Articles

Fluorescent Quantum Dot−Polymer Nanocomposite Particles by Emulsification/Solvent Evaporation

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CdSe/ZnS quantum dots (QDs) were incorporated into biocompatible polyisoprene (PI) particles by microencapsulation through emulsification/solvent evaporation, a technique that is facile, robust, and inexpensive. Emulsification/solvent evaporation results in QDs encapsulated into the particle core without requiring chemical modification of the as-prepared QDs. The PI can be easily cross-linked after encapsulation to enhance the fluorescence stability of the QDs. The resulting PI−QD nanocomposite particles form as colloidally stable suspensions in water that exhibit stable fluorescence for months. The surface of the PI−QD nanocomposite particles was functionalized with carboxylates during the QD encapsulation to facilitate subsequent bioconjugation. Streptavidin-coated PI−QD particles displayed selective binding to biotin-conjugated polystyrene spheres, thus demonstrating the potential application of these particles in biolabeling.

Introduction

Semiconductor quantum dots (QDs) are ideal fluorophores for biological imaging:[1,2] their emission wavelength can be tuned by simply changing the particle size, and simultaneous excitation of different-sized QDs can be achieved using only a single wavelength,[3] allowing facile multicolor coding for high-throughput assays. However, the surface of QDs, as synthesized, is hydrophobic,[4−6] making them unsuitable for direct use in biological environments. Examples of strategies used to make QDs more biocompatible include exchanging the organic ligands on the QD surface with a more polar species,[1,2,7] coating with a silica layer,[8] and encapsulating the hydrophobic QD in lipid micelles.[9] However, none of these methods completely prevent individual QDs from photooxidation, and thus fluorescence efficiencies and the shelf life of biocompatible QDs are typically reduced several fold relative to their organic-capped counterparts.[10]

Recently, the encapsulation of QDs into polymer colloids has seen growing interest as a route to improve photostability and for development of colorimetric optical bar codes for biological sensing.[11−15] Several different approaches have been taken including solvent swelling of aqueous polymer colloids,[11] entrapping QDs in cross-linked reverse micelles,[13] covalently bonding QDs with polymerizable ligands to polystyrene during suspension polymerization,[14,16] entrapment through strong noncovalent interactions such as hydrogen bonding and ionic attraction,[17−19] and physically entrapping QDs into particles formed by emulsion polymerization[15] or

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sol−gel synthesis.[20] In the present study, we chose to investigate QD/polymer nanocomposite formation through emulsification/solvent evaporation, a technique commonly used to create pharmaceutical/polymer composite particles for drug delivery.[21,22] Encapsulation of QDs through emulsification/solvent evaporation has several potential advantages over other encapsulation methods. Emulsification/solvent evaporation can encapsulate QDs without chemical modification of the QD surface, unlike methods that copolymerize reactive QDs.[14,16] The reactive QDs can also adversely affect the nucleation and growth of polymer particles during copolymerization, leading to a widening of the particle size distribution.[16] The QDs are encapsulated in the core of the polymer particles during emulsification/solvent evaporation. Methods that incorporate QDs by solvent swelling of preformed aqueous polymer colloids[11] generally lead to a large fraction of surface-adsorbed QDs, with a relatively small fraction actually encapsulated in the core of the polymer particles.[23] Finally, emulsification/solvent evaporation also allows the surface chemistry of the nanocomposite particles to be easily controlled through the choice of surfactant used to form the emulsion. Control of surface chemistry is important to allow subsequent bioconjugation to the polymer−QD particles.

In the emulsification/solvent evaporation technique, the polymer and desired additive are first dissolved in a mutual solvent. Then, the polymer/additive solution is emulsified into an aqueous surfactant solution and the polymer solvent is evaporated, leaving a polymer/additive composite colloid stabilized by surfactant. In the present study, the additive to be encapsulated is CdSe QDs coated with alkane ligands. The alkane ligands stabilize the QDs in hydrophobic solvents such as chloroform and hexane. To form QD/polymer composite particles by emulsification/solvent evaporation, the polymer must dissolve in a solvent compatible with the QDs. *trans*-Polyisoprene was chosen because it is soluble in many of the same solvents as the QDs, including alkanes, so it is expected that polyisoprene (PI) should interact favorably with the alkane-coated quantum dots. The polymer chain is unsaturated so that it is easy to chemically cross-link after processing, ensuring that the QDs remain entrapped after encapsulation. PI is also highly desirable because it is biocompatible, nontoxic, and noncarcinogenic.[24]

Experimental Section

Materials. Tetradecylphosphonic acid (TDPA) was purchased from Alfa Aesar. Trioctylphosphine oxide (TOPO), tri-*n*-octylphosphine (TOP), CdO, selenium powder, stearic acid (SA), diethyl zinc, hexamethyldisilathiane, *trans*-polyisoprene (PI), acrylic acid, lauric acid, 2,2′-azobisisobutyronitrile (AIBN), and sodium hydroxide (NaOH) were purchased from Aldrich. Streptavidin, 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and sulfo-NHS were obtained from Peirce Bio. Hexane (AR grade) and methanol (SpectrAR grade) were obtained from Mallinckrodt Chemicals. Sodium dodecyl sulfate (SDS) was purchased from J. T. Baker. Deionized water was used in the experiments.

Synthesis of CdSe/ZnS Quantum Dots. The core CdSe QDs were synthesized in various sizes, coated with a thin shell of ZnS, and capped with TOP and TOPO following procedures similar to those in the literature.[4,5,25] In a typical synthesis, 0.05 g of CdO, 7.0 g of TOPO, 1.3 g of SA, and 0.16 g of TDPA were first loaded into a three-neck flask. The mixture was then dried and degassed in the reaction vessel by heating to 190 °C for about an hour, flushing periodically with N_2. Then the temperature was raised to 320 °C for selenium injection. At that temperature, 2.4 mL of 0.42 M selenium in TOP was quickly injected into the reaction flask. The size of the CdSe nanocrystals was adjusted by varying the reaction time.

After CdSe nanocrystals were synthesized, they were further capped with a semiconductor shell (ZnS).[5] In the capping experiment, 6.0 g of TOPO in a flask was first purified under vacuum at 190 °C for 1 h. Then the flask was cooled down to 90 °C, and 2 mL of TOP and 0.1 mmol of CdSe QDs in hexane were injected into the reaction vessel. Hexane was pumped off immediately and then the vessel was heated up to 130 °C under nitrogen. Diethyl zinc and hexamethyldisilathiane were used as Zn and S precursors, respectively, and were dissolved in 4.0 mL of TOP at an equimolar ratio inside a N_2 atmosphere glovebox. The precursor solution was added to the flask dropwise over a period of 30−40 min at 130 °C using a syringe pump. After injection, the QDs were annealed at 100 °C for approximately 1 h and then cooled down. The capped QDs were stored in hexane. Prior to microencapsulation, methanol was added to precipitate the QDs. The precipitated QDs were separated by centrifugation and then dried in a vacuum oven.

Microencapsulation of QDs in Un-cross-linked PI Particles. The dried QDs were dissolved in 5 mL of chloroform to make a QD solution, and its concentration was determined by absorption spectroscopy. Then 0.15 g of PI was dissolved in 5 mL of chloroform to form a polymer solution. The QD solution and the polymer solution were mixed together. In a separate flask, 0.15 g of lauric acid and 0.045 g of NaOH were dissolved in 50 mL of water to produce an aqueous surfactant solution. The polymer/QD solution was poured into the aqueous surfactant solution and bubbled with Ar for 20 min. The mixture was homogenized at 24000 rpm for 1 min (IKA WORKS, T25 basic ULTRA-TURRAX homogenizer) and then stirred using a magnetic stirrer for 5 h under argon to evaporate the organic solvent. After solvent evaporation, polymer particles formed and entrapped the QDs. During the experiment, the system was shaded from light to avoid any possible photobleaching of QDs.

Microencapsulation of QDs in Cross-linked PI Particles. A polymer solution containing QDs and an aqueous surfactant solution were made as stated above. Then 0.020 g AIBN and 0.020 g acrylic acid were added into the polymer solution. The solution thus obtained was poured into the aqueous surfactant solution and bubbled with Ar for 20 min. The mixture was homogenized at 24000 rpm for 1 min and then transferred to a round-bottom flask into which 0.050 g of SDS had been added. The flask was sealed and purged with Ar for 15 min and then put into an oil bath at 70 °C. The polymerization was carried out for 6 h. The cross-linked latex produced was transferred to a beaker after cooling and stirred overnight under argon to allow the organic solvent to evaporate.

Coupling of Streptavidin to PI Particle Surface. Streptavidin was coupled to carboxylic acid groups on the surface of QD-loaded

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cross-linked PI (PI−QD) particles through EDC activation.[26,27] One hundred microliters of PI−QD latex (approximately 3×10^{12} microspheres per mL) were centrifuged in a centrifugal filter (Millipore Microcon, 100000 MWCO) and then the separated PI−QD particles were dispersed in 500 μL of MES buffer solution (50 mM 2-(*N*-Morpholino) ethanesulfonic acid, sodium salt, pH = 6.15). Twenty microliters of 0.1 M EDC and 1.8 mg of Sulfo-NHS were added into the particle suspension under mild stirring. Thirty minutes later, the particles were separated by centrifugation and redispersed in 500 μL of MES buffer (pH = 8.0). The suspension produced was mixed with 100 μL of streptavidin (1.0 mg/mL) and the reaction mixture was incubated for 4 h at room temperature. The streptavidin-coupled PI−QD particles were then washed with MES buffer three times by centrifugation/redispersion, followed by dispersion in 50 mM borate buffer (pH = 8.3, 2% BSA).

Testing Bioconjugation with Biotin-Coated PS Microspheres. Polystyrene (PS) microspheres (11 μm in diameter) with biotin on their surface (Bangs Laboratories Inc.) were washed with 50 mM borate buffer three times by centrifugation/redispersion and then mixed with freshly prepared streptavidin-coupled PI−QD particles under mild stirring for 3 h. The PI−QD particles attached to the surface of the PS microspheres through the biotin−streptavidin interaction.[27] The resulting suspension was centrifuged to separate the two classes of particles. PS microparticles precipitated from the suspension because their density is larger than that of water, while the unbound PI−QD particles were suspended in the supernatant because their density is lower than that of water. This separation procedure was performed at least three times to ensure that all free PI−QD particles were removed.

Particle Size and Morphology. The hydrodynamic particle diameter and polydispersity were determined by dynamic light scattering (Brookhaven Instruments model 90 Plus) using cumulant analysis for polydispersity determination. The morphology of the particles was characterized by transmission electron microscopy (TEM) (Hitachi H-7100 Electron Microscope).

Fluorescence Imaging and Spectroscopy. Particle fluorescence and dark field scattering images were acquired on a Nikon inverted confocal microscope (Eclipse TE300) illuminated with 488 nm (Ar laser) and white light, respectively, and detected with either a cooled CCD camera (Versarry 512B, Princeton Instruments) or a digital camera (Nikon Coolpix 995). Colloidal QD fluorescence spectra were taken with a modular Acton Research fluorometer. Particle fluorescence spectra were acquired by coupling an Acton spectrometer (SpectraPro 300i) to the output port of the microscope.

Results and Discussion

Polyisoprene particle formation was first investigated in the absence of QDs using SDS as the emulsifier. The significant factors determining particle size and size polydispersity during emulsification/solvent evaporation are the energy input during emulsification and the starting concentration of the polymer solution, as shown in Tables 1 and 2. We found the particle size and size polydispersity decreased when the homogenizing speed was increased because of the increase in the energy of emulsification. As the concentration of PI dissolved in chloroform was increased, both the particle size and size polydispersity increased due to the increase in solution viscosity, as listed in Table 2. The trends in particle size and polydispersity with changing viscosity and energy of emulsification are as expected.[22,28] The average particle size can be controllably tuned between 200 and 500 nm by adjusting the homogenizing speed and the concentration of PI. The size and size polydispersity did not change significantly with the concentration of surfactant provided that the amount of surfactant was above the minimum amount needed to stabilize the polymer colloid. For SDS, the minimum surfactant concentration was found to be ~0.030 g of SDS in 50 mL of water when the polymer solution was 0.20 g of PI in 15 mL of chloroform.

Table 1. Effect of Homogenizing Speed on Mean Particle Size and Polydispersity as Measured by Dynamic Light Scattering Using the Method of Cumulants[a]

homogenizing speed (rpm)	particle size (nm)	polydispersity
17500	490	0.26
21500	413	0.23
24000	293	0.15

[a] Polymer solution, 0.20 g of PI in 15 mL of chloroform; aqueous surfactant solution, 0.050 g of SDS in 50 mL of water; homogenization time, 1 min.

Table 2. Effect of Concentration of Polymer Solution on Mean Particle Size and Polydispersity as Measured by Dynamic Light Scattering Using the Method of Cumulants[a]

polymer solution	particle size (nm)	polydispersity
0.10 g of PI in 15 mL of chloroform	300	0.18
0.15 g of PI in 15 mL of chloroform	368	0.21
0.20 g of PI in 15 mL of chloroform	413	0.23

[a] Aqueous surfactant solution, 0.050 g of SDS in 50 mL of water; homogenizing speed, 21500 rpm; homogenization time, 1 min.

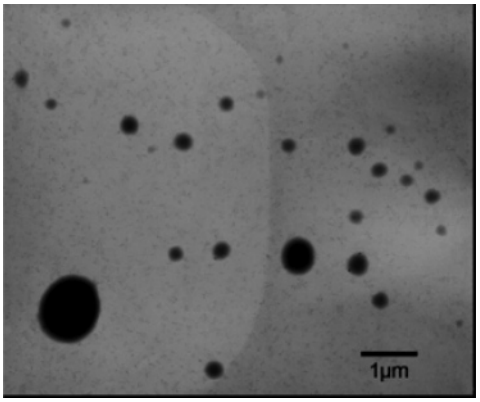

Figure 1. TEM image of un-cross-linked PI−QD particles.

Figure 1 shows a TEM image of a typical PI−QD particle sample with an average particle size of 375 ± 10 nm and size polydispersity of 0.20 as measured by dynamic light scattering. Even though the mean particle size is submicrometer, the broad distribution in particle size results in some particles larger than 1 μm, as seen in the TEM image. The average particle size obtained by measuring the 27 particles visible in the TEM image in Figure 1 is 294 nm with a standard deviation of 186 nm. The average diameter from TEM is smaller than that measured by DLS due to the much smaller sample size in TEM images and the fact that DLS measures the hydrodynamic diameter with an average

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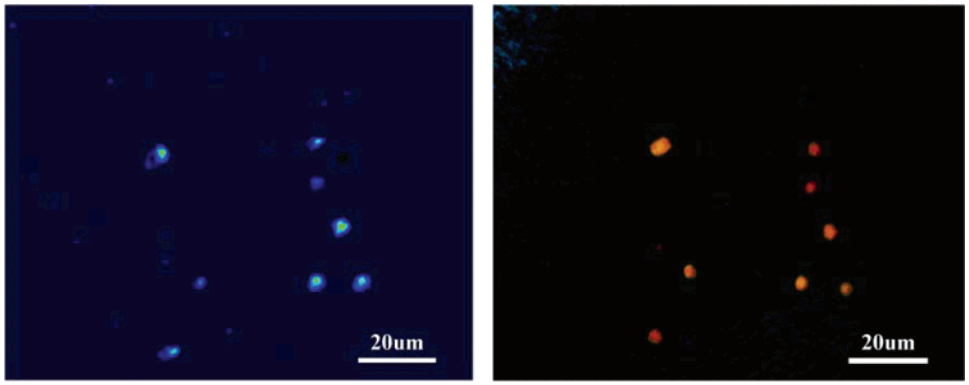

Figure 2. Optical microscopic images of un-cross-linked PI−QD particles: (A) dark-field scattering and (B) true-color fluorescence.

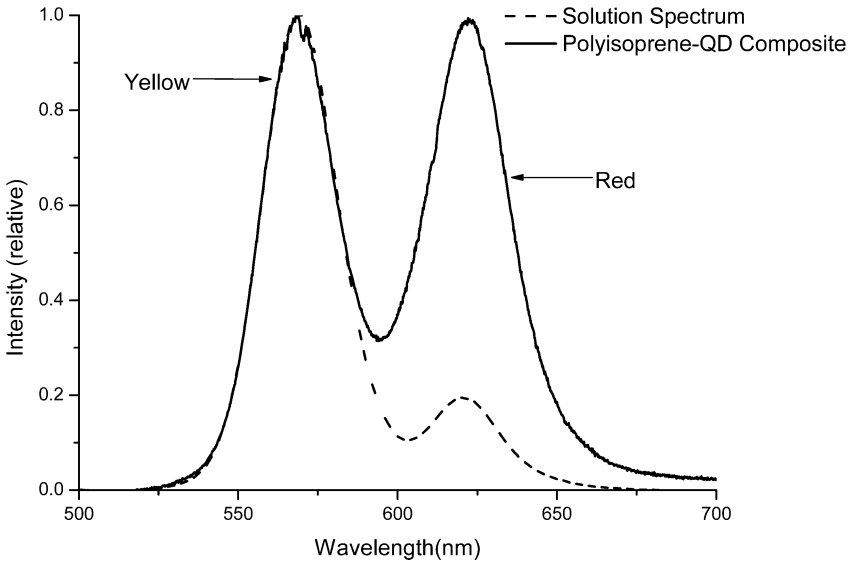

Figure 3. Normalized single-particle fluorescence spectrum of un-cross-linked PI containing two different-colored QDs (solid line) and the fluorescence emission spectrum of the same mixture of QDs in hexane (dashed line).

weighted by larger sized particles.[29] For this initial study, all particles were used as made by simple emulsification with a homogenizer to investigate the encapsulation of QDs and no effort was made to reduce the particle size distribution. The broad particle size distribution is a result of the broad size distribution of the precursor emulsion and is not an inherent limitation of the emulsification/solvent evaporation technique. Uniform polymer particles can be made by emulsification/solvent evaporation starting from a monodisperse emulsion created by microfluidic flow focusing,[30] membrane emulsification,[31−33] or microchannel emulsification.[34]

Emulsification/solvent evaporation was carried out to encapsulate a mixture of yellow and red quantum dots with a yellow:red number ratio of 2:1. The resulting nanocomposite particles were 403 ± 10 nm in diameter as measured by dynamic light scattering with a polydispersity index of 0.25. The estimated theoretical QD loading was approximately 1270 QDs per PI−QD polymer particle assuming a particle size of 403 nm and that all of the QDs added were incorporated evenly into the particles. The polymer colloid was diluted 10 fold by water and the particles were placed onto a glass slide by spin-coating one drop (∼20 μL) of the resulting suspension. White light scattering and fluorescence images of the hybrid particles are shown in Figure 2. Figure 2A is a white-light dark-field scattering intensity image obtained by the CCD, and Figure 2B is the corresponding true color fluorescence image obtained for the same sample. The fluorescence image shows that the location of the QD fluorescence corresponds to the location of the large PI−QD nanocomposite particles. However, the relative brightness of each nanocomposite particle, as expected, was highly nonuniform due to the particle size polydispersity. It appears that QDs were distributed evenly inside the particles, and not simply adsorbed on the surface. The fluorescence intensity of particles can be adjusted by changing QD concentration before solvent evaporation.

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Figure 4. Digital photographs of aqueous suspensions of 0.3 wt % PI−QD particles under UV illumination. Both samples have yellow and red QDs encapsulated in a number ratio of 2:1, respectively. The sample on the left is cross-linked, while the sample on the right is un-cross-linked. Pictures were taken (A) immediately after preparation, (B) 45 days after preparation, and (C) 261 days after preparation.

Figure 3 shows the fluorescence spectrum (solid line) of a single PI−QD particle from the same sample shown in Figure 2. The spectrum has two sharp peaks resulting from yellow QDs (fluorescence maximum = 560 nm) and red QDs (fluorescence maximum = 620 nm). Compared to the fluorescence spectrum of a hexane solution of yellow and red QDs with the same molar ratio of 2:1, respectively, the relative fluorescence intensity of the larger (red) QDs was enhanced relative to the smaller (yellow) QDs when entrapped in the PI−QD particles. (Note that the yellow QDs have a fluorescence quantum yield (QY) of 12% compared to only 1% for the red QDs.) The changes in the fluorescence spectrum are consistent with nonradiative (Förster) energy transfer from the yellow to the red QDs.[35] If the QDs were uniformly distributed in the PI, the average separation would be ∼30 nm, much too far apart for efficient energy transfer. Therefore, our observation of inter-QD energy transfer suggests aggregation of the QDs in the polymer particle likely due to micro phase separation during the encapsulation process. Local close-packing was also recently observed for QDs with polymerizable ligands incorporated into polystyrene during dispersion polymerization.[16] The spatial distribution of QDs inside the PI−QD particles is important for multicolor optical labeling. Because of the changes in fluorescence emission caused by micro phase separation in the polymer, the resulting multicolor fluorescence spectrum from the particle will not represent the relative concentrations of the QDs inside. Even for samples in which the fluorescence spectrum of the QDs is altered within the polymer, it is still possible to achieve multicolor coding by adjusting the number ratio of QDs, as demonstrated recently.[36]

The emulsification/solvent evaporation technique is a facile route to encapsulate the QDs into the core of the polymer particle without free radical polymerization. However, the PI particle is in the rubber state at room temperature (glass transition temperature of *trans*-polyisoprene is −68 °C) and thus the QDs have potentially high mobility in the polymer that would allow them to aggregate. Polymerization can optionally be employed after microencapsulation to cross-link the polyisoprene to reduce QD mobility. Lower mobility will inhibit QD aggregation and prevent migration to the surface of the polymer particles. In addition to preventing QD migration, cross-linking may also improve the barrier properties of polyisoprene with respect to oxygen. Previous studies have demonstrated that highly cross-linked polymers display improved barrier properties to oxygen when compared to un-cross-linked polymers.[37] Since oxygen plays a major role in degradation of QD fluorescence, it is likely that cross-linking the polymer will result in increased stability to oxidation and longer shelf life.

Cross-linked PI−QD particles were made containing a mixture of yellow and red QDs with the same yellow:red number ratio as in the un-cross-linked sample shown in Figure 2. Figure 4 shows digital photographs of two vials, each containing 0.3 wt % aqueous suspensions of PI−QD nanocomposite particles under UV light illumination. The vial on the left contained cross-linked PI−QD particles and that on the right contained un-cross-linked particles. Both types of PI−QD particles were loaded with the same yellow and red QDs. A series of images are shown for both vials, taken immediately after preparation, 45 days later, and 261 days later. It is apparent that the cross-linked samples display

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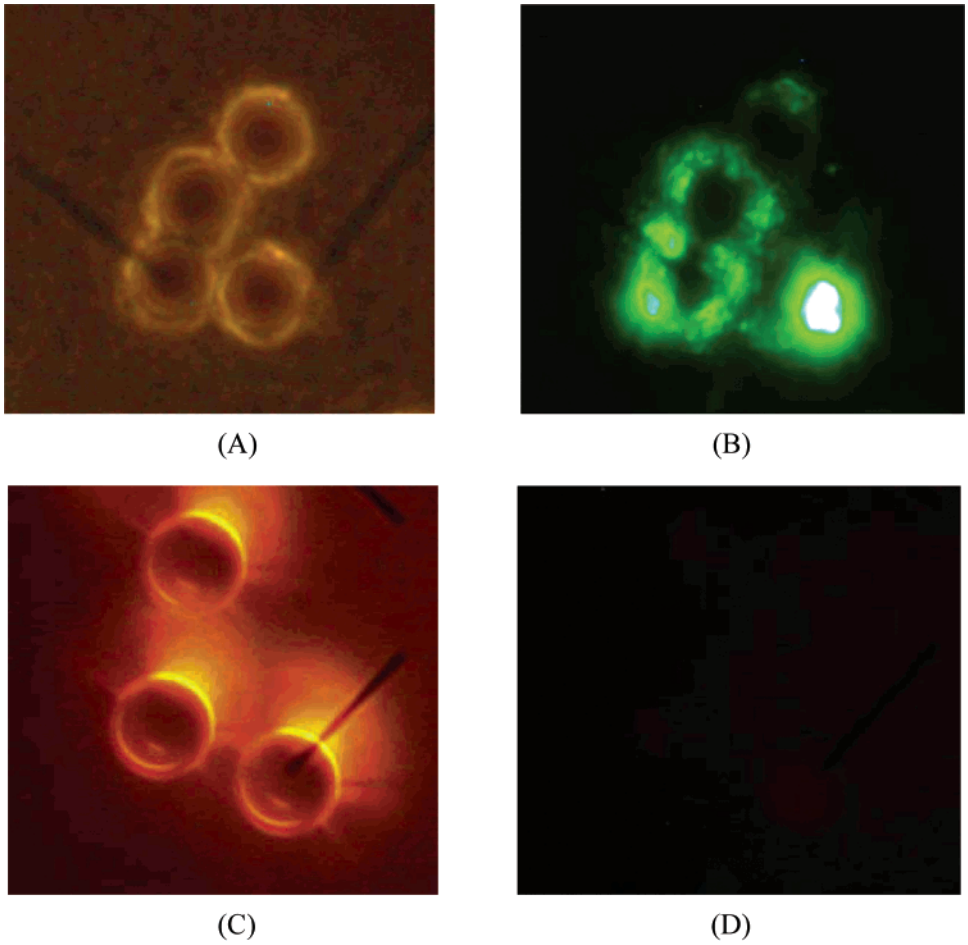

Figure 5. Microscopy images of biotin-coated PS particles exposed to PI−QD particles. (A) and (B) are true-color white light scattering and fluorescence images, repectively, of the PS particles exposed to streptavidin-coated PI−QD. (C) and (D) are true-color white light scattering and fluorescence images, repectively, of the PS particles exposed to PI−QD particles without streptavidin.

enhanced stability against photooxidation when compared to the un-cross-linked sample. The un-cross-linked sample appears to completely lose fluorescence after 261 days in aqueous suspension and also appears to change color from orange to red over time. The same mixture of yellow and red QDs in hexane looks similar in color to the cross-linked sample. The un-cross-linked sample is orange just after preparation, possibly due to higher polymer chain mobility that allows QDs to aggregate during the evaporation process, resulting in increased energy transfer to the red QDs. The shift over time to red color for the un-cross-linked sample is likely due to differences in the rates of oxidation for the yellow versus red QDs. The images of fluorescence decay are consistent with visual observations of the samples. While the cross-linked sample is much more stable after preparation, we observed an approximately 10 fold decrease in the fluorescence intensity of the PI−QD nanocomposite caused by the cross-linking reaction. Others have also reported that free radical polymerization with AIBN is detrimental to QD fluorescence.[38] Even though some QDs were quenched by the free radical polymerization, the remaining fluorescent QDs were stable for extended periods. To increase overall fluorescence from the cross-linked samples, the concentration of QDs incorporated into the PI−QD particles could be increased.

To demonstrate the potential application of the cross-linked PI−QD particles for biological assays, a preliminary study of biomolecule conjugation to the hybrid particles was performed. Streptavidin was coupled to carboxylic acid groups on the surface of cross-linked PI−QD (green) particles through EDC activation.[26,27] To verify the success of the coupling raction, the streptavidin-coupled particles were mixed with commercially available polystyrene (PS) particles functionalized with biotin. The diameter of the PS particles was on the order of typical eukaryotic cell dimensions (11 μm), and thus much larger than the diameter of the PI−QD particles. If the surface of the PI−QD particle was successfully conjugated with streptavidin, the PI−QD particles will attach on the surface of the PS particles due to strong streptavidin−biotin interaction, and the PS particles will then become fluorescent at their outer edges.

Figures 5A and 5B show the white-light scattering and fluorescence images, respectively, of the PS particles exposed to streptavidin-functionalized PI−QD particles. The PS particles scatter very strongly from their edges, and thus look like rings in the image (Figures 5A and 5C). The PS particles also display a bright green fluorescence around their circumference when exposed to streptavidin-coated PI, indicating the PI−QD particles attached to the PS particle surface through the streptavidin−biotin interaction. A control experiment was carried out in which PI−QD particles

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without streptadvidin were mixed with the PS particles, and the result is shown in Figures 5C and 5D. The PS particles are not fluorescent, indicating that the PI−QD particles did not attach to the PS particles when streptavidin was not present. Thus, we can conclude that streptavidin was coupled to the surface of the PI−QD particles, and nonspecific adsorption between the PS and PI−QD particles does not occur. The simple bioconjugation method used to successfully attach streptavidin to the acidic groups on the surface of the PI−QD particles suggests that different biomolecules can be similarly coupled and thus the PI−QD particles have the potential to function as a general biological label.

Conclusions

Fluorescent CdSe(ZnS) quantum dots (QDs) were successfully encapsulated into biocompatible polyisoprene (PI) particles using an emulsification/solvent evaporation method. The simple encapsulation method results in QDs encapsulated into the core of the PI particles without requiring surface modification of the QDs. The PI can be surface-modified with carboxyl groups during encapsulation to facilitate subsequent bioconjugation. The polyisoprene is easily cross-linked by adding a free radical initiator during encapsulation. Cross-linking was shown to greatly enhance the long-term fluorescence stability of the encapsulated QDs. The cross-linked PI−QD nanocomposite particles displayed strong and stable fluorescence emission after months in aqueous suspension. The fluorescence spectra of mixtures of two different-sized QDs change in PI as compared to their solution spectra, suggesting energy transfer between QDs due to their aggregation during the encapsulation. Even though there appears to be some QD aggregation, different emission peaks were clearly resolved, indicating that the particles are suitable for multicolor coding. Streptavidin was coupled to the surface of PI−QD particles and the bioconjugated particles demonstrated specific binding to biotin-coated polystyrene. These fluorescent hybrid nanocomposite particles have potential application in genomics, drug discovery, and other applications that could exploit the high-throughput afforded by multicolor coding.

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