Articles

Design and Characterization of Lysine Cross-Linked Mercapto-Acid Biocompatible Quantum Dots

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Received June 27, 2005. Revised Manuscript Received November 25, 2005

Semiconductor quantum dots (QDs) are a new generation of inorganic probes with advantageous properties over traditional organic-only probes for biological applications. A major hurdle in the use of QDs for biology is the inability of the hydrophobically synthesized QDs to interface with aqueous environments. There have been tremendous advances in the surface modification of hydrophobic QDs. However, none of the current techniques fits all of the criteria for an ideal QD coating for biological applications (e.g., maintain the small size and optical properties of QDs, have low nonspecific binding) while providing cost-effective, easy preparation on a large scale. We developed a highly stable biocompatible coating for the surface of ZnS-capped CdSe QDs that maintains all of the hydrophobic-coated QD optical properties. These QDs are prepared by first coating them with mercaptoundecanoic acid and are further cross-linked with the amino acid lysine in the presence of dicyclohexylcarbodiimide to form a stable hydrophilic shell. The surface contains carboxylic acid and amino functional groups for conjugation to biomolecules. Using a dynamic light scattering method, we found that the hydrodynamic diameter of these surface-modified QDs is approximately 20 nm. We demonstrated the feasibility of preparing >400 mg of the biocompatible QDs and the successful conjugation of proteins onto their surface. Finally, we characterized the QD stability and optical properties in various biologically relevant environments.

Introduction

Semiconductor nanocrystals, or quantum dots (QDs) have received great interest from the biological and medical communities in the last few years.[1−4] This interest can be largely attributed to the unique optical and electronic characteristics of QDs and their advantageous properties over organic-based fluorophores for many biological applications. QDs have high luminescence (1 QD = 10 to 20 fluorophores), high resistance to photobleaching, narrow spectral line widths, and tunable emission that can be excited using a single wavelength.[5,6] As QDs become broadly applied for in vitro (e.g., cell labeling, DNA assays) and in vivo (e.g., animal injections) biological research, there is a need to scale-up the production of water-soluble and biocompatible QDs. Recent advances in QD synthesis have led to the development of novel methods for the large-scale preparation of organic-soluble QDs.[7]

The emergence of QD surface-modification chemistry has provided several unique strategies that render the dots useful as biological probes. Molecules that have been utilized to alter the polarity of QDs include amphiphilic polymers,[8,9] phospholipids,[10] dendrimers,[11] oligomeric ligands,[12] bifunctional molecules,[5,6]and genetically modified proteins.[13] However, the transformation of organic-soluble QDs into useful biocompatible probes has suffered from poor conversion yields, complexity in surface modification, and high precursor costs. The use of polymer coating, for instance, produces stable, water-soluble QDs that can preserve the fluorescence yield; however, such an approach is complex and requires the polymer to be either modified or synthesized. Second, the conversion yield for producing single, mono-

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10.1021/cm051393+ CCC: $33.50
Published on Web 01/21/2006

disperse, and water-soluble QDs is relatively low. Furthermore, there are few published manuscripts that characterize the surface-modification process of these QDs (leading one to speculate on the final size of the QDs after cross-linking). Water-soluble QDs produced using bifunctional ligands such as mercaptoacetic acid are easy to produce, and large quantities of the QDs can be made in one reaction with a very high conversion yield. But the major limitation with such a coating is its instability. Precipitation of the QDs occurs after 1−2 days in aqueous buffer because of ligands desorbing from the QD surface.[26,27] In this manuscript, we describe an advance in the design of a surface-coating strategy that can produce stable, monodisperse, luminescent, and bioconjugatable QDs in large quantities. The quality of the surface coating is strongly dependent on the selection of surface ligands, cross-linking agents, and their relative concentrations. These surface-modified QDs (denoted as LM-QDs) were tested in various biologically relevant environments and maintained their physical and optical properties before and after surface modification. We also demonstrated that these LM-QDs can be conjugated to biomolecules such as proteins for specific cellular fluorescence-staining applications.

Experimental Section

Nomenclature. The following denotations are used: QDs refers to quantum dots, TOPO-QDs refers to tri-*n*-octylphosphine coated quantum dots, MUA-QDs refers to mercaptoundecanoic acid-coated quantum dots, and LM-QDs refers to mercaptoundecanoic acid-coated quantum dots that are cross-linked with the molecule lysine and dicyclohexylcarbodiimide.

Sample Preparation of MUA-QDs. Mercaptoundecanoic acid (MUA, 1 g; Aldrich, 95%) was added to a three-neck flask and melted at 65 °C under Ar. Afterward, approximately 0.6 μmol of 560 nm emission tri-*n*-octylphosphine oxide (TOPO)-coated QDs, prepared by using an organometallic procedure, was injected into the mercaptoundecanoic acid solution. The temperature was raised to 80 °C. After 2 h, the solution appeared cloudy but became optically clear when 25 mL of dimethyl sulfoxide (EMD Chemicals Incorporated, 99.9%) was injected into the three-neck flask. This solution was stirred for another 2 h. Afterward, the solution was cooled to room temperature, and chloroform was added to precipitate out the MUA-QDs. They were centrifuged at 3700 rpm to remove unbound mercaptoundecanoic acid. MUA-QDs were redissolved in dimethyl sulfoxide for cross-linking. The key to the successful ligand exchange of mercaptoundecanoic acid for tri-*n*-octylphospine oxide on the surface of the TOPO-QDs was the concentration ratio of mercaptoundecanoic acid to TOPO-QD. We found that 8000× molar excess of mercaptoundecanoic acid to TOPO-QDs was needed to successfully coat yellow-emitting TOPO-QDs (λ_{em} = 580 nm) with mercaptoundecanoic acid. We estimated the molar concentration of QDs using the molar absorptivity value from a published report.[15] The concentration of mercaptoundecanoic acid to TOPO-QDs had to be adjusted for different sizes.

Sample Preparation of LM-QDs. The following solutions were prepared prior to cross-linking: (A) DL-Lysine (Aldrich, 98%) was dissolved in phosphate-buffered saline (10 mM, pH 7.4) at a concentration of 16 000 lysine molecules/MUA-QD and (B) dicylcohexylcarbodiimide (Aldrich, 99%) was dissolved in DMSO at 5 times the concentration of lysine. Solutions A and B were directly added to the MUA-QD solution in dimethyl sulfoxide and stirred for 2 h at room temperature. The solution became cloudy immediately upon mixing. After approximately 30 min, MUA-QDs started to form large aggregates. The solution was centrifuged (3700 rpm, 5 min), and the solvent was decanted. The precipitate was washed with tetrahydrofuran to remove weakly adsorbed mercaptoundecanoic acid (MUA). These LM-QDs were redissolved in double-distilled water and purified by either using a gel filtration column or dialyzing overnight to remove uncross-linked mercaptoundeacanoic acid. Mercaptoundecanoic acid is insoluble in double-distilled water, and desorbed mercaptoundecanoic acid from the surface of the LM-QDs appeared as a white precipitate inside the dialysis tube. In a slower but simpler approach to dialysis for removing uncross-linked or weakly cross-linked mercaptoundecanoic acid, we incubated LM-QDs at ambient temperatures for 7 days in phosphate-buffered saline (pH 7.4, 10 mM). The actual pH of this solution becomes greater than 7.4 because of the LM-QDs. This solution became milky, indicating desorption of mercaptoundecanoic acid from the surface of the LM-QDs. When we placed this solution at 4 °C, we observed the settling of the desorbed mercaptoundecanoic acid at the bottom of the vial. A syringe filter (Sigma, 0.22 μm pore diameter) or centrifugation was used to remove the white precipitates (which are insoluble mercaptoundecanoic acid) from LM-QDs. Finally, tetrahydrofuran was added to the LM-QD solution and then centrifuged to precipitate out the LM-QDs from solution. The precipitate was left in the fume hood at room temperature overnight to dry. Powdered LM-QDs should be stored at room temperature under nitrogen. If needed, the LM-QDs can be further cross-linked in phosphate-buffered saline (10 mM, pH 7.4) in the presence of excess lysine and 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide. Other types of small organic molecules, such as diaminopimelic acid (Sigma-Aldrich), can be used to cross-link mercaptoundecanoic acid carboxylic acid groups to form a stable QD-coating. The use of excess lysine or diaminopimelic acid should lead to a highly cross-linked shell on the surface of the MUA-QDs. If the MUA-QDs are not fully cross-linked, mercaptoundecanoic acid desorption from the surface of QDs (observed by a white precipitate) is apparent ∼7 days (depending on the buffer) after the day of resolubilization. The white precipitates can be removed by using a syringe filter.

Measuring the Hydrodynamic Diameter of QDs. A dynamic light lightface scattering method (Malvern Zeta Sizer S) was used to measure the hydrodynamic diameter of the QDs.

Gel Electrophoresis of QDs. LM-QDs of various emissions were added to 0.5× agarose gel made with 0.8× tris-borate EDTA (TBE) buffer. TBE buffer (0.8×) was also used as the buffer for

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electrophoresis measurements (Fischer gel electrophoresis, 100 V). For transferrin- and bovine serum albumin-conjugated LM-QDs, the conjugates were first purified using G75 Sphedex (Sigma) centrifugation columns to remove reactants and byproducts.

Temperature Study. LM-QDs were dissolved in water and heated. Sample aliquots of the QD solution were taken out at different temperatures (i.e., 20 and 30 °C, etc.), and the fluorescence was measured using a spectrofluorometer (Fluoromax, Jobin−Yvon, $\lambda_{ex} = 350$ nm, $\lambda_{em} = 580$ nm).

pH Study. LM-QDs were dissolved in water. The LM-QD solutions with various pH levels were prepared by the dropwise addition of NaOH or HCl to different samples. The pH was monitored with a pH meter (InoLab). For each solution, the quantum yield was compared to the organic dye Rhodamine 6G (Aldrich, dissolved in ethanol).

Salt Concentration Study. LM-QDs were dissolved in double-distilled water and injected into a solution with different concentrations of NaCl salt. We used an epifluorescence microscope (Olympus) and the image program ImagePro (Carsen) to evaluate the monodispersity of LM-QDs at varying salt concentrations. The NaCl concentration of the solvent ranges from 10 to 750 mM, with the concentration of LM-QDs remaining constant at 1 μM.

Conjugation with the Protein Transferrin and Cell Studies. A LM-QD stock solution was made by dissolving 10 mg/ml of a LM-QD solution in double-distilled water. The LM-QD stock solution (15 μL) was mixed with 20 μL of a 10 mg/ml solution of the protein transferrin (Sigma-Aldrich) in phosphate-buffered saline (10 mM, pH 7.4). Stock solution of 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (10 μL, 50 mM) dissolved in double-distilled water was added to this mixture and shaken at room temperature for 2 h to allow the reaction to occur. After the reaction was complete, the solution mixture was purified using a gel filtration column with Sephadex G-75 beads (Sigma) spun at 3700 rpm for 5 min to remove any unbound proteins and reaction byproducts. Transferrin−LM-QD conjugates (10 pmol) were incubated with HeLa cells (30−50% confluent cultured in a 15 mm × 100 mm tissue culture dish) overnight at 37 °C in 5% CO_2. HeLa cells were cultured in Dulbecco's minimum essential media (Gibco) with 10% fetal bovine serum (Sigma), 1% penicillin (Sigma), and 1% amphotericin B (Sigma). For control studies, we incubated either a solution of LM-QDs or bovine serum albumin-conjugated LM-QDs to HeLa cell cultures with the same experimental conditions (i.e., transferrin−LM-QDs concentration, cell populations, etc.). Prior to imaging, the cells were washed with phosphate-buffered saline multiple times to remove any unbound LM-QDs in solution.

Results and Discussion

Surface Modification of QDs. Panels a and b of Figure 1 show the schematic for the mass-scale preparation of highly stable and luminescent LM-QDs. ZnS-capped CdSe QDs are synthesized using an organometallic reaction scheme.[14] The tri-*n*-octylphospine oxide molecules on the surface of the ZnS-capped CdSe QDs are initially displaced from the QD's surface with mercaptoundecanoic acid (MUA). These MUA-coated QDs are then centrifuged and resolubilized in an intermediate polar solvent (e.g., dimethyl sulfoxide). The amino acid lysine or small molecule diaminopimelic acid and cross-linking agent dicyclohexylcarbodiimide are then added to MUA-coated QDs in the solvent dimethyl sulfoxide. Within 30 min, the MUA-coated QDs precipitated out of solution; the solution was then centrifuged, and the precipitates were redissolved in double-distilled water (with a concentration as high as 400 mg/mL). Figure 1c shows that the LM-QDs (of different fluorescence emission and size) are soluble in the water layer (top) and not the chloroform layer (bottom). The improvement in the solubility of the LM-QDs in comparison to the MUA-coated QDs in aqueous solvents could be attributed to the formation of polar amide bonds and excess polar functional groups (e.g., carboxylic acids and amines) on the MUA-coated QD surface from the lysine or diaminopimelic acid. Fourier transform infrared (FTIR) spectroscopic measurements show the formation of amide bonds on the surface of LM-QDs, as we observed a broad peak with a maximum wavenumber of 3100 cm^{-1} (See the Supporting Information, Figure S1). We also observed a peak at 1570 cm^{-1}, which corresponds to carboxylic acid functional groups. Although we expected the amines from the lysine or diaminopimelic acid to be completely conjugated to the carboxylic acid during cross-linking, our FTIR spectra suggest that not all of the amino groups are cross-linked, as shown by a small broad peak at 1600 cm^{-1}. Nevertheless, we showed that a highly cross-linked polar−organic capsulelike structure was created on the MUA-coated QD surface. This cross-linking stabilizes mercaptoundecanoic acid against desorption and prevents QDs from aggregating.

We used several conventional techniques ((1) optical microscopy, (2) electron microscopy, (3) gel electrophoresis, and (4) light scattering) to determine whether LM-QDs are monodisperse and nonaggregated after surface modification. Optical microscopy shows the LM-QDs are blinking on and off, a property that is associated with single fluorescent molecules and particles (Figure 2a and the Supporting Information, video V1).[18] These LM-QDs were also analyzed using electron microscopy and showed discrete dark spots that are approximately the same size (Figure 2b). Gel electrophoresis also confirmed the LM-QDs are monodisperse, because we observed a narrow gel band (Figure 2c). If LM-QDs are aggregated, the gel band would be broad or stationary. Finally, a single size distribution of LM-QDs is obtained using a dynamic light scattering method (Figure 3); if LM-QDs are aggregated, we would observe a multimodal sizing distribution. On the basis of these characterizations, we conclude that the LM-QDs are single and monodisperse after surface modification.

Typically, water-soluble mercaptoacetic acid-coated QDs tend to aggregate after 1−2 days in double-distilled water. However, we monitored our LM-QDs in double-distilled water for two months and did not observe aggregation. If the cross-linking is incomplete, we do observe a milky solution at room temperature or a white precipitate at the bottom of the vial (at 4 °C) and aggregation within a short period of time. The milky solution is more readily apparent when the LM-QDs are dissolved in a buffer rather than in double-distilled water. To alleviate these problems, we could further cross-link the LM-QDs with lysine or diaminopimelic acid and 1-ethyl-3-[3-dimethylpropyl]carbodiimide (in phosphate-buffered saline, pH 7.4, 10 mM).

There are two major requirements for successfully producing monodisperse LM-QDs: (1) the alkyl chain length of the bifunctional molecule (with one end containing thiol and the other end containing carboxylic acid functional groups)

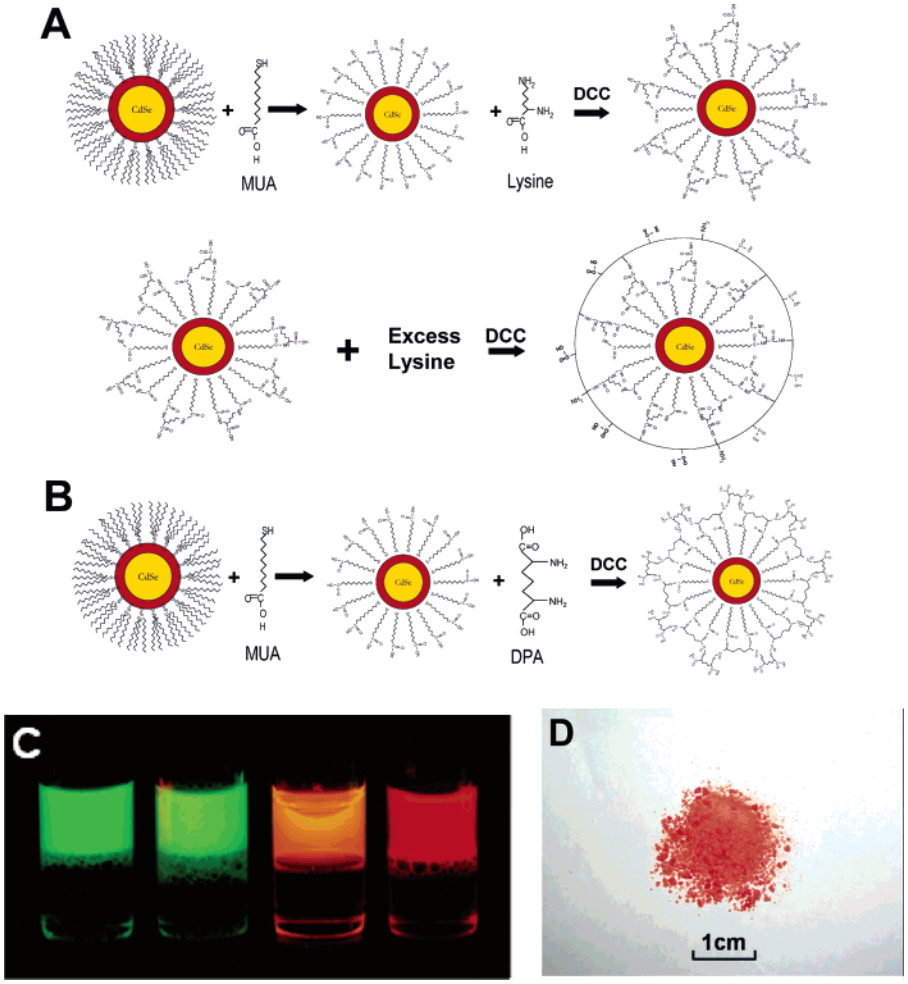

Figure 1. Reaction schematic of the surface modification of tri-*n*-octylphosphine oxide (TOPO)-coated organic-soluble QDs. (a and b) The TOPO molecules on the surface of the QDs are replaced with the thiolated bifunctional ligand mercaptoundecanoic acid. The thiol group is attached to the surface of the QDs, and the carboxylic acid groups are cross-linked by either the amino acid lysine or diaminopimelic acid in the presence of carbodiimide. The outcome of the modification is water-soluble LM-QDs that can be conjugated to biorecognition molecules. (c) Vials of four different colors of QDs under UV excitation. The LM-QDs are soluble in the water layer (top) and not the chloroform layer (bottom) after surface modification and cross-linking. (d) Photo image of a large amount (>400 mg) of LM-QDs. In any given reaction, >90% of the organic-soluble QDs can be converted and recovered after surface modification. These surface-modified LM-QDs can be prepared as a powder for easy and prolonged storage. MUA is mercaptoundecanoic acid, DPA is diaminopimelic acid, and DCC is dicylcohexylcarbodiimide.

must be between 8 and 13 carbons in length. QDs coated with bifunctional molecules with less than 8 carbons in the alkyl chain (e.g., mercaptoacetic acid and mercaptoproprionic acid) are soluble in aqueous solvents, but they are extremely difficult to cross-link with 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide, the only water-soluble version of dicyclohexylcarbodiimide commercially available. Uncross-linked mercaptoacid-coated QDs have a tendency to aggregate in the presence of 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide in aqueous solvents. QDs with bifunctional molecules that have more than 13 carbons in the alkyl chain (e.g., mercaptohexadecanoic acid) are soluble in intermediate (e.g., dimethyl sulfoxide) and nonpolar solvents (e.g., chloroform). After cross-linking, the increase in polarity from the formation of amide bonds and the carboxylic acid groups is enough to overcome the nonpolar alkyl chains. (2) The cross-linking molecule must be a small organic molecule that contains two primary amino groups and at least one carboxylic acid group (preferably two). These molecules must be miscible or soluble in an intermediate polar solvent. Large molecules such as the polylysine polymer do not produce monodisperse water-soluble QDs because they contain excessive amounts of reactive sites (e.g., 7 reactive site for a 5-mer). Multiple carboxylic acid-functionalized QDs can react onto a string of polylysine, leading to large aggregation (See Supporting Figure S2). Furthermore, polylysine is insoluble in dimethyl sulfoxide and therefore is not useful for this procedure.

After modifying the surface chemistry of the TOPO-coated QDs, we dried and stored the dots in powdered form, as shown in Figure 1d. Powdered LM-QDs are prepared by first precipitating LM-QDs out of solution using either high salt concentrations or intermediate solvents (e.g., tetrahydrofuran); they are then centrifuged, washed in tetrahydrofuran, and air-dried overnight at room temperature. LM-QDs in powdered form are relatively easy to store and transport. After four months of storage in ambient temperatures, LM-QDs were readily soluble in double-distilled water or basic buffers (pH > 9.0) and they maintained all of their properties from the first day of surface modification. Furthermore, the ability to produce large amounts of LM-QDs permits the use of grams as a measurement unit for LM-QDs (from the same batch), which can improve the overall quantitation for conjugating biomolecules onto their surface and for quantitation in biological experiments. Many

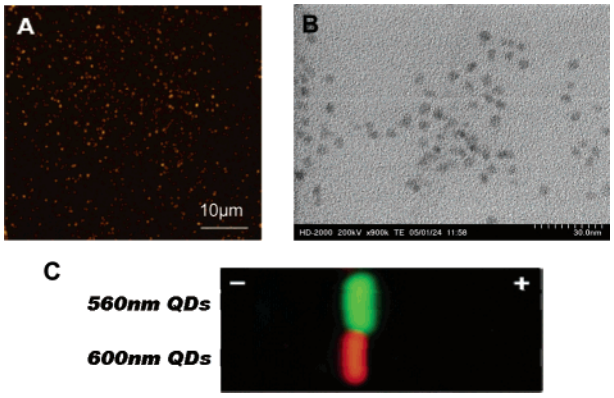

Figure 2. Characterization of LM-QDs in aqueous buffer to determine monodispersity. (a) Under epifluorescence microscopy, the on−off (blinking) behavior of single LM-QDs is observed (see the Supporting Information, video V1). The QDs were dissolved in double-distilled water, spread on a polylysine-covered glass cover slip, and imaged. (Olympus IX71, 60× objective, 100 W Hg lamp, 525/25 excitation, 610/27 emission filters). (b) Transmission electron microscopy of LM-QDs. The spreading of LM-QDs on a carbon-coated copper grid resulted in monolayer distribution and minimal stacking of LM-QDs. This indicates that a majority of the LM-QDs are single. (Hitachi HD2000 STEM, 900 K magnification, 200 kV). (c) Gel electrophoresis of two different emitting LM-QDs. (Fisher gel electrophoresis, 0.8× agarose gel, 0.5× TBE buffer, 100 V). These data show that the LM-QDs are monodisperse and single in aqueous buffer.

researchers have relied on the use of published molar absorptivity coefficients (ϵ) to determine concentrations.[15−17] However, the reported ϵ value has been inconsistent and because of this, experimental results using QD probes could be inaccurate.

Characterization of QD Optical and Size Properties before and after Surface Modifications. The use of mercaptoundecanoic acid and lysine/dicyclohexylcarbodiimide cross-linking did not alter the optical properties of the initial TOPO-coated QDs (for TOPO-coated QDs we tested, emission wavelength of 480 to 600 nm). The quantum yield, fluorescence spectra, and absorbance profile are similar before and after surface modifications (see the Supporting Information, Figure S3). It is worth mentioning that the initial surface modifications of some QD batches produced a low quantum yield of aqueous-soluble QDs because they can be photobrightened after exposure to room light at ambient temperature for 1−2 days. The final quantum yield of these aqueous soluble QDs is similar to that of the initial TOPO-coated QDs. Using this approach, we typically prepared 400 mg of LM-QDs per conversion, but the preparation of gram-sized quantities of water-soluble LM-QDs is feasible. The yield for conversion of organic-soluble to aqueous-soluble LM-QDs is typically >95%.

We further probed the change in the hydrodynamic diameter of the QDs before and after modification. When dynamic light scattering was used, the hydrodynamic diameter of QDs (maximum emission of 600 nm) was not altered when mercaptoundecanoic acid replaced the tri-*n*-octylphosphine oxide on the surface of the QDs (8.3 vs 8.1 nm, Figure 3a,b); however, the size of the QDs increased after the addition of lysine and dicyclohexylcarbodiimide. The average size changed from 8.7 to 20.3 nm. The hydrophilic organic shell adds a thickness of 5.8 nm to the surface of the QDs. Figure 3 shows the particle-size distribution for the QDs pre and post surface modifications. The increase in the hydrodynamic diameter is a clear indication of the formation of a shell structure surrounding the QD surface. The use of excess lysine in cross-linking does increase the overall hydrodynamic diameter of the QDs. Shown in Figure 3d, the particle diameter increased significantly when excess lysine is added to form the hydrophilic shell but reached a plateau when the particles became too large. In the case of the 600 nm emitting QDs, the maximum size of the QD, using this coating strategy, is 50−60 nm in diameter. In these experiments, all parameters were kept constant (i.e., QD concentration, dicyclohexylcarbodiimide concentration, etc.), with the exception of the lysine concentration. Beyond 50−60 nm (using excess lysine concentration), the QDs tend to precipitate out of solution.

Characterization of Biocompatible LM-QDs in Biological Conditions. We then examined the stability and optical properties of LM-QDs in various biologically relevant environmental conditions (e.g., different pH, temperature, plasma, and salt concentrations). When the LM-QDs are dissolved in double-distilled water, the pH of the solution is 9.0. The LM-QDs maintained their monodispersity in solution within the pH range 4−8 for ∼1 day. This allowed for the measurement of the quantum yield of the LM-QDs in acidic-to-neutral pH environments (see Figure 4a). Interestingly, they remained monodisperse for more than 1 day in plasma, which has a pH of 7.4. However, at pH 8−12, the LM-QDs are stable for months. Figure 4a shows that the quantum yield of LM-QDs fluctuated greatly between a pH of 4 and 8 and remained constant at pH of 8−12. At pH < 4, LM-QDs had lost ∼80% of their fluorescence. Similar results are observed with other types of QD surface coatings, and this effect may be attributed to the acid etching or breakdown of QDs.[19,20] This highly acidic environment is generally not common with most biological experiments. The stability of LM-QDs was further tested in double-distilled water with different NaCl concentrations. Aggregation was not observed for the solution with an NaCl concentration below 250 mM, whereas above a 500 mM NaCl concentration, the aggregation of the LM-QDs is more evident. At low salt concentration (<500 mM), the LM-QD solutions are stable for weeks. The precipitates of the LM-QDs at high salt concentrations can be resuspended in solution when the salt concentration is reduced (i.e., by adding double-distilled water).

Temperature is another important parameter for biological experiments (e.g., cell-incubation studies, polymerase chain reaction (PCR), DNA sensors). The optical properties of LM-QDs are highly dependent upon temperature (see Figure 4b). The fluorescence and absorbance spectra shift to the red and the quantum yield dramatically decreases upon heating (∼30 to 40%, from 25 °C to 70 °C). However, the quantum yield increases back to the original level when the solution is cooled. The rate of increase in quantum yield is much slower than the rate of decrease. These results are in agreement with published work by McLendon and co-workers and Weller and co-workers.[23−25] There are various explanations for the cause of this decrease in the quantum yield and shift in the fluorescence. One explanation is that

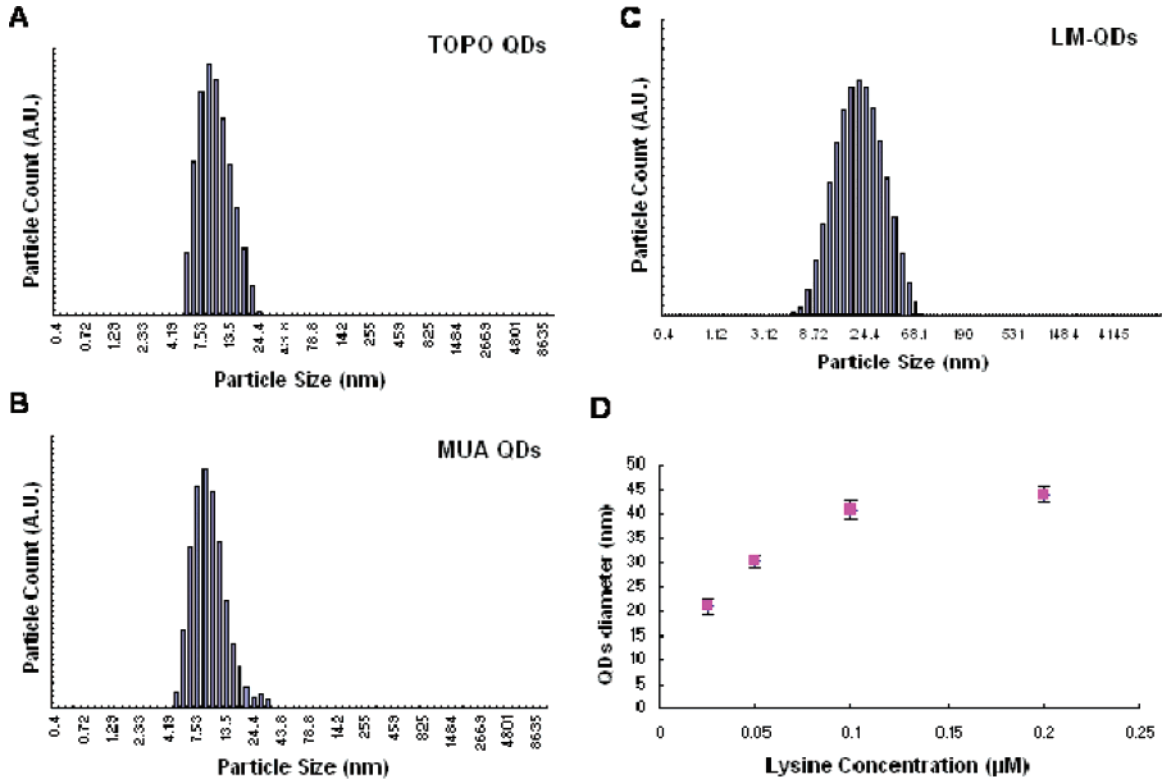

Figure 3. Characterization of QD size pre and post surface-modification using a dynamic light backscattering method. (a) TOPO-coated, (b) MUA-coated, and (c) LM-coated QDs had an average hydrodynamic diameter of 8.3, 8.1, and 22.9 nm, respectively. The broadness of the peak indicates the size distribution of the QDs. (d) The change in the hydrodynamic size of the LM-QDs is highly dependent upon lysine concentration. As the concentration of lysine doubles (from 0.05 μM to 0.10 μM), the average hydrodynamic diameter of the LM-QDs increases from 30 to 40 nm.

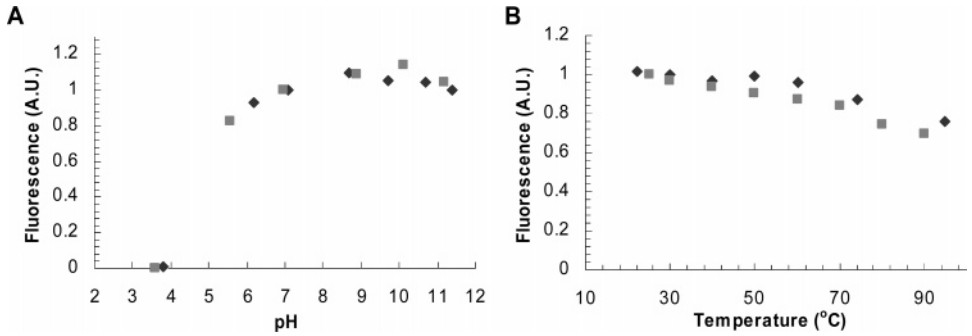

Figure 4. Characterization of the optical properties of LM-QDs in various pH and temperatures. (a) The LM-QDs maintained their fluorescence intensity within physiological pH range. Below pH 5, the QD fluorescence decreased dramatically, possibly because of the acid etching of the QD core. (b) The fluorescence intensity of LM-QDs does not drop significantly as the temperature increases. At 90 °C, the LM-QDs lost ~20% of the original fluorescence intensity.

temperature determines the fate of mobile carriers trapped in shallow states and, therefore, this affects the recombination process. Another explanation is that the high temperatures create a greater population of defective sites to trap mobile carriers. Future investigations should provide more insights into the cause of this effect.

Demonstration of the Conjugation of QDs to the Protein Transferrin. Unlike previous mercaptoacid-coated QDs, we obtained an improved consistency in conjugating biomolecules onto the surface of LM-QDs using carbodiimide catalysis. To date, we have successfully conjugated transferrin, antibodies, and other proteins, as well as oligonucleotides, onto LM-QDs. In this reaction scheme, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide reacts with a carboxylic acid group on the surface of the LM-QDs; this leads to the formation of an amine-reactive O-acylisourea intermediate. If an amine-containing molecule is present, this reactive intermediate will react with the primary amine group, yielding a conjugate of the two molecules joined by a stable amide bond.[21−23]

A key parameter in conjugating proteins onto the surface of LM-QDs is varying the LM-QD to protein concentration ratio. Proteins generally have multiple reactive sites and can act as a bridge between LM-QD if the reaction concentrations are not optimized. In these experiments, we carefully selected and optimized the correct reagent concentrations to produce single, unaggregated LM-QDs after conjugation. The measurement unit of moles of proteins to grams of LM-QDs for conjugation is used. Prior to the use of transferrin-conjugated LM-QDs in cell assay, we used the same criteria as described in this paper to verify they are monodisperse after conjugation. It is difficult to measure amide

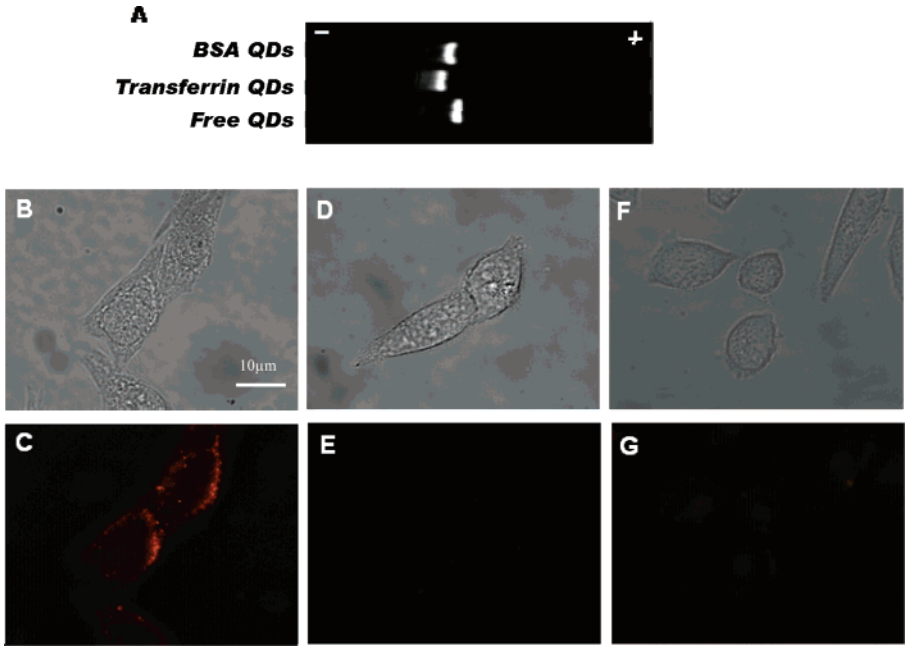

Figure 5. Fluorescence labeling of HeLa cells via transferrin-conjugated LM-QDs. (a) Gel electrophoresis band showing that protein (either transferrin or bovine serum albumin)-conjugated LM-QDs migrate at a slower rate. The gel band remains fairly narrow after conjugation, indicating no significant aggregation occurred during conjugation. (b) Differential interference contrast and (c) fluorescence images of HeLa cells stained with 600 nm emitting transferrin-conjugated LM-QDs. There are minimal fluorescence stains in the HeLa cells incubated with either (d, e) LM-QDs or (f, g) bovine serum albumin-conjugated LM-QDs. All fluorescence images were processed using the software ImagePro 5.0 in a similar manner.

bond formation (because the organic shell contained amide bonds) using FTIR spectroscopy, so we used gel electrophoresis to indicate successful conjugation. The electrophoretic mobility of the transferrin- and bovine serum albumin-conjugated LM-QDs is slower than that of the LM-QDs. This is likely due to the larger size of the particles after conjugation (Figure 5a).

We used the classic QD-transferrin study to demonstrate the successful conjugation of the protein transferrin onto the surface of LM-QDs and demonstrated the successful staining of HeLa cells with transferrin-conjugated LM-QDs (Figure 5B and C). These transferrin-conjugated QDs did not enter the cell nucleus, as determined by the lack of orange emission in the DAPI-stained nucleus (See the Supporting Information, Figure S4). In the control study, we can clearly see that neither LM-QDs (Figure 5d,e) nor bovine serum albumin-conjugated LM-QDs (Figure 5f,g) entered the cells because we did not observe much fluorescence. These results showed minimal nonspecific staining of any part of the HeLa cells; this is different than the silanized-coated QDs, which were reported to stain cells in a highly nonspecific manner.[5] However, we will need to investigate cell types other than HeLa cells. The nonspecific adsorption or uptake of QDs may be dependent on cell type.

Conclusions

We described the large-scale preparation of water-soluble and biocompatible QDs that can be conjugated to biomolecules. This coating strategy is simple to conduct and cost-effective. The biocompatible organic shell does not require any complex synthetic techniques to prepare. All of the precursor reagents for preparing the biocompatible organic shell are commercially available at a reasonable price, and the conversion yield from organic to water-soluble is near unity. This coating strategy could be adapted to QDs synthesized by methods other than the organometallic strategy. These biocompatible LM-QDs can be processed into a powdered form, which simplifies the storage and conjugation of biocompatible QDs. We characterized the stability and optical properties of LM-QDs in various biologically important conditions and showed there are optimum conditions for the use of QDs in biological applications; these results suggest that for quantitative analysis using QD-probes, control studies and standards will be both required and important. In conclusion, our QD surface-modification technique is simpler to conduct and more cost-effective than other techniques and leads to the large-scale preparation of high-quality biocompatible LM-QDs; this paves the way for broader use of QDs for small- and large-scale biological research.

Acknowledgment. This work was made possible by funding from the University of Toronto (start-up fund), Canadian Foundation for Innovation, Ontario Innovation Trust, and Connaught Foundation. W. J. and H.F. thank OGS and S. M. thanks NSERC for graduate fellowships. Also, we thank Dr. P. Zandstra for the kind donation of HeLa cells, Dr. C. Yip for assistance in FTIR measurements, Mr. O. Couture and Dr. S. Foster for use of their dynamic light scattering system, and Anupam Singhal for interesting technical discussions.

Supporting Information Available: FTIR spectra, fluorescence and absorbance spectra of pre- and postsurface-modified QDs, optical image of polylysine cross-linked mercaptoundecanoic acid-coated QDs, fluorescence images of transferrin−QD-labeled HeLa cells (pdf), and a video showing the blinking behavior of single monodispersed LM-QDs (avi). This material is available free of charge via the Internet at http://pubs.acs.org.

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