considered as 100% cell survival. A 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the compound that reduced the absorbance of the control samples by 50%.

Intracellular Uptake of QDs. Confluent HeLa cells seeded in 96-well plates were incubated with six QDs for 12 h at 37 °C. The QD solution was removed and the cells were washed five times with growth media to remove any extracellular QDs. The cells were trypsinized and resuspended in PBS(-). The cell concentration was determined by standard hemocytometry. The cells were then transferred into 96-well plates and measured by using a fluorometric imaging plate reader. The fluorescence per cell was calculated by dividing the measured fluorescence by the number of cells.

Cell Imaging. All fluorescence images were incubated with antibody-conjugated QDs in virus- or mock-infected HeLa cells and were recorded *via* fluorescence microscopy (Axiovert 200, Carl Zeiss, Thornwood, NY). For fluorescence microscopy, the cultured cells were washed with PBS(-) and fixed with 2% phosphate-buffered paraformaldehyde at RT for 10 min. The cells were rinsed with PBS(-) and permeabilized using absolute methanol at −20 °C for 20 min. After washing, blocking was performed with 0.4% bovine serum albumine (Sigma) in PBS(-) at RT for 10 min. Each Ab-QD was added to the cells at RT for 1 h. After washing, the nuclei of cells were stained with DAPI solution and were mounted in a glycerol buffer. Fluorescence microscopy was used to detect the fluorescence in the cells.

Statistical Analysis. The statistical evaluations of the experiments were performed by ANOVA followed by a Newman-Keuls Multiple Comparison Test.

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