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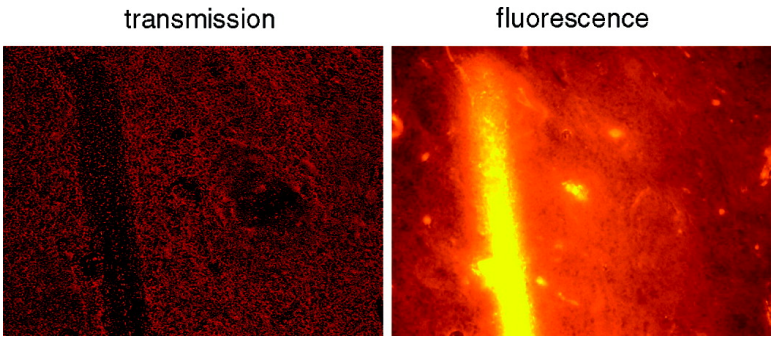

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Synthesis of Water-Dispersible Fluorescent, Radio-Opaque, and Paramagnetic CdS:Mn/ZnS Quantum Dots: A Multifunctional Probe for Bioimaging

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Over the past several years, fluorescent quantum dots (Qdots) have been well studied and have shown tremendous potential in labeling biological entities such as cells, tissues, and biohazard particles (bacteria, viruses, etc.). Qdots stand out from conventional organic-based dyes in at least two aspects: photostability and sensitivity.[1] Because of their hydrophobic surface property, an appropriate surface coating is necessary to disperse Qdots in aqueous solution. Coating also protects them from photoinitiated surface degradation, which is directly related to fading of fluorescence intensity and toxicity. Despite recently reported toxic effects of quantum dots,[2] both in vitro[1,3] and in vivo[4] studies have been reported in favor of using Qdots for biolabeling applications, including in vivo disease diagnosis.[5]

In the visible range, because of the limitation of low signal penetration capability of Qdot fluorescence through living tissue, other types of optical probes, such as near infrared (NIR) dyes,[6] NIR Qdots,[7] and up-converting phosphors,[8] have attracted attention recently. It is expected that by using these probes, a signal from a few millimeter deep tissue (such as skin cancers) could be detected noninvasively. These NIR probes, however, will not be suitable for the detection of brain tumors. It is unlikely that an optical signal will pass through the skull, severely limiting any brain-related application of these optical probes.

For in vivo biolabeling applications, it is desired to incorporate additional properties such as radio-opacity and paramagnetism in the same probe (multifunctional probe). This will allow noninvasive tumor diagnosis using computer tomography (CT) scan and/or magnetic resonance imaging (MRI) scan before performing the surgery. Multifunctional probes with both fluorescence and paramagnetic properties have been reported recently in the literature.[9] These probes were synthesized by incorporating fluorescent and magnetic components separately into a biodegradable polymer matrix. In these cases, organic dyes were used as the fluorescent component, which may not be stable in an in vivo environment.[10]

We report a single-step synthesis protocol for producing novel, highly water-dispersible, multifunctional, 3.1-nm-size CdS:Mn/ZnS core−shell Qdots using water-in-oil (W/O) microemulsion method. These Qdots are fluorescent, radio-opaque, paramagnetic, and suitable for attaching biomolecules such as DNA, proteins, peptides, antibodies, etc. and are extremely stable in an in vivo environment.[11] We engineered these Qdots specifically for in vivo applications, considering a few important imaging aspects: (i) endovascular administration of Qdot formulation could be monitored under fluoroscopic guidance using X-ray, (ii) after the administration of Qdot formulation, CT scan and/or MRI scan will allow pre-operative diagnosis of the labeled target, and (iii) for certain applications (such as tumor resection), Qdot fluorescence would allow real-time visualization of labeled target tissue (such as tumors).

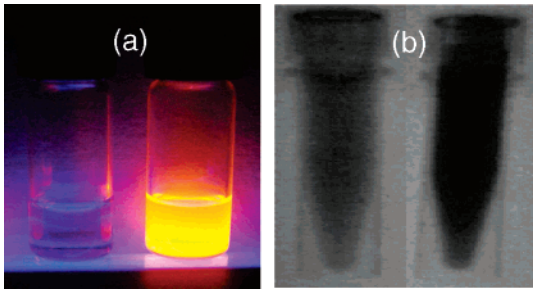

Figure 1. (a) Fluorescence image of yellow-emitting Qdots (right) and DI water (left) under a 366-nm multiband handheld UV source (Spectroline, model UV-4B). (b) Fluoroscopy images of Qdots (left) and Omnipaque (right) of equal concentration (20 mg/mL) under the same magnification and X-ray power (OEC 9600 C-Arm).

The Qdots were first synthesized following Yang and Holloway's synthesis protocol.[12] To make Qdots suitable for bioconjugation, a further modification[13] was made to obtain primary amine groups on the Qdot surface. The hydrolysis and co-condensation reaction of tetraethyl orthosilicate, 3-(aminopropyl) triethoxysilane (APTS), and 3-(trihydroxysilyl)propyl methylphosphonate (THPMP) produced a highly water-dispersible silica layer around each Qdot. Pure silica-coated Qdots are water-dispersible. At neutral pH, the zeta potential (ξ) of silica-coated Qdots is about −42 mV. The negative potential is due to the presence of deprotonated silanol groups ($Si-O^-$) on the Qdot surface ($pK_a = 7.0$). Upon amine modification with APTS, the ξ value drops close to the isoelectric point ($\xi \approx 0.5$). This is due to the protonation of primary amine groups ($pK_a = 9.0$) resulting in severe particle aggregation. The addition of THPMP ($pK_a = 2.0$) recovers the Qdot aqueous dispersibility by increasing ξ value to about −36 mV.

Multifunctional properties of Qdots were characterized by fluorescence, radio-opacity, and magnetic measurements. Bright yellow emission is clearly observed by using a 366-nm multiband handheld UV light source (Figure 1a).[14] For radio-opacity (X-ray contrast) measurement, a Qdot sample was placed under a fluoroscope that uses X-ray excitation. To compare radio-opacity, the Qdot sample was compared with a conventional radio-opaque material (Omnipaque, also called iohexol, a contrast agent for CT scan and angiography) at the same concentration. From Figure 1b, it was estimated that the X-ray absorption of Qdots is less than that of Omnipaque, which provides a sufficient contrast for current practice, and we are attempting to improve the contrast. We expect

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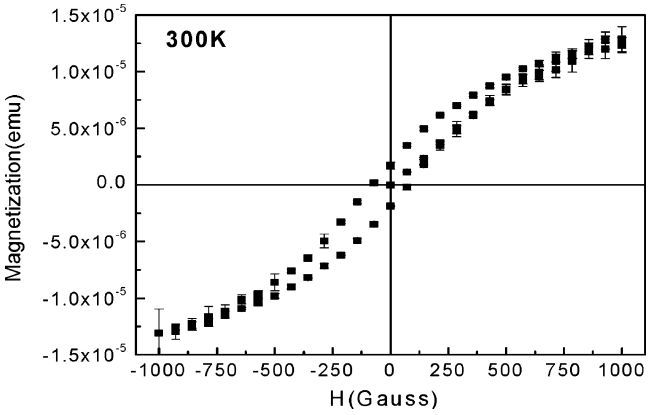

Figure 2. Magnetization curve for Qdots.

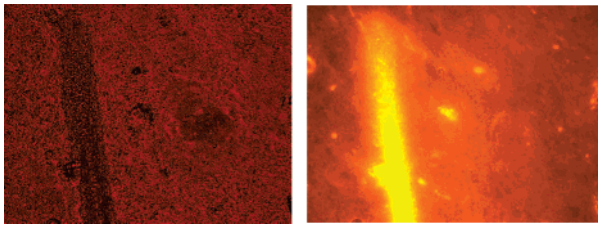

Figure 3. Branches of right middle cerebral artery: Transmission (left) and fluorescence (right) images of a cross section of rat brain (magnification 40×). A quantity of 0.75 mL (10 mg/mL) of TAT-conjugated Qdots was administered intra-arterially over a 3-min time period.

that similar contrast will also be seen under a CT scan. Using a superconducting quantum interference device (SQUID) magnetometer, we have performed magnetic measurement of Qdots. At room temperature, Qdots showed paramagnetic property (Figure 2) with a typical hysteresis curve for paramagnetic material at room temperature. The paramagnetic property of Qdots can be utilized to obtain contrast in an MRI scan in an in vivo setup.

Qdot fluorescence has been well-explained previously.[12,15] The bright yellow fluorescence is a result of an efficient surface passivation by the epitaxially matched ZnS crystalline layer around the CdS:Mn crystalline core. Radio-opacity of the Qdot is due to the presence of the electron dense cadmium atoms, which interact with the X-ray beam. Manganese ions (Mn^{2+}) present in Qdots are paramagnetic and responsible for the magnetic property.

To demonstrate in vivo bioimaging capability, amine-modified Qdots were conjugated to TAT peptide (a cell penetrating peptide) by modifying Josephson et al.'s protocol.[16] TAT-conjugated Qdots in phosphate buffer saline (pH 7.4) were then administered through the right common carotid artery that supplies blood only to the right part of the rat's brain. After completing the procedure, we then sliced the whole brain into four pieces and sent the pieces for histological analysis. Figure 3 confirmed labeling of branches of the right middle cerebral artery, a distal branch of the internal carotid artery, in the brain and confirmed the efficacy of the labeling protocol. No labeling occurred on the left brain hemisphere (control experiment). In this study, we aimed to selectively label brain blood vessels using an endovascular approach. It is well-understood that the blood−brain barrier (BBB, a tight junction of endothelial cells) protects the brain from toxic substances that are present in the blood circulation. The TAT peptide can penetrate the BBB without disruption. Using a TAT-mediated delivery system, it is thus possible to deliver diagnostic and therapeutic agents to the brain without compromising the BBB. From our in vivo experiment, it was clearly seen that TAT-conjugated Qdots stained brain parenchyma and blood vessels with higher concentration at the vessel wall.

In conclusion, we have shown for the first time that ultra-small (3.1 nm) multifunctional semiconductor quantum dots (Qdots), which possess fluorescent, radio-opacity, and paramagnetic properties in a single unit, may be synthesized. For bioconjugation, we have aminated the Qdot surface, completely prevented particle agglomeration, and formed a stable aqueous suspension. We have conjugated Qdots with a TAT peptide. Using a rat model, we have successfully administered a Qdot suspension endovascularly. Histological analysis (using a fluorescence microscope) of the microtome-sliced brain tissue clearly demonstrated the labeling efficacy of the Qdots. Because of their multifunctional properties, we believe that these Qdots will be widely used for in vivo biolabeling. Soon, we will explore their abilities to target tumors, including brain tumors, as well as real-time tracking of cells, such as stem cells or other engineered cells, for certain disease diagnosis and therapy.

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Supporting Information Available: Protocols for Qdot synthesis, water-dispersible amine functionalization, TAT peptide conjugation, and procedure for the animal experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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