Imaging Pancreatic Cancer Using Surface-Functionalized Quantum Dots

Jun Qian,¹,‡ Ken-Tye Yong,¹ Indrajit Roy,¹ Tymish Y. Ohulchanskyy,⁶ Earl J. Bergey,⁷ Hoon Hi Lee,¹ Kenneth M. Tramposch,¹ Sailing He,³ Anirban Maitra,³ and Paras N. Prasad*,†,‡

Institute for Lasers, Photonics, and Biophotonics, University at Buffalo, The State University of New York, Buffalo, New York 14260-4200, Centre for Optical and Electromagnetic Research, Zhejiang University, Hangzhou 310058, China, and Department of Pathology and Oncology, The Sol Goldman Pancreatic Cancer Research Center, CRB-2, Suite 345, Johns Hopkins University School of Medicine, 1550 Orleans Street, Baltimore, Maryland 21231

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In this study, CdSe/CdS/ZnS quantum dots (QDs) were used as optical contrast agent for imaging pancreatic cancer cells in vitro using transferrin and anti-Claudin-4 as targeting ligands. CdSe/CdS/ZnS was chosen because the CdSe/CdS/ZnS QDs have better photoluminescence (PL) efficiency and stability than those of CdSe/ZnS. The transferrin-mediated targeting is demonstrated in both a cell-free coprecipitation assay as well as using in vitro confocal microscopy. Pancreatic cancer specific uptake is also demonstrated using the monoclonal antibody anti-Claudin-4. This targeted QD platform will be further modified for the purpose of developing as an early detection imaging tool for pancreatic cancer.

Pancreatic cancer is the fourth most common cause of cancer-related mortality in the United States and a disease of near-uniform lethality. The vast majority of patients present with locally advanced or distant metastatic disease, rendering their malignancy surgically inoperable. The best option currently available for ameliorating pancreatic cancer survival is to diagnose the neoplasm at an early, and therefore, potentially curable stage.¹,² It is crucial that new generation of robust image contrast agents be developed, which can be specifically targeted to pancreatic cancer in vivo, leading to improved diagnosis at an early stage. Nanosized materials, containing one or more diagnostic probes, and linked with cancer-specific targeting ligands, are ideally suited for this purpose.³–⁵ Targeting of these imaging modalities to pancreatic cancer, even at an early stage of development, can be achieved with the help of proteins/peptides directed against overexpressed surface receptors on the cancer cells/tissues such as the transferrin receptor and the antigen Claudin-4.

Semiconductor quantum dots (QDs) are slowly replacing molecular fluorophores as optical contrast agents by virtue of their significantly improved spectral stability as well as tenability.⁶ Ever since the first reports of their use as biomarkers nearly eight years ago, a wide variety of biological applications have been demonstrated using quantum dots, including those with emission in the near infrared (NIR) range. The biological applications of QDs include robust tumor targeting in vitro and in vivo, long-term in vivo observation of cell-trafficking, and study of intracellular events within the resolution of a single molecule, to name a few. The main reason for their versatility is their rich surface chemistry in the aqueous phase, which makes it possible to incorporate a wide spectrum of biomolecules (protein, peptide, DNA, etc.) designed for performing a specific function.

In this paper, we report the use of bioconjugated CdSe/CdS/ZnS QDs as contrast agent for labeling human pancreatic cancer cells. CdSe/CdS/ZnS QDs were chosen because they possess superior photoluminescence (PL) efficiency and stability than those of CdSe/ZnS QDs.⁷,⁸ Transferrin (Tf), an iron-transporting protein, was utilized for the synthesis of QD bioconjugates. The lysine-coated CdSe/CdS/ZnS QDs were conjugated with Tf by using carbodiimide chemistry, as reported previously.⁹ To date, no study has been reported demonstrating the use of CdSe/CdS/ZnS QD–Tf conjugates as targeted optical probes for live pancreatic cancer cell imaging. With confocal microscopy, we demonstrate the receptor-mediated uptake of QD–Tf conjugates into pancreatic cancer cell lines, which are known to overexpress the transferrin receptor (TfR). More importantly, we have also demonstrated specific targeting of human pancreatic cancer cells by conjugating QDs with a pancreatic cancer specific monoclonal antibody, anti-Claudin-4.

CdSe/CdS/ZnS QDs were prepared by growing a CdS/ZnS graded shell on a CdSe core.¹⁰–¹² Briefly, the reaction was carried out with CdSe core QDs corresponding to ~0.4 g, using a Cd:Zn:S ratio of 1:3:4 (0.5 mmol Cd, 1.5 mmol Zn, and 2 mmol S), and 10 mL of oleic acid at ~280 °C. The CdS initially prefers to grow on the CdSe dots because the CdSe lattice mismatch with CdS is less than that of ZnS. The CdS layer will mediate the growth of the more strained ZnS.¹² The shell growth is uniform and epitelial and eventually coats the CdSe core. The QDs were separated from the surfactant solution by the addition of ethanol and centrifugation. The reddish QD precipitate could be readily dispersed in various organic solvents (hexane, toluene, and chloroform). The dissolution of
QDs in aqueous phase was accomplished by adapting the method of Jiang et al. First, the aqueous-compatible surfactant mercaptoundecanoic acid (MUA) was used to displace the existing hydrophobic surfactant molecules on the QD surface, thereby rendering them soluble in dimethyl sulfoxide (DMSO). Then, lysine was used to cross-link with the carboxylic acid terminating ends of the MUA on the QD surface, which generates an organic shell around the QDs. These lysine-coated QDs served as a multifunctional platform for further conjugation of biomolecules. The details of the preparation procedure can be found in the Supporting Information.

Both organically soluble and water-soluble lysine-coated CdSe/CdS/ZnS QDs were characterized by transmission electron microscopy (TEM), powder X-ray diffraction (XRD), and energy-dispersive X-ray spectroscopy (EDS). TEM samples were prepared by drop-coating the sample dispersion onto an ultrathin carbon-coated copper grid. A concentrated QD dispersion was drop-cast onto a glass plate for XRD measurement. Figure 1a shows the TEM image of uncoated CdSe/CdS/ZnS QDs. The particles are monodispersed, with a typical diameter of ~6.2 nm. Figure 1b shows the TEM image of the lysine-coated QDs. As seen in the image, the size of the QDs remains unchanged upon aqueous dispersion. Furthermore, no aggregation was observed after cross-linking the lysine with the MUA-coated QDs. Figure 1c shows the XRD patterns of the as-synthesized (uncoated) CdSe/CdS/ZnS QDs. The particles are monodispersed, with a typical diameter of ~6.2 nm. The particles are monodispersed, with a typical diameter of ~6.2 nm. Figure 1a shows the TEM image of uncoated CdSe/CdS/ZnS QDs. The particles are monodispersed, with a typical diameter of ~6.2 nm. Figure 1b shows the TEM image of the lysine-coated QDs. As seen in the image, the size of the QDs remains unchanged upon aqueous dispersion. Furthermore, no aggregation was observed after cross-linking the lysine with the MUA-coated QDs. Figure 1c shows the XRD patterns of the as-synthesized (uncoated) CdSe/CdS/ZnS QDs. All the diffraction peaks can be readily indexed to the wurtzite structure of CdSe. The intensity of the (002) diffraction peak is much stronger than that of all other peaks, suggesting that the CdSe/CdS/ZnS QDs have a strong preferential orientation along the [001] direction. Further analysis of the QDs using EDS has confirmed the presence of Cd, Zn, Se, and S elements (see Supporting Information).

The optical properties of these QDs were examined by UV-vis absorbance and PL spectroscopy (see Supporting Information). The absorption spectrum features an excitonic peak around 610 nm, and the PL spectrum shows a band edge emission at 624 nm. The fluorescence quantum yield of the QDs is estimated to be 55%. The quantum yield of the QDs was obtained by comparing their emission with absorption-matched solution of rhodamine 6G. The obtained QY values are sufficiently high for cell-labeling studies. The lysine-coated CdSe/CdS/ZnS QD solution did not show any significant decrease in the fluorescence intensity even after storing them for three weeks. These stored QDs can be used for bioconjugation and cell labeling without showing any sign of quenching effects.

To perform cell-labeling studies, the lysine-coated QDs were first conjugated with Tf, as described in the Supporting Information. To validate the presence of Tf on the surface of the QDs in a cell-free assay, the QD bioconjugates were coprecipitated with immunobeads made up of Sepharose-conjugated anti-transferrin affibody. The preparation of these immunobeads is described in the Supporting Information. The lysine-coated QDs, with and without conjugated Tf, were mixed and incubated with the immunobeads for 1 h at room temperature, followed by centrifugation at 3000 rpm for 5 min to precipitate the beads (it is known that free QDs do not settle down at such a low-speed centrifugation). The pellets were washed three times with PBS and photographed following irradiation with an UV torch, as shown in the Supporting Information. The specific attachment of the Tf-coated QDs with the immunobeads can be observed from the strong emission of the pellets (left tube). In comparison, the emission from the pellets obtained from reacting the immunobeads with lysine-coated QDs are weak (right tube), indicating some weak nonspecific interaction with the immunobeads.
Figure 2. Confocal microscopic images of MiaPaCa-2 cells treated with (a) lysine-coated CdSe/CdS/ZnS QDs and (b,c) Tf-conjugated CdSe/CdS/ZnS QDs. Cells in (c) were saturated with free Tf prior to treatment with Tf-QD bioconjugates. The panel on the left displays the transmission images, and their corresponding fluorescence images are shown in the middle panel. The panel on the right shows the overlays of fluorescence and transmission images.

Figure 3. Confocal microscopic images of MiaPaCa-2 cells treated with (a) lysine-coated CdSe/CdS/ZnS QDs and (b) anti-Claudin-4-conjugated CdSe/CdS/ZnS QDs. The panel on the left displays the transmission images, and their corresponding fluorescence images are shown in the middle panel. The panel on the right shows the overlays of fluorescence and transmission images.
For in vitro imaging with quantum dots, we used three human pancreatic cancer cell lines (Panc-1, MiaPaCa-2, and CoLo-357), obtained as described previously\cite{16} and maintained in DMEM medium with 10% fetal bovine serum (FBS) and appropriate antibiotic. The day prior to treatment, cells were seeded in 35 mm cell culture dishes at 70–80% confluence. On the day of treatment, the cells were incubated with the Tf-conjugated quantum dots at a final concentration of ~1.4 μg/mL for 1 h at 37°C. Control dishes were treated with free Tf (~2 mg/mL) for 1 h before treatment with the Tf-conjugated QDs in order to “minimize” available TIRs on the cell surface. Unconjugated QDs was incubated with the cells and served as another control. After 1 h, the cells were washed thrice with PBS and directly imaged using a laser scanning confocal microscope (MRC-1024, Bio-Rad, Richmond, CA), which was attached to an upright microscope (Nikon model Eclipse E800).

Figure 2 demonstrates representative confocal images of MiaPaCa-2 pancreatic cancer cells stained with QD–Tf bioconjugates. From the confocal microscopic images, the Tf-mediated targeting can be observed by the robust optical signal from the cells treated with Tf-conjugated QDs (Figure 2b) as opposed to the complete absence of signal from the cells treated with the nonconjugated QDs (Figure 2a). Furthermore, the specific nature of the Tf-mediated targeting is demonstrated, where the blocking of the Tf receptors on the cells by pretreatment with free Tf resulted in significantly low uptake of the Tf-coated QDs (Figure 2c). Comparable trends in terms of preferential uptake of surface-functionalized QDs were also observed for Panc-1 and CoLo-357 pancreatic cancer cell lines (data not shown).

In addition to transferrin conjugation, QDs were also conjugated with a monoclonal antibody for specific targeting of human pancreatic cancer cells. The antibody used here is anti-Claudin-4, which is known to be overexpressed in both primary and metastatic pancreatic cancer.\cite{17} Figure 3 shows confocal images of MiaPaCa-2 pancreatic cancer cells labeled with QD–anti-Claudin-4 bioconjugates. As clearly seen on the images (panel b), a strong uptake of the QD–anti-Claudin-4 bioconjugates was observed, as opposed to the unconjugated QDs, which showed no uptake. The developed bioconjugates may serve as a potential targeted nanoprobe to specifically diagnose human pancreatic cancer cells.

In summary, we demonstrate that CdSe/CdS/ZnS QDs can be used for specifically targeting pancreatic cancer cells in vitro following conjugation with targeting molecules like transferrin and anti-Claudin-4. This work will serve as a solid foundation for future studies involving detection of primary and metastasized tumors in an orthotopic mouse model of pancreatic cancer.

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**Supporting Information Available:** Detailed materials and experimental methods. EDS spectrum from CdSe/CdS/ZnS Quantum dots. UV–Vis absorbance and fluorescence spectra of quantum dots. Emission from immunobeads coupled with quantum dot bioconjugates. This material is available free of charge via the Internet at http://pubs.acs.org.

**References and Notes**