Silica-coated quantum dots and magnetic nanoparticles for bioimaging applications (Mini-Review)\textsuperscript{a)}

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Fluorescent quantum dots (e.g., CdSe–ZnS) and magnetic nanoparticles (e.g., Fe\textsubscript{3}O\textsubscript{4} or Fe\textsubscript{2}O\textsubscript{3}) are two important candidate systems that have been emerging as potential probes for bioimaging applications. This review focuses on the development of silica-coated inorganic probes (optical and magnetic) that are originated mainly from the author’s laboratory for bioimaging applications. The recent developments in the synthesis of rare earth nanoparticles for multimodality imaging are also delineated. © 2010 American Vacuum Society. [DOI: 10.1116/1.3516492]

I. INTRODUCTION

Inorganic nanoparticles (NPs) for biomedical applications have advanced rapidly in recent years due to their excellent optical and magnetic properties. Over the past several years, quantum dots\textsuperscript{1–9} (QDs) and magnetic nanoparticles\textsuperscript{10–13} (MPs) have been emerging as inorganic based optical and magnetic bioprobes, respectively. The magnetofluorescent NPs are potential candidate systems for in vivo imaging, which can be detected by imaging techniques such as magnetic resonance imaging (MRI), fluorescence imaging, tomography, confocal microscopy, and flow cytometry.

Highly fluorescent semiconductor QDs (e.g., CdSe–ZnS) have emerged as a potential fluorescent label owing to their remarkable optical properties. Compared to fluorescent dyes, QDs do not have the setback of photobleaching, and their emission colors can be tuned from visible to near-infrared (NIR) region by varying the size or composition of QDs. A significant research effort has been devoted to prepare core-shell type QDs with a cross-linked shell that would protect the QDs much better than thiol-based coating. The most widely used approach is silica coating\textsuperscript{14–19} among other methods such as ligand or polymer bridging.\textsuperscript{20} Although a variety of functional NPs or QDs have been synthesized by silica or polymer coating, each coating method has inherent advantages and limitations. It would be ideal to have a thin, cross-linked coating that could protect the core, improve colloidal stability, and introduce chemical functionality for bioconjugation.\textsuperscript{21} Efforts are still underway in various groups in order to make a library of robust functional NPs.

Conversely, MPs are used as contrast agents in MRI applications. Generally, there are two types of contrast agents (positive and negative), which are dictated by the shortening of longitudinal or transverse relaxation times of water protons, resulting in either brightening or darkening of magnetic resonance images for T\textsubscript{1} and T\textsubscript{2} weighted imaging, respectively. The typical examples of T\textsubscript{2} contrast agents are γ-Fe\textsubscript{2}O\textsubscript{3} (maghemite), Fe\textsubscript{3}O\textsubscript{4} (magnetite), Co, and MFe\textsubscript{2}O\textsubscript{4} (M=Ni, Co, Fe, and Mn), and those of T\textsubscript{1} contrast agents are gadolinium (Gd)-chelates [Gd-diethylene triamine penta-acetic acid (DTPA)] and Gd\textsubscript{2}O\textsubscript{3}.

The bifunctional probes offer great advantages for optical and magnetic based imaging applications. The magnetofluorescent NPs, or in short magnetic QDs, are useful for fluorescence based labeling applications, magnetic based cell harvesting, tracking, and drug targeting, and MRI applications. The synthesis of these bimodal magnetic-fluorescent probes has received great interest in recent years. Over the years, we have been interested in the assemblies of QDs or MPs within a shell consisting of either silica or polymers. Herein, we briefly review our recent work on the progress of silica-coated QDs and MPs for biolabeling and MRI applications. We also highlight the recent demonstration of rare earth (RE) based down- and up-conversion NPs (UCNPs) for multimodality imaging.

II. FLUORESCENT QUANTUM DOTS FOR CELL LABELING

There are three important stages before the QDs can be utilized for biological applications: (a) synthesis, (b) coating, and (c) surface functionalization or bioconjugation, as depicted in Scheme 1. This scheme is common for metal NPs (e.g., Au and Ag) and semiconducting QDs (CdSe/ZnS) for applications in biosensing and bioimaging, respectively.

A. Synthesis and coating strategies

In general, the high-temperature organometallic route produces high-quality QDs. The as-synthesized QDs are not water-soluble as they are capped with organic surfactants such as trioctylphosphine oxide (TOPO) and hexadecyl amine. Nevertheless, for practical biological applications, the hydrophobic QD surface needs to be rendered hydrophilic and amenable to surface modification and functionalization. Silica surface meets these requirements. However, because of the ultrasmall sizes of QDs, it is extremely difficult to achieve silica coating of single QDs via the Stöber process.

Water-soluble functional NPs are indispensable for various biomedical applications. However, the synthesis of ro-
bust functional NPs is very challenging, since most of the
good synthetic methods available for noble metal, QD, and
magnetic oxides produce hydrophobic NPs because of hy-
drophobic surfactant coating. Thus, water solubilization and
functionalization are the key issues prior to their application
and here lies the significance of coating.\textsuperscript{20,22} The coating
helps convert hydrophobic NPs into hydrophilic water-
soluble particles and introduces chemical functionality to the
particle surface so that different chemicals and biomolecules
can be covalently attached. There are two common coating
strategies to convert hydrophobic NPs into hydrophilic and
functional NPs. The first approach involves the ligand ex-
change of the original surfactant by hydrophilic ligands such
as thiols or other functional groups.\textsuperscript{23} Thiol-based ligand ex-
change is most common for noble metal NPs compared to
other systems. This is because thiol makes a strong chemi-
sorption on noble metal surface. In addition, various ap-
proaches of thiol-based methods were developed to make a
stable coating, which involves the use of ligands with either
multiple thiols, thiolated dendrimers, dendrons, or cross-
linking of surface ligands.\textsuperscript{1,10}

The second approach involves the interdigitated bilayer for-
mation between amphiphilic molecules/polymer and the
passivating surfactant layer surrounding NPs.\textsuperscript{24} These
approaches have been successfully applied to noble metal NPs,
in comparison with iron oxide MPs and QDs. Several meth-
ods exist in the literature on the design of water-soluble
QDs.\textsuperscript{24–27} One method involves an organic coating using ei-
ther polymers,\textsuperscript{25} micelles,\textsuperscript{26} or thiols\textsuperscript{27} as the linker mol-
ecules. Another method is based on the well-known silica
chemistry developed for coating metal NPs.\textsuperscript{28}

\section*{B. Direct coating of hydrophobic semiconductor QDs}

Silica coating is one of the facile approaches to render the
QDs with characteristic properties such as water solubility,
m moderate buffer stability (more stable under alkaline condi-
tions), and photostability.\textsuperscript{14} There are two ways to prepare
silica-coated QDs: (a) thin silica coating (<10 nm) by si-
lanization and (b) thick silica coating (>10 nm) by the
Stöber method. Earlier, we synthesized 20–30 nm silica-
coated CdSe/ZnS QDs via a reverse microemulsion.\textsuperscript{5}

The as-synthesized hydrophobic ZnS-capped CdSe QDs
can be coated with silica in a direct one-pot reverse micro-
emulsion method, employing Igepal as the nonionic surfactant.\textsuperscript{5} Initially, reverse micelles were synthesized using
Igepal and cyclohexane as nonionic surfactant and solvent,
respectively. We have developed a simple strategy for mak-
ing plain CdSe QDs (without ZnS capping) water-soluble by
silica coating. The surfactant interaction prior to silica coat-
ing allows the hydrophobic QDs to be encapsulated within the
aqueous domains of the reverse microemulsion (Fig. 1). This
involves the hydrophilic groups of TOPO and Igepal
CO-520, which are surfactants present on the QD surface and
in the reverse microemulsion, respectively.

This direct silica coating approach has enabled a wide
variety of hydrophobic NPs or QDs (e.g., CdSe and PbSe),
magnetic NPs (e.g., Fe\textsubscript{3}O\textsubscript{4}), and bifunctional NPs or het-
erodimers (e.g., CdSe–ZnS/Fe\textsubscript{3}O\textsubscript{4}) to be encapsulated within
spherical silica particles.\textsuperscript{3,6,19,26} At first, NPs have to be trans-
ferred to the hydrophilic interior of micelles where silica
growth takes place. The particle bearing a hydrophobic sur-
face has to be exchanged with a hydrophilic ligand. The
mechanism of transfer is clearly elucidated in a recent
article.\textsuperscript{30} The TOPO-capped QDs were introduced to the
reverse micelles, where Igepal could be exchanged partially or
completely with TOPO. The addition of a base, ammonia,
forms a reverse microemulsion [Fig. 1(a)]. The QDs are still
present in the oil phase. After the addition of a silane [teta-
ethoxy silane (TEOS)], hydrolysis and condensation occur,
resulting in the encapsulation of QDs within a silica shell, as
depicted in Fig. 1(b). This simple silica coating strategy en-
abled us to prepare water-soluble, plain CdSe QDs (without
ZnS capping). The typical transmission electron microscopy
(TEM) image of silica-coated QDs is shown in Fig. 1(c).

\section*{C. Live cell imaging}

Recently, we developed silica-coated QDs for live cell imag-
ing.\textsuperscript{6} Silanization in reverse microemulsion produced a
thin silica coating on bare CdSe QDs with surface NH\textsubscript{2}
groups [Fig. 2(a)]. The addition of aminopropyl triethoxysi-
lane (APS) introduced amine groups that were attached to
the surface of QDs. With the sequential addition of tetram-
ethylammonium hydroxide in 2-propanol/methanol and wa-
ter, a reverse microemulsion was formed. The methoxy
groups of APS were hydrolyzed and condensed with another APS, exposing surface amine groups on the silanized QDs for conjugation with oleyl-O-poly(ethylene glycol) succinyl-N-hydroxysuccinimidyl ester, denoted as bioanchored membrane (BAM). The reaction between the amine group and NHS ester resulted in a covalent amide bond formation, leaving the exposed oleyl group for the effective targeting of cell membrane. Figures 2(b) and 2(c) show the confocal laser scanning microscopy (CLSM) images of different cell membranes labeled with BAM/SiO$_2$/CdSe QDs. The labeling of live cell membranes (HepG2 human liver cancer cells and NIH-3T3 mouse fibroblast cells) indicated the successful conjugation of silica-coated QDs with BAM.6

III. ADVANCES IN MULTIFUNCTIONAL NANOPIARTICLES

Multifunctional NPs have been actively explored for the enhancement of imaging, targeting, and delivery. In the field of biological and biomedical imaging, QDs and MPs have been enjoying greater roles in biolabeling and MRI, respectively. A combination of optical and magnetic properties in a single material would enable the simultaneous biolabeling/imaging and cell sorting/separation.

MPs less than 15 nm in size display superparamagnetic characteristics, which are important for applications such as MRI, magnetically guided site-specific drug delivery, and magnetic field assisted cancer therapy.33 In recent years, researchers have focused on the synthesis of highly uniform size iron oxide NPs, particularly magnetite (Fe$_3$O$_4$) and maghemite ($\gamma$-Fe$_2$O$_3$). We have reported a gram-scale synthesis of nearly monodispersed $\gamma$-Fe$_2$O$_3$ (magnemite) nanoclusters with a less expensive oxidant and without a hazardous iron pentacarbonyl [Fe(CO)$_5$] or iron acetylacetonate precursor.34 Biomagnetic NPs consisting of ferritin protein were conjugated to carboxyl-coated QDs (QD525, QD655, and QD800 from Invitrogen) using 1-ethyl-3-(3-
dimethylaminopropyl) carbodiimide hydrochloride coupling. To improve the biocompatibility of silica NPs, multiple MPs can be encapsulated within silica shells. Adapted from Ref. 29 with permission. Copyright 2005, ACS.

We have developed several approaches for the fabrication of multifunctional NPs. In the following sections, we describe the synthesis and application of (a) silica-coated nanocomposites of fluorescent QDs and MPs, (b) seed-mediated synthesis of fluorescent QDs and MPs, and (c) rare earth probes as multimodal contrast agents.

A. Silica-coated nanocomposites of fluorescent QDs and MPs

In 2005, we reported an elegant method to incorporate QDs and MPs within silica using a reverse microemulsion method. The scheme in Fig. 3(a) depicts the synthesis of silica-coated nanocomposites of MPs and QDs. Single or multiple MPs (Fig. 3(b)) can be encapsulated within each silica shell. The silica shell thickness can be altered by varying the TEOS concentration. Varying the amount of water and ammonia in the microemulsion also affected the silica shell thickness, since this altered the aqueous domain size. The encapsulated MPs are monodispersed (11.8 ± 1.3 nm in diameter) and multiple (single, double, and triple) MPs are clearly seen in the image [Fig. 3(b)].

B. Seed-mediated synthesis of fluorescent QDs and MPs

Bifunctional NPs consisting of QDs and MPs, known as magnetic quantum dots (MQDs), are emerging as a versatile system for both fluorescence and magnetic based applications. Recently, we have developed a seed-mediated synthesis of MQDs by growing CdSe QDs on Fe2O3 cores, yielding either heterodimers or a homogeneous dispersion of QDs around Fe2O3. This method allows for flexibility in tuning the optical and magnetic properties separately. At first, Fe2O3 MPs were synthesized in oleic acid and diocyl ether by the decomposition of iron pentacarbonyl. In the growth solution, CdSe QDs were allowed to grow for different time periods (1–5 min) to yield different dot sizes that corresponded to green, yellow, orange, and red emissions. The synthesis and purification procedures were detailed in Ref. 6.

In another interesting work, we have demonstrated a versatile one-pot approach for the synthesis of Fe2O3–CdSe MQDs with a high quantum yield of up to 42%. The as-synthesized nanocomposite particles remained stable in non-polar solvents, such as chloroform and hexane. Addition of methanol destabilized the suspension, and both QDs and MPs were attracted to a magnet placed close to the suspension [Figs. 4(a) and 4(b)]. When methanol was added, both the MPs and QDs were believed to be aggregated and separated by the magnet, due to either the presence of dimers or the formation of the hydrophobic bilayer, utilizing the interaction of the surfactants (octadecyl amine on the MP surface and TOPO on the QD surface). The aggregated particles, which were both fluorescent and magnetic, could be dispersed in chloroform [Fig. 4(c) inset]. The emission peaks became broader with an increase in growth time from 1–12 to 25–30 min [Fig. 4(c)], indicating that the particle aggregation was induced by either bilayer or heterodimer formation. It is important to note that different QD growth rates during the reaction period might also be responsible for emission-peak broadening.

C. RE NPs as multimodal contrast agents

For a wide range of biological applications, silica coating is an effective means to protect or modify the surface of RE
NPs. Reverse microemulsion has been conveniently employed to coat hydrophobic Y2O3,38 YF3,39 and NaYF4 (Ref. 40) NPs with silica. Multicolor spheres were produced by encapsulating organic dyes or QDs into the silica shell, and up-conversion fluorescence was generated based on fluorescence resonance energy transfer from the NaYF4 cores to organic dyes or QDs. The silica-coated NPs were dispersible in water and showed good colloidal and photochemical stability.

Silica coating of RE NPs also provides a facile route to construct multicolor NPs when organic dyes or other fluorescent QDs are encapsulated within the silica shell. Due to their sharp emission peaks and long life time, RE NPs act as good energy donors to organic dyes or QDs. A similar reverse microemulsion system was used to construct multicolor dye/QD doped NaYF4:Yb,Er/Tm at SiO2 NPs.40 In such NPs, NIR radiation absorbed by the up-conversion NPs (the core) at a single wavelength (980 nm) is converted to visible fluorescence, which in turn is absorbed by the down-conversion materials (embedded in the shell) to emit multicolor fluorescence. The characteristic visible colors from the dyes and QD605, which are generally excited under blue light, could clearly be seen when these NPs are excited with a 980 nm NIR laser.

Very recently, UCNPs are emerging as a new type of multimodal imaging probe for both optical imaging and MRI.40,41 The deep penetration depth of NIR excitation, excellent photostability, nonblinking, and absence of autofluorescence of UCNPs make them attractive imaging probes for applications such as targeting of tumor tissues in vivo and long-term cellular and animal imaging.

Very recently, we have reported a simple strategy for synthesizing paramagnetic-fluorescent ultranarrow gadolinium oxide nanorods (NRs) as multimodal contrast agents.12 The room temperature photoluminescence spectra of the Tb-doped Gd2O3 NRs excited at 235 nm showed down-conversion emission. The characteristic emission peaks of Tb ions appeared at 489, 545, 585, and 619 nm. To demonstrate the versatility of the RE ion doping approach for up-conversion emission, we doped the NRs with Yb and Er ions. The up-conversion luminescence of Yb-/Er-codoped Gd2O3 NRs showed green emissions at 520 and 539 nm at 980 nm excitation. The room temperature magnetization of Gd2O3:RE (RE=Tb, Yb/Er), as a function of applied field (from −10 to +10 kOe), showed a linear correlation with a magnetization value of 2.46 emu/g (at 10 kOe), suggesting that the Gd2O3:RE NRs are paramagnetic.

Down-conversion and up-conversion fluorescence can be achieved by changing the lanthanide dopants, as shown in Figs. 5(a) and 5(b). Furthermore, the Yb-/Er-codoped Gd2O3 NPs exhibited good T1-weighted MRI contrast, comparable to the commercial product Gadovist [Fig. 5(c)].

IV. CONCLUDING REMARKS

Over the decade, the application of QDs in biolabeling has been emerging as a matured technology especially in cell based imaging. Cadmium is basically carcinogenic, and therefore, the in vivo applications still concern the toxicity of QDs. Robust coating methods have shown the nontoxicity of QDs in in vitro studies. However, the size, charge, and coating material of QDs dictate the cell uptake and clearance as demonstrated in recent studies.43,44 Multifunctional NPs possessing fluorescent, magnetic, and targeting functionalities are useful in biomedical research. Although there are considerable advances in recent years, the application of multifunctional NPs in in vivo imaging is still in its infancy. This invites the development of more multifunctional systems with less toxic fluorescent probes, excluding carcinogenic elements such as Cd and Pb. The size of the NPs plays a critical role in cellular uptake and tumor-targeting. If the size is small (<10 nm), the NPs could be excreted from the animal body easily. In order to minimize the overall size of QDs or multifunctional NPs, a thin hydrophilic shell should be grown. The reverse microemulsion method can be employed to achieve a thin silica or polymer shell, which can be further functionalized with biomolecules of interest. The RE-doped
UCNPs are emerging as an alternative candidate system for bioimaging applications. The challenge that we face in the future would involve the development of “smart” contrast agents that are capable of monitoring specific cellular and molecular events in vivo. Our current work is focused on the development of versatile multifunctional paramagnetic NPs for multimodality imaging in a number of clinical pathologies such as early cancer diagnosis and cellular trafficking in stem cell therapy and immunological interventions. The targeted delivery of drugs is an important area in health care. By conjugating multiple components such as fluorescent QDs or dyes or UCNPs, tumor-targeting groups, anticancer drugs, or siRNA to the MPs, future work would seek to provide solutions to early cancer diagnosis and targeted delivery of therapeutics.

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