J|A|C|S
COMMUNICATIONS

Published on Web 02/07/2006

Size Series of Small Indium Arsenide−Zinc Selenide Core−Shell Nanocrystals and Their Application to In Vivo Imaging

John P. Zimmer,[†] Sang-Wook Kim,[§] Shunsuke Ohnishi,[‡] Eichii Tanaka,[‡] John V. Frangioni,[‡] and Moungi G. Bawendi*,[†]

Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, Division of Hematology/Oncology and Department of Radiology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, Massachusetts 02215, and Department of Molecular Science and Technology, Ajou University, 5 Wonchun-Dong Yeongtong-Gu Suwon, Korea 443-749

Received November 23, 2005; E-mail: mgb@mit.edu

Semiconductor nanocrystals (or quantum dots, QDs) are excellent fluorophores due to their continuous absorption profiles at wavelengths to the blue of the band edge, high photostability, and narrow, tunable emission peaks. For in vivo biological imaging applications, the QD emission wavelength should ideally be in a region of the spectrum where blood and tissue absorb minimally but detectors are still efficient, approximately 700−900 nm in the near-infrared (NIR).[1] In addition, the hydrodynamic size of the QD should be appropriately matched to the biological experiment of interest.[2] In previous work, for example, we described the efficacy of Type II QDs with hydrodynamic diameters (HD) of 15.8−18.8 nm to map sentinel lymph nodes (SLN) selectively.[2a] Here we report the synthesis of a size series of (InAs)ZnSe (core)shell QDs that emit in the near-infrared and exhibit HD < 10 nm. We demonstrate their utility in vivo by imaging multiple, sequential lymph nodes and showing extravasation from the vasculature in rat models, neither of which has been achieved before with QDs to our knowledge.

While InAs QDs are known, most studies report emission wavelengths longer than 800 nm.[3] Until now, only Battaglia and Peng have shown well-defined InAs first absorption peaks at wavelengths below 800 nm.[4] Their work, however, primarily concerned InP QDs. We have developed a procedure for the synthesis of a well-characterized size series of small InAs cores (diameters < 2 nm). Moreover, we have extended the work to show the overcoating of these very small cores with a second, higher band gap semiconductor shell. Zinc selenide was chosen as the ideal shell material due to its reasonably small lattice mismatch with zinc blende InAs (6.44%), its high bulk band offsets (1.26 and 0.99 eV for the conduction and valence bands, CB and VB, respectively), and its reported ability to increase the quantum yield (QY) of InAs cores by more than an order of magnitude.[3e] Longer emission wavelengths, particularly the biologically desirable 800−840 nm range, can be achieved by increasing (1) the core size or (2) the shell thickness, or (3) by altering the band offsets between core and shell such as by adding a small amount of Cd to the ZnSe shell. Therefore, by varying the core size, and the shell thickness or composition, a wide tunability of the final emission wavelength was obtained, ranging from 750 to 920 nm. Due to the perceived undesirability of Cd for in vivo imaging, however, we generally obtained longer wavelengths by the first two options. In addition, these materials were water solubilized and exhibited stable emission in serum at 37 °C over 6 h. Quantum yields for (InAs)ZnSe (core)-shell QDs were 7−10% in hexane and 6−9% in water. While these QYs were lower than typical QYs for II−VI semiconductor QDs,

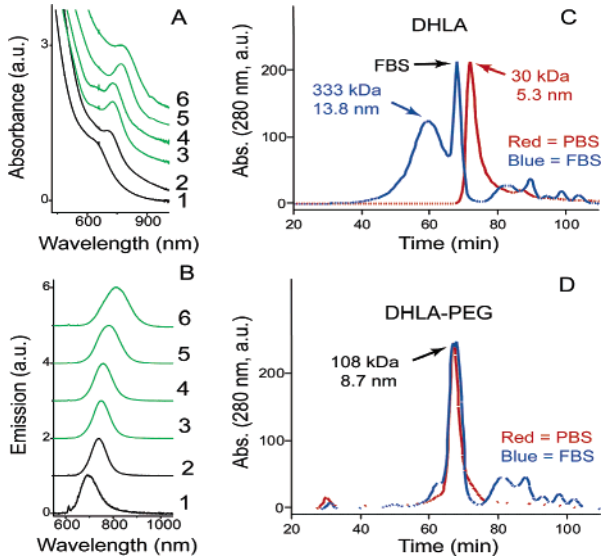

Figure 1. (A) Absorption and (B) emission spectra of InAs (black) and (InAs)ZnSe (green) QDs, where the numbered spectra correspond to QDs with first absorption, emission peak, and fwhm of (1) 645, 694, 85 nm; (2) 710, 739, 77 nm; (3) 726, 749, 82 nm; (4) 731, 757, 85 nm; (5) 768, 781, 109 nm; (6) 771, 812, 121 nm. C and D show gel filtration data for QDs coated with DHLA and DHLA−PEG, respectively.

such as (CdSe)ZnS, they were not unusual for III−V QDs, which tend to be less bright.[3e] In addition, lower QYs are expected for smaller QDs due to the relatively high percentage of the total volume contributed by surface unit cells vis-à-vis larger QDs of the same material. For in vivo imaging, we found that QYs as low as 1−2% were sufficient. Figure 1(A, B) shows optical data for a size series of InAs and (InAs)ZnSe QDs. Without the ZnSe shell, the InAs QDs were very unstable to air or water and rapidly lost quantum efficiency even in hexane solutions stored under ambient laboratory conditions. The addition of a ZnSe shell permitted the maintenance of emission intensity, and its presence was confirmed by wavelength dispersive spectroscopy (WDS) and transmission electron microscopy (TEM).

For their use in vivo, the (InAs)ZnSe QDs were water solubilized with either dihydrolipoic acid (DHLA) or dihydrolipoic acid conjugated to a short poly(ethylene glycol) ($n = 8$; DHLA-PEG) through a carbodiimide coupling scheme.[5] With DHLA alone, the HD in phosphate buffered saline (PBS) was only 5.3 nm, but nonspecific protein binding in fetal bovine serum (FBS) increased the HD to 13.8 nm, according to gel filtration chromatography (Figure 1C). The DHLA−PEG ligand, however, afforded aqueous solutions of QDs which did not aggregate or bind protein in serum,

[†] Massachusetts Institute of Technology.
[‡] Beth Israel Deaconess Medical Center.
[§] Ajou University.

10.1021/ja0579816 CCC: $33.50

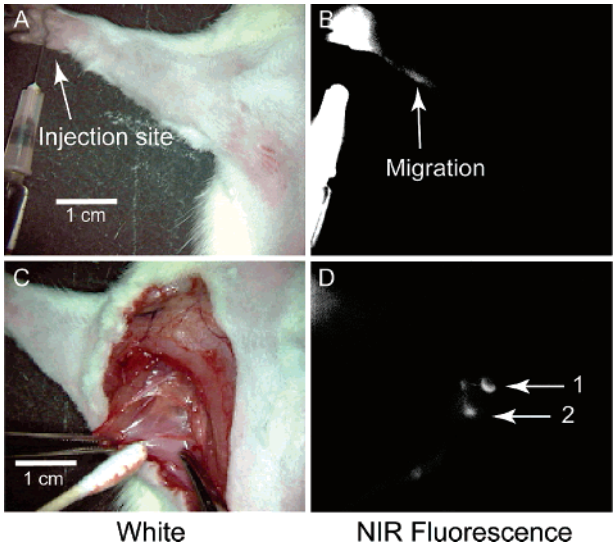

Figure 2. Sequential lymph nodes (1 and 2) and the lymphatic channel between them were imaged (C, D) in a rat by white light and NIR fluorescence 5 min after injection of the QDs (A, B).

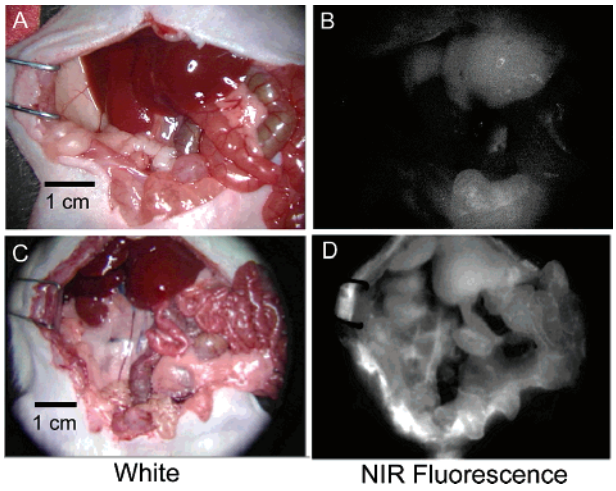

Figure 3. No fluorescence was seen in the interstitial fluid surrounding the incision in the rat model with DHLA-coated (InAs)ZnSe QDs (A, B). With DHLA−PEG, however, fluorescence was observed from extravasated QDs (C, D).

giving a total HD of 8.7 nm (Figure 1D). This is much smaller than the sizes reported for other QDs used in vivo, which range from about 15 to 30 nm in buffer.[6−9] Any additional or nonspecific protein binding would further increase the effective sizes of these QD probes in vivo.

The unusually small HD obtained with our combination of small QDs and ligands resistant to nonspecific protein binding allowed us to observe new in vivo behavior. When injected subcutaneously into the paw of a mouse or rat, the QDs rapidly migrated to the SLN, as we previously reported for two other QD formulations.[2a,10] As shown in Figure 2, however, the new QDs [(InAs)$Cd_xZn_{1-x}Se$ QDs in the figure, with similar results without Cd in the shell] did not get trapped completely in the SLN, but instead migrated further into the lymphatic system, imaging up to 5 lymph nodes sequentially, as well as the channels between nodes. We attribute this dramatic difference to the exceptionally small size of the DHLA or DHLA−PEG QDs.

A second new in vivo observation was the extravasation of the QDs from the vasculature when injected intravenously. The QDs, coated with DHLA−PEG, circulated for many minutes and were able to migrate out of the blood vessels and into the interstitial fluid, as seen in Figure 3. This is an important milestone toward the successful use of QDs as efficient and specific in vivo labels delivered intravenously because the first step in tissue penetration and labeling is extravasation. This critical behavior was not observed with QDs coated with DHLA alone, despite similar circulation times. We believe the increased size of DHLA QDs caused by nonspecific protein binding (Figure 1C) was responsible.

In summary, we have developed a size series of unusually small, water-soluble (InAs)ZnSe (core)shell QDs that emit in the NIR and demonstrate new behavior in vivo. The biological utility of these fluorescent probes resulted from our intentional choice to match the semiconductor material and water-soluble ligand with a desired final HD and emission wavelength.

Acknowledgment. This work was supported by NIH Grants R33-EB-000673 and R01-CA-115296, a CIMIT Application Development Award, the NSF-MRSEC and -CRC programs, and the DOE and ARO through the Institute for Collaborative Biotechnologies. We thank Neel Chatterjee for collecting WDS data, David Oertel for helpful discussions about the manuscript, and Alec De Grand for assistance with the figures.

Supporting Information Available: Experimental procedures, transmission electron microscopy data, and additional optical characterization, including emission stability in serum. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Lim, Y. T.; Kim, S.; Nakayama, A.; Stott, N. E.; Bawendi, M. G.; Frangioni, J. V. *Mol. Imaging* **2003**, *2*, 50−64.
(2) (a) Kim, S.; Lim, Y. T.; Soltesz, E. G.; De Grand, A. M.; Lee, J.; Nakayama, A.; Parker, J. A.; Mihaljevic, T.; Laurence, R. G.; Dor, D. M.; Cohn, L. H.; Bawendi, M. G.; Frangioni, J. V. *Nat. Biotechnol*. **2004**, *22*, 93−97. (b) Osaki, F.; Kanamori, T.; Sando, S.; Sera, T.; Aoyama, Y. *J. Am. Chem. Soc*. **2004**, *126*, 6520−6521.
(3) (a) Uchida, H.; Matsunaga, T.; Yoneyama, H.; Sakata, T.; Mori, H.; Sasaki, T. *Chem. Mater*. **1993**, *5*, 716−719. (b) Guzelian, A. A.; Banin, U.; Kadavanich, A. V.; Peng, X.; Alivisatos, A. P. *Appl. Phys. Lett*. **1996**, *69*, 1432−1434. (c) Peng, X.; Wickham, J.; Alivisatos, A. P. *J. Am. Chem. Soc*. **1998**, *120*, 5343−5344. (d) Cao, Y.; Banin, U. *Angew. Chem., Int. Ed*. **1999**, *38*, 3692−3694. (e) Cao, Y.; Banin, U. *J. Am. Chem. Soc*. **2000**, *122*, 9692−9702. (f) Green, M.; Norager, S.; Moriarty, P.; Motevalli, M.; O'Brien, P. *J. Mater. Chem*. **2000**, *10*, 1939−1943. (g) Lu, J.; Wei, S.; Yu, W.; Zhang, H.; Qian, Y. *Inorg. Chem*. **2004**, *43*, 4543−4545.
(4) Battaglia, D.; Peng, X. *Nano Lett*. **2002**, *2*, 1027−1030.
(5) (a) Mattoussi, H.; Mauro, J. M.; Goldman, E. R.; Anderson, G. P.; Sundar, V. C.; Mikulec, F. V.; Bawendi, M. G. *J. Am. Chem. Soc*. **2000**, *122*, 12142−12150. (b) Uyeda, H. T.; Medintz, I. L.; Jaiswal, J. K.; Simon, S. M.; Mattoussi, H. *J. Am. Chem. Soc*. **2005**, *127*, 3870−3878.
(6) Wu, X.; Liu, H.; Liu, J.; Haley, K. N.; Treadway, J. A.; Larson, J. P.; Ge, N.; Peale, F.; Bruchez, M. P. *Nat. Biotechnol*. **2003**, *21*, 41−46.
(7) Ishii, D.; Kinbara, K.; Ishida, Y.; Ishii, N.; Okochi, M.; Yohda, M.; Aida, T. *Nature* **2003**, *423*, 628−632.
(8) Guo, W.; Li, J. J.; Wang, Y. A.; Peng, X. *Chem. Mater*. **2003**, *15*, 3125−3133.
(9) (a) Jaiswal, J. K.; Mattoussi, H.; Mauro, J. M.; Simon, S. M. *Nat. Biotechnol*. **2003**, *21*, 47−51. (b) Pellegrino, T.; Manna, L.; Kudera, S.; Liedl, T.; Koktysh, D.; Rogach, A. L.; Keller, S.; Rädler, J.; Natile, G.; Parak, W. J. *Nano Lett*. **2004**, *4*, 703−707. (c) Gao, X.; Cui, Y.; Levenson, R. M.; Chung, L. W. K.; Nie, S. *Nat. Biotechnol*. **2004**, *22*, 969−976.
(10) Kim, S.-W.; Zimmer, J. P.; Ohnishi, S.; Tracy, J. B.; Frangioni, J. V.; Bawendi, M. G. *J. Am. Chem. Soc*. **2005**, *127*, 10526−10532.

JA0579816