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Encapsulation of CdSe/ZnS Quantum Dots in Poly(ethylene glycol)-Poly(D,L-lactide) Micelle for Biomedical Imaging and Detection

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Abstract: Luminescent CdSe/ZnS QDs, with emission in the red region of the spectrum, were synthesized and encapsulated in poly(ethylene glycol)-poly(D,L-lactide) diblock copolymer micelles, to prepare water-soluble, biocompatible QD micelles. PEG-PLA diblock copolymers were synthesized by ring opening polymerization of D,L-lactide, in the presence of methoxy PEG as a macroinitiator. QDs were encapsulated with PEG-PLA polymers using a solid dispersion method in chloroform. The resultant polymer micelles, with encapsulated QDs, were characterized using various analytical techniques, such as UV-Vis measurement, light scattering, fluorescence spectroscopy, transmission electron microscopy (TEM) and atomic forced microscopy (AFM). The polymer micelles, with encapsulated QDs, were spherical and showed diameters in the range of 20-150 nm. The encapsulated QDs were highly luminescent, and have high potential for applications in biomedical imaging and detection.

Keywords: CdSe/ZnS QDs, PEG-PLA diblock copolymer, polymer micelle, encapsulation.

Introduction

Organic dyes have been widely used as fluorophores in biomedical imaging and detection. However, organic dyes are generally vulnerable to the physiological environment and are quickly photobleached under normal imaging conditions. Thus, the long-term biomedical imaging has recently been one of the hottest research topics.[1-6] Nanometer-scale semiconductor nanocrystals, known as quantum dots (QDs) have attracted significant attentions during the last decades because they can dramatically improve the use of fluorescent markers in biological imaging.[3,7-10] Due to their unique characteristics such as tunable fluorescence wavelength by size, sharp and symmetrical fluorescence peak, strong and stable emission, high quantum yield, and broad excitation spectra, the QDs can provide distinct advantages over conventional organic dyes *in vitro* and *in vivo*.

QDs could be made directly in water but often have narrow available size ranges and wide size distribution, leading to wide FWHM, full width at half maximum of the emission spectrum.[11-14] On the contrary, QDs produced from high temperature organic solvent synthetic strategies are monodisperse (leads to narrow FWHM) with very wide emission color ranging from ultraviolet to near infrared (300-1,200 nm) by simply changing the size, composition and/or structure.[15-23] However, these QDs are hydrophobic and so usually aggregated in aqueous media. Their luminescences are easily impaired or even last those foreign body-like QDs are targeted by the mononuclear phagocyte system (MPS) and quickly removed from blood circulation.[24]

For more extensive and effective biological applications, QDs have been encapsulated or surface modified to prevent aggregation and make them biocompatible. The various strategies have been used to make them water-soluble, such as surface functionalization with water-soluble ligands,[3,10] silanization,[25] and encapsulation within block-copolymer micelles.[26] The strategy of using amphiphilic polymers is generally superior to the surface modification, because (a) there is no direct interaction with the QD surface atoms and therefore can preserve the original quantum efficiency to a highest extent; (b) the presence of hydrophobic polymer domains around QDs may strengthen the hydrophobic inter-

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action to form more steady structures and consequently more stable water-soluble QDs; and (c) these amphiphilic polymers can be tailor-made to have good stability in aqueous media and introduce other functional moieties on the surface of QDs.

Polymer micelles have been extensively studied for solubilization of hydrophobic drugs and bioactive agents due to their unique properties including the nano-scaled size, high water-solubility, high structural stability, high carrying capacity of hydrophobic agents, and easiness in introducing functional moieties on the outer shell.[27] Especially, poly(ethylene glycol)-poly(D,L-lactide) diblock copolymer micelles are the most frequently used system because of their biocompatibility and biodegradability.[27,28] In this study, luminescent CdSe-ZnS QDs were synthesized and encapsulated in biodegradable amphiphilic poly(ethylene glycol)-poly(D,L-lactide) (PEG-PLA) diblock copolymers for preparation of water-soluble and biocompatible QDs nanoparticles. The hydrophobic QDs were encapsulated into the PEG-PLA micelle by a solid dispersion method. The polymer micelles with QDs encapsulated were characterized by UV-Vis measurement, light scattering measurement, fluorescence spectroscopy, transmission electron microscopy (TEM) and atomic forced microscopy (AFM).

Experimental

Materials. Trioctylphosphine oxide (TOPO), trioctylphosphine (TOP), hexadecylamine (HAD), hexamethyldisilathiane ($(TMS)_2S$), methoxy poly(ethylene glycol) (MPEG, M_n = 2,000), and stannous octoate were purchased from Sigma-Aldrich (St. Louis, MO). D,L-lactide (LA), purchased from Polyscience Inc. (Warrington, PA), was recrystallized from dry ethyl acetate and dried under vacuum for 12 h, prior to use. Dimethylcadmium ($CdMe_2$) and diethylzinc ($ZnEt_2$) were purchased from Fluka (Seelze, Germany). The other chemicals and solvents were of reagent grade and used as received without further purification.

Synthesis of PEG-PLA Diblock Copolymers. PEG-PLA diblock copolymers were synthesized by ring opening polymerization of LA in the presence of methoxy PEG as shown in the Figure 1. A predetermined amount of PEG was placed in the two neck flask containing magnetic needle and dried under vacuum condition at 80 °C for overnight. The temperature was increased to 110 °C and 0.4 wt% of the stannous octoate in toluene was added as a catalyst with N_2 purge. After a desired amount of LA was added into

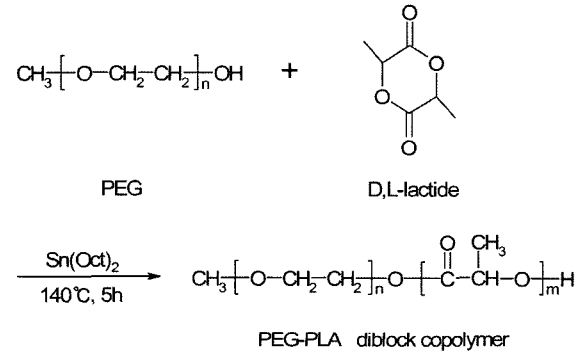

Figure 1. Synthesis of PEG-PLA diblock copolymers.

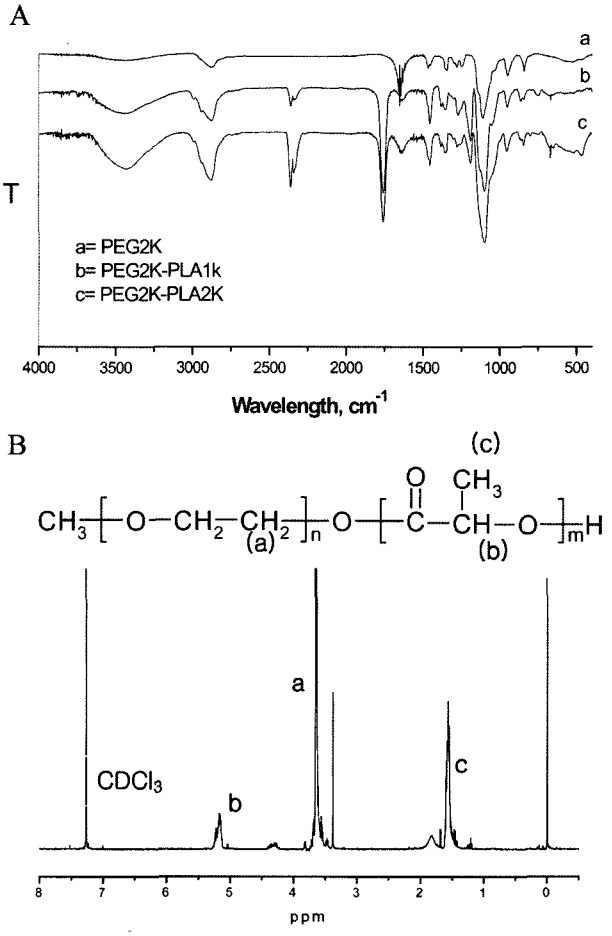

Figure 2. Characterization of PEG-PLA diblock copolymers: A. FT-IR spectra of (a) PEG2k block, (b) PEG2k-PLA2k, and (c) PEG2k-PLA1k diblock copolymers. B. ^{1}H-NMR spectrum of PEG-PLA diblock copolymers.

Table I. The Synthetic Results of PEG-PLA Diblock Copolymers

	Sample Name	[LA]/[PEG][a]	Molecular Weight		M_w/M_n	Yield (%)
			M_n^b	M_n^c		
1	PEG2k-PLA1k	14.0/1.0	3,140	3,000	1.16	74
2	PEG2k-PLA2k	35.0/1.0	4,140	3,740	1.29	70

[a]Feed molar ratio of LA to PEG. [b]Molecular weight value determined from ^{1}H-NMR measurements. [c]Molecular weight value measured by gel permeation chromatography.

the flask, N_2 purge was done at least 3 times to make an oxygen- and moisture-free environment. The reaction mixture was heated up to 140 °C with stirring for 5 h. The resultant product was dissolved in dichloromethane and precipitated with an excess of cold diethyl ether. The prepared diblock copolymers were characterized by FT-IR, ^{1}H-NMR, and GPC as shown in the Figure 2 and Table I.

Synthesis of CdSe/ZnS QDs. Luminescent CdSe/ZnS QDs were synthesized based on the method developed by Peng *et al.*[29] with minor modifications. Cadmium oxide (12.7 mg) and lauric acid (160 mg) were mixed in a 100 mL three-neck flask. The mixture was heated to 200 °C in a mantle to fully dissolve the cadmium oxide in the lauric acid solution. Then, TOPO (1.94 g) and HAD (1.94 g) were added to the solution. The mixture was heated to a temperature higher than 280 °C and kept under a dry nitrogen atmosphere. Upon reaching the desirable temperature the mantle was removed and 2 mL of TOP solution containing 80 mg of selenium powder was rapidly injected into the solution under vigorous stirring. To form ZnS coating on the CdSe QDs, the mixture was cooled to 200 °C, and a solution containing 0.25 mL of $(TMS)_2S$ and 1 mL of $Zn(Et)_2$ premixed in 2 mL TOP was gradually injected into the solution over a minute. The reaction mixture was kept at 180 °C with stirring for 1 h. The solution was cooled to room temperature and the resulting product of CdSe/ZnS QDs were washed three times with methanol and chloroform.

Characterization of PEG-PLA Diblock Copolymers. ^{1}H-NMR and FT-IR spectra of the synthesized polymers were obtained on a JNM-AL400 spectrometer (Jeol Ltd, Akishima, Japan) at 400 MHz and FT-IR spectrometer (Nicolet, USA) respectively. The molecular weights and molecular weight distributions were determined using a GPC equipped with an Agilent 1100 series RI detector, quaternary pump, and two PLgel 5 μm MIXED-D & E columns in set, and calculated with PEG standards. The flow rate was 1 mL/min and the temperature (both the column compartment and the flow cell of the refractive index detector) was kept at 35 ± 0.1 °C.

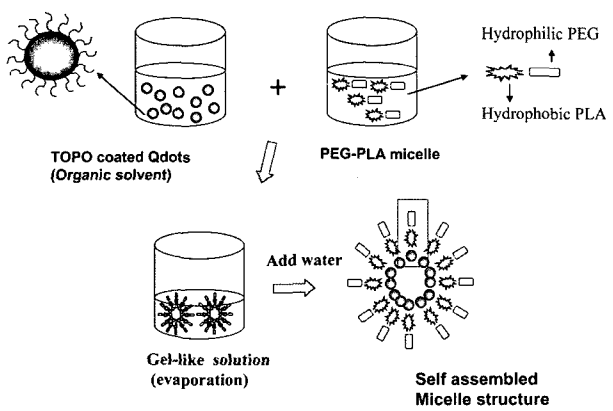

Figure 3. QDs encapsulation by solid dispersion method.

Encapsulation of QDs in PEG-PLA Diblock Copolymer Micelles. The prepared QDs were encapsulated with PEG-PLA diblock copolymers by solid dispersion technique as shown in the Figure 3. QDs and the PEG-PLA diblock copolymers were dissolved in $CHCl_3$ separately and then the solutions were mixed together with gentle stirring for 15 min. The solvent was evaporated under reduced pressure at 60 °C to obtain a gel-like matrix, followed by addition of an appropriate amount of preheated water with gentle stirring. There was no precipitation observed in the aqueous solution, indicating that all QDs were successfully encapsulated in the polymer micelle. The resultant aqueous solution of the polymer micelle with QDs encapsulated was freeze-dried for 3 days.

Optical Spectroscopy. UV-vis absorption spectra were recorded in a quartz cuvette with a 1-cm path length using Shimadzu UV-2501PC UV-vis scanning spectrophotometer (Shimadzu, Japan). Fluorescence spectra were taken on a photoluminescent spectrometer (LS-50B, Perkin Elmer, Wellesley, MA). For the single-photon emission spectra, the excitation wavelength was fixed to 570 nm with slit widths of 1 nm each, and the spectra were recorded from 400 to 800 nm.

Measurement of Size and Morphology. Atomic force microscopy (XE-100, PSIA Inc, Santa Clara, CA) was performed on wafer to determine the morphologies of QDs and encoded QDs in micelle at the cellular micron scale. Nitrogen-dried surfaces were analyzed using a Digital Instruments equipped with an utralever non-contact mode tip (PSIA Inc.) and titanium cantilever. Three areas per sample were imaged under non-contact mode and analyzed with Proscan 1.9 and Image Analysis 2.1 to obtain a line scan section profile used to measure morphology. Using dynamic light scattering (DLS), the effective size and size distribution of the QDs and encoded QDs in micelles were estimated by Zeta Sizer 3000 (Malvern Instruments, Worcestershire, UK). Micelles and encapsulated QDs in micelles were dispersed in water at a concentration of 1-10 mg/mL.

Transmission Electron Microscopy (TEM). The size and the morphology of the encapsulated QDs in micelles were examined by using a JEOL JEM-100CX TEM (Tokyo, Japan). One drop of encapsulated QDs in micelles was mounted on a thin copper grid. After light staining, the dried sample was observed under high vacuum.

Results and Discussion

Synthesis of PEG-PLA Diblock Copolymers. Two kinds of PEG-PLA diblock copolymers with different block lengths were successfully synthesized by ring opening polymerization of LA in the presence of methoxy PEG as a macroinitiator. The PLA block length was controlled by varying

the feed molar ratio between LA and PEG. The synthetic results are summarized in Table I. The molecular weight of the diblock copolymers increased with the increasing feed ratio of LA to PEG. Their molecular weights determined by the peak integration of ^{1}H-NMR spectra were close to those from GPC measurements. Both polymer products showed narrow molecular weight distribution (PDI < 1.3) and reasonable yield as shown in Table I. The presence of ester groups corresponding to the PLA block was confirmed by the characteristic band at 1760 cm^{-1} (C=O, PLA block) in the FT-IR spectra as shown in Figure 2A. The ^{1}H-NMR spectrum from the diblock copolymers exhibited several characteristic peaks at 1.59 (-CH_3, PLA), 3.65 (-CH_2, PEG), and 5.17 ppm (-CH, PLA) (Figure 2B). The peaks of methyl group in PLA and methylene group in PEG were used to calculate the molecular weight of the prepared diblock copolymers.

Optical Properties of QDs. The experimental results revealed that the modified QDs preparation method generated most QDs emitting well in yellow, orange, and red, respectively. The luminescent color of QDs was changed with reaction temperature in the range of 240 and 320 °C. Figure 4 shows the emission and absorption spectra of QDs suspensions in $CHCl_3$. The selected QDs among four QDs prepared were 605 nm emission QDs (maximum peak), with FWHM of 95 nm. On the basis of the UV-vis spectrum, the absorption peak was in the range of 550-600 nm and the florescence quantum efficiency of QDs (Quantum yield (QY) = 0.7) was higher than coumarin 540A (QY = 0.4). The photoluminescence (PL) properties of the QDs, including quantum efficiency, the peak position, and the PL FWHM, did not show any detectable change upon aging in air for several months. Occasionally, when the QDs precipitated in the solution and dried under vacuum was kept for months, it took several hours to redisperse the solid QDs completely in the solvents such as $CHCl_3$. However, by pouring the additional $CHCl_3$ in the solution of QDs or

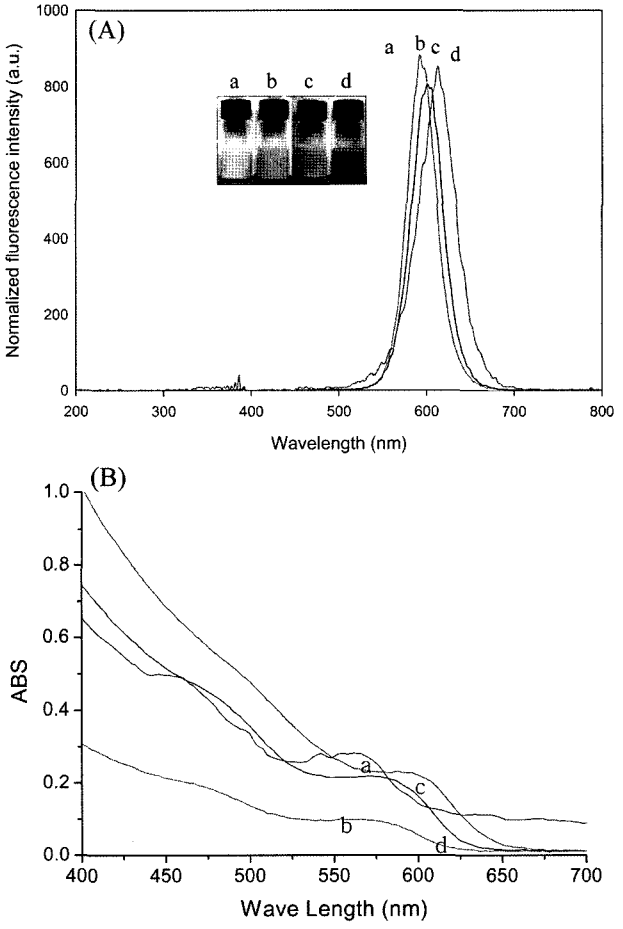

Figure 4. Emission spectrum (A) and UV absorption curve (B) of QDs. Fluorescent intensities of QDs prepared at different temperatures: (a) 240 °C, (b) 260 °C, (c) 270 °C, and (d) 320 °C.

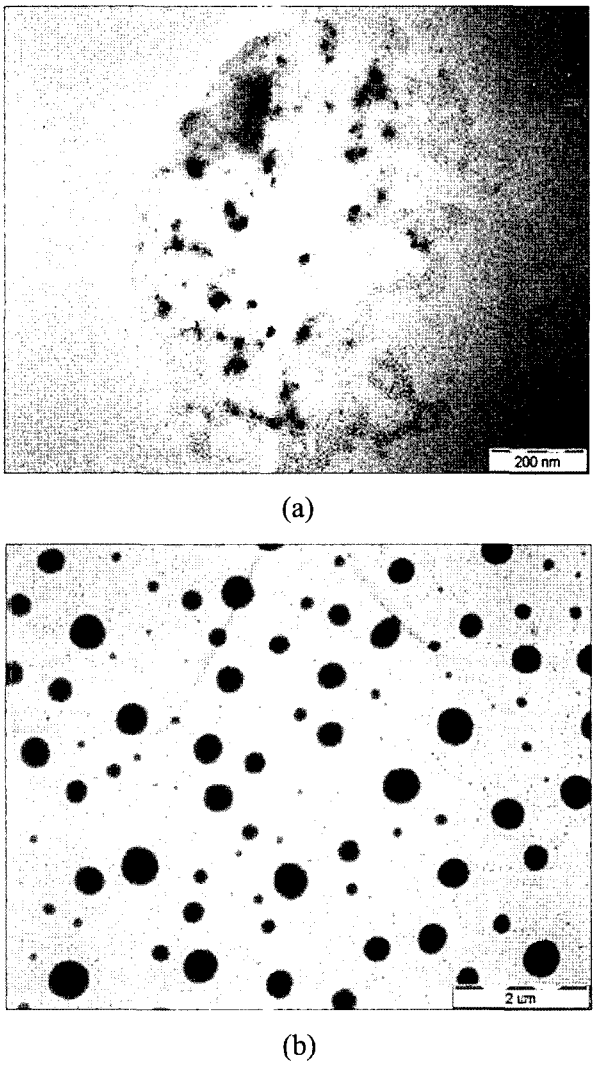

Figure 5. Transmission electron microscope (TEM) image of QDs (a) and encapusulated QDs (b).

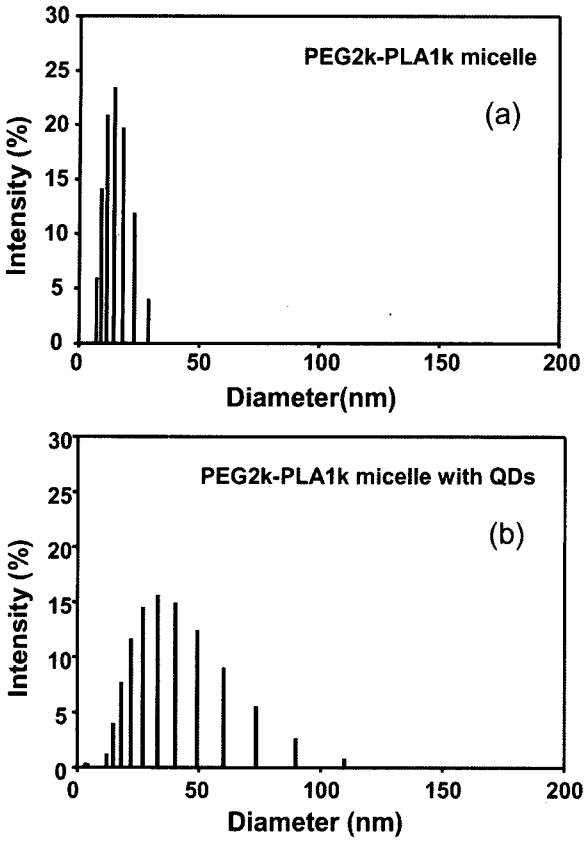

Figure 6. Size distribution of PEG2k-PLA1k micelle (a) and PEG2k-PLA2k micelle with QDs encapsulated (b).

doing ultrasonication, the precipitation of the QDs from their concentrated solutions could be performed without damaging the optical properties of the QDs.

Size and Morphology of QDs and Encapsulated QDs in PEG-PLA Micelles. The morphology of QDs and encapsulated QDs in micelles was observed from TEM measurements to have a round shape (Figure 5). The size of the polymer micelles with or without QDs encapsulated was measured by dynamic light scattering (Figure 6). The average diameter of polymeric micelles and QD-encapsulated polymeric micelles were 17.3 nm ± 11.0 and 41.3 nm ± 5.8, respectively. While the diameter of PEG-PLA micelle showed a size range of about 5-30 nm, the QDs encapsulated micelle showed an increased size range (about 10-100 nm). AFM images (Figure 7) of the polymer micelles with QDs encapsulated also showed the spherical morphology and showed a diameter range of 20-150 nm in size. The size of QDs encapsulated micelle increased slightly when the block length of PLA increased from 1,000 to 2,000 (Figure 7). Based on the micellar structure and size it may be concluded that many QDs are located and encapsulated in the hydrophobic core of the polymer micelle, of which surface are made up of hydrated PEG shell that have an important role for good colloidal properties and biological stability.

Comparison of PL Intensities of QDs and QDs Encapsulated Micelles. Because QDs are encapsulated in the hydrophobic core of the micelles, it is reasonable to expect that the optical properties of the entrapped QDs in aqueous solution would be similar or superior to the optical properties of free single QD in chloroform. Figure 8 shows the emission of free quantum dots in chloroform and encapsulated QDs in water under the same QDs concentration (1 mg/mL). The sharp emission spectra and the emission band of both the red emission dots were well preserved following their encapsulation in the micelles.

In brief, a solid dispersion method was successfully utilized to prepare luminescent QDs encapsulated in the polymer micelles. This method has been broadly used to encapsulate various hydrophobic molecules, such as hydrophobic drugs (example: anticancer drug), in polymer micelles. The hydrophilic PEG outer layer may increase the aqueous

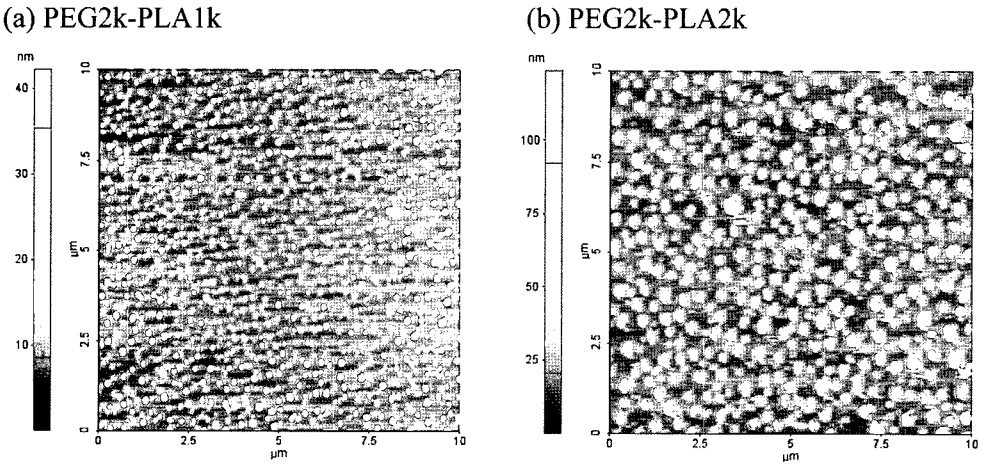

Figure 7. AFM images of PEG-PLA micelles with QDs encapsulated: (a) PEG2k-PLA1k and (b) PEG2k-PLA2k.

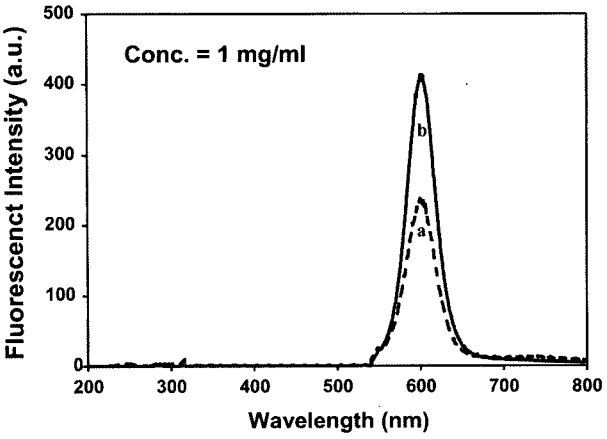

Figure 8. Comparison of emission spectra of free QDs in chloroform (a) and encapsulated QDs in micelles (b). The concentration was fixed to 1 mg/mL.

stability of the luminescent micelles and have an important role in preserving QDs biocompatible and stable against enzyme attack or metabolism. These newly developed stable luminescent micelles could be used as bright fluorescent labels in biological applications involving the conjugation of biomolecules such as enzymes, antibodies, and DNA molecules to the micelles. The luminescence intensity of single QD is low and cannot be observed with sufficient signal-to-background ratio using an ordinary fluorescence microscope. A laser is generally needed to excite the quantum dots. However, the intense laser beam required for excitation of the individual QD is likely to damage biological samples. The collective emission intensity from the polymer micelles with encapsulated QDs may increase the signal and the signal-to-background ratio considerably without significantly affecting the spatial resolution in the image, as the nanometric size of the micelle is well below the optical limit of diffraction.

Conclusions

There is a great deal of ongoing work to improve the synthesis of QDs and to develop a novel method for delivering QDs into the body or living cells. Our study showed that luminescent CdSe-ZnS QDs were successfully encapsulated in biodegradable amphiphilic PEG-PLA diblock copolymers for preparation of water-soluble and biocompatible QDs micelles. The critical point in our study is that we have prepared potentially a less toxic and more efficient QD formulation based polymer micelle for cellular imaging application. Future work within our group will be focused on biological application and single cell imaging *in vitro* and *in vivo*.

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References

(1) M. Han, X. Gao, J. Z. Su, and S. Nie, *Nat. Biotechnol.*, **19**, 631 (2001).
(2) X. Gao, C. W. Chan Warren, and S. Nie, *J. Biomed. Opt.*, **7**, 532 (2002).
(3) W. C. W. Chan, D. J. Maxwell, X. Gao, R. E. Bailey, M. Han, and S. Nie, *Curr. Opin. Biotechnol.*, **13**, 40 (2002).
(4) X. Wu, H. Liu, J. Liu, K. N. Haley, J. A. Treadway, J. P. Larson, N. Ge, F. Peale, and M. P. Bruchez, *Nat. Biotechnol.*, **21**, 41 (2003).
(5) H. Xu, M. Y. Sha, E. Y. Wong, J. Uphoff, Y. Xu, J. A. Treadway, A. Truong, E. O'Brien, S. Asquith, M. Stubbins, N. K. Spurr, E. H. Lai, and W. Mahoney, *Nucleic Acids Res.*, **31**, e43 (2003).
(6) P. S. Eastman, W. Ruan, M. Doctolero, R. Nuttall, G. D. Feo, J. S. Park, J. S. F. Chu, P. Cooke, J. W. Gray, S. Li, and F. F. Chen, *Nano Lett.*, **6**, 1059 (2006).
(7) A. P. Alivisatos, *Science*, **271**, 933 (1996).
(8) C. B. Murray, D. J. Norris, and M. G. Bawendi, *J. Am. Chem. Soc.*, **115**, 8706 (1993).
(9) M. A. Hines and P. Guyot-Sionnest, *J. Phys. Chem.*, **100**, 468 (1996).
(10) M. Bruchez, M. Moronne, P. Gin, S. Weiss, and A. P. Alivisatos, *Science*, **281**, 2013 (1998).
(11) Y. Wang, Z. Tang, M. A. Correa-Duarte, I. Pastoriza-Santos, M. Giersig, N. A. Kotov, and L. M. Liz-Marzan, *J. Phys. Chem. B.*, **108**, 15461 (2004).
(12) J. Zhuang, X. Zhang, G. Wang, D. Li, W. Yang, and T. Li, *J. Mater. Chem.*, **13**, 1853 (2003).
(13) R. Kho, C. L. Torres-Martinez, and R. K. Mehra, *J. Colloid Interf. Sci.*, **227**, 561 (2000).
(14) J. O. Winter, T. Y. Liu, B. A. Korgel, and C. E. Schmidt, *Adv. Mater.*, **13**, 1673 (2001).
(15) C. B. Murray, D. J. Norris, and M. G. Bawendi, *J. Am. Chem. Soc.*, **115**, 8706 (1993).
(16) Z. A. Peng and X. Peng, *J. Am. Chem. Soc.*, **123**, 183 (2001).
(17) W. W. Yu, Y. A. Wang, and X. Peng, *Chem. Mater.*, **15**, 4300 (2003).
(18) W. W. Yu and X. Peng, *Angew. Chem. Int. Ed.*, **41**, 2368 (2002).
(19) D. Battaglia and X. Peng, *Nano Lett.*, **2**, 1027 (2002).
(20) W. W. Yu, J. C. Falkner, B. S. Shih, and V. L. Colvin, *Chem. Mater.*, **16**, 3318 (2004).
(21) R. E. Bailey and S. Nie, *J. Am. Chem. Soc.*, **125**, 7100 (2003).
(22) S. Kim, Y. T. Lim, E. G. Soltesz, A. M. De Grand, J. Lee, A. Nakayama, J. A. Parker, T. Mihaljevic, R. G. Laurence, D. M. Dor, L. H. Cohn, M. G. Bawendi, and J. V. Frangioni, *Nat. Biotechnol.*, **22**, 93 (2004).
(23) X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir, and S. Weiss, *Science*, **307**, 538 (2005).

(24) X. Peng, J. Wickham, and A. P. Alivisatos, *J. Am. Chem. Soc.*, **120**, 5343 (1998).
(25) H. Mattoussi, *et al.*, *J. Am. Chem. Soc.*, **122**, 12142 (2000).
(26) D. Gerion, *et al.*, *J. Phys. Chem. B*, **105**, 8861 (2001).
(27) C. Allen, D. Maysinger, and A. Eisenberg, *Colloid Surface B*, **13**, 3 (1999).
(28) Y. P. Jung, Y. K. Son, and J. H. Kim, *Macromol. Res.*, **15**, 82 (2007).
(29) L. Qu and X. Peng, *J. Am. Chem. Soc.*, **124**, 2049 (2002).