Photochemistry and Photobiology, 2006, 82: 617–625

Invited Review

Quantum Dot-based Energy Transfer: Perspectives and Potential for Applications in Photodynamic Therapy

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Received 11 May 2005; accepted 10 February 2006; published online 13 February 2006 DOI: 10.1562/2005-05-11-IR-525

ABSTRACT

Quantum dots have emerged as an important class of material that offers great promise to a diverse range of applications ranging from energy conversion to biomedicine. Here, we review the potential of using quantum dots and quantum dot conjugates as sensitizers for photodynamic therapy (PDT). The photophysics of singlet oxygen generation in relation to quantum dot-based energy transfer is discussed and the possibility of using quantum dots as photosensitizer in PDT is assessed, including their current limitations to applications in biological systems. The biggest advantage of quantum dots over molecular photosensitizers that comes into perspective is their tunable optical properties and surface chemistries. Recent developments in the preparation and photophysical characterization of quantum dot energy transfer processes are also presented in this review, to provide insights on the future direction of quantum dot-based photosensitization studies from the viewpoint of our ongoing research.

INTRODUCTION

Over the past years semiconductor quantum dots have attracted great interest due to their unique photophysical properties, which are attractive to a diverse range of applications that extends from energy conversion to biomedicine (1–10). In the biomedical arena quantum dots are now heavily studied for their usefulness as imaging agents (11–20). Photodynamic therapy is thereby a newer application that lends itself for the exploration of quantum dots as photosensitizers (21–23).

Since its discovery in the early 1900s (24–28), PDT has developed to an emerging cancer treatment that grew to an FDA-approved therapy for different malignancies (29–34) and has demonstrated potential in the treatment of other ailments such as coronary heart disease, AIDS and psoriasis (35–36). The growing popularity of this therapeutic method can be attributed to its highly selective nature of eradicating diseased tissues, which is based on the localized generation of cytotoxic singlet oxygen, following the activation of a non-toxic photosensitizer with light (37). A first-generation photosensitizer that has been accepted for clinical use is the hematophorphyrin derivative, Photofrin® (38). Despite the clinical success of Photofrin, some of its disadvantages like prolonged cutaneous photosensitivity, chemical impurity and weak absorption at therapeutic wavelengths (39), have inspired the development of new PDT photosensitizers with improved optical and chemical properties. Many second-generation photosensitizers related to chlorins, bacteriochlorins and phthalocyanines have been reported and are undergoing clinical trials. A number of excellent reviews have been written discussing the advantages and disadvantages of these newly developed molecular photosensitizers (39–43). In this review, we focus our discussion on conjugates with one of these promising second-generation photosensitizers, the phthalocyanines (Pcs), which are currently being evaluated for quantum dot-based PDT applications in our research context (21).

Pc derivatives have favorable photophysical and chemical properties, which include strong absorbance at long wavelengths and chemical tunability through substituent addition on the periphery of the macrocycle or on the axial ligands (34,37). However, like most photosensitizing agents, Pcs have poor solubility in water and tend to aggregate in aqueous solutions, which can result in loss of photochemical activity and affect their cell penetrating properties (37,44,45). To resolve such issues nanoparticles are currently being explored as potential delivery systems for PDT photosensitizers or directly as PDT agents (21,44–46). Gold nanoparticles coated with a zinc-Pc photosensitizer were prepared to facilitate the development of a hydrophilic three-component nanoparticle system (photosensitizer-gold-transfer agent), which was shown to generate singlet oxygen with enhanced efficiency as compared to the free Pc (44). Along this direction, silica-based nanoparticles were recently developed to entrap water-insoluble photosensitizing agents and were shown to be an effective PDT drug carrier in aqueous media (45). These developments underline the potential of nanoparticle-based PDT for cancer therapy applications.

Among the different nanomaterials that offer great promise in PDT applications are semiconductor quantum dots. Quantum dots (QDs) in the size range of 1–6 nm are not quite clusters or bulk materials/crystals, and their size compare well to biological molecules. Furthermore, the size of QDs give them unique optical properties that can be tuned from the UV to the infrared region by changing their size, shape and composition (5). The tunability of emission properties results from quantum-confinement effects that

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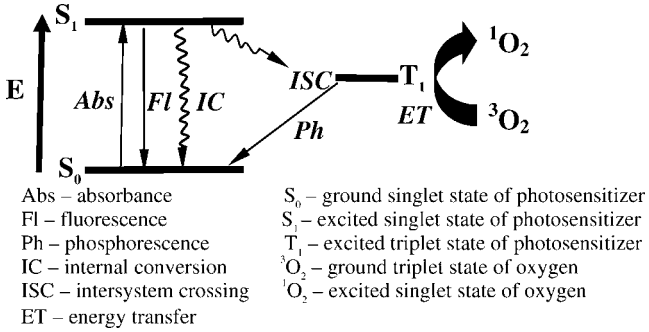

Figure 1. Simplified Jablonski diagram of singlet oxygen generation in PDT.

can be translated into the Near-IR region as opposed to most conventional photosensitizers. Since there is minimal light scattering and absorption in the Near-IR region of the spectrum, light of relatively low intensity can be used to penetrate tissue to depths of several centimeters, thereby allowing access to deeper-seated tumors. Furthermore, due to their large transition dipole moment, QDs are strong absorbers, making them suitable agents for PDT applications (21). More importantly, the surface coating of QDs can be functionalized to make them water soluble, biocompatible, target specific (47–49), and overall good materials for biomedical applications.

To date, most of the articles written about semiconductor QDs in the area of biomedicine have focused on their use in fluorescence imaging applications (49). Here, we aim to present QDs in light of their potential as photosensitizers in PDT-related applications. Due to the continuously growing interest in QD research, it is thereby possible that we have overlooked some recent contributions to the field and we apologize to anybody that we were not able to mention in this paper.

PHOTOSENSITIZED SINGLET OXYGEN GENERATION

Singlet oxygen ($^1O_2$) is regarded as the main mediator of photo-induced cytotoxicity in PDT, which causes oxidation and degradation of cellular components, and ultimately cell apoptosis (31–33). During PDT, $^1O_2$ is generated in the diseased cells by a simple and controllable light-activated process, which involves a photosensitizer that is capable of absorbing light of an appropriate wavelength and utilizing that energy to excite oxygen to its singlet state. The photophysics of this $^1O_2$ generation process is illustrated in a simplified Jablonski diagram in Fig. 1.

Following irradiation with light of suitable wavelength, the photosensitizer is excited from the ground state (S_0) to the first excited singlet state (S_1). Intersystem crossing subsequently generates the sensitizer's excited triplet state (T_1). The lifetime of the T_1 state (μs) is longer than that of the S_1 state (ns), which favors the interaction of this excited state with surrounding molecules (50–52). Generally, the T_1 state reacts in one of two ways, classified as Type I and Type II mechanisms (53). A Type I mechanism involves hydrogen-atom abstraction or electron-transfer between the excited sensitizer and a substrate, a solvent or another sensitizer, to yield free radicals. These radicals can react with molecular oxygen to generate reactive oxygen species such as the superoxide radical anion. By contrast, the Type II mechanism, which is regarded as the main process occurring in PDT, involves an energy transfer between the excited T_1 state of the sensitizer and the triplet ground state of molecular oxygen ($^3O_2$), to generate $^1O_2$ that is in turn used in therapeutic treatments. Following its generation, $^1O_2$ can cause irreversible damage to various cell constituents including mitochondria, lysosomes and membranes (32,33), which often result in protein modifications (33). Ultimately, the target tissue may be eradicated with little or no damage to the surrounding normal tissues.

The ability of a photosensitizer to generate singlet oxygen is measured by a quantum yield, which is denoted as Φ_Δ and defined as $\Phi_\Delta = \Phi_T\phi_{en} = \Phi_T(k_{en}[^3O_2]/k_r + k_{nr} + k_q[^3O_2])$ where, Φ_T is the quantum yield of the triplet formation; ϕ_{en} is the efficiency of energy transfer; k_{en} is the rate constant of energy transfer; k_r and k_{nr} are the rate constants of radiative and non-radiative relaxation processes of the T_1 state, respectively; and k_q is the sum of the rate constants from the quenching of the photosensitizer's T_1 state by oxygen (53).

To date several methods have been developed to enable the measurement of the quantum yield of singlet oxygen generation. Commonly used techniques involve the direct detection of the luminescence produced upon radiative relaxation of singlet oxygen at 1240 nm, which includes both steady-state and time-resolved infrared luminescence techniques; calorimetric methods such as photoacoustic calorimetry and time-resolved thermal sensing; and quantitative analysis of photooxidation reactions that involves the measurement of oxygen uptake or the loss of absorbance or the fluorescence of probe molecules (54,55). For detailed description of these techniques the readers are referred to the excellent reviews written by Wilkinson *et al.* (54) and by Redmond and Gamlin (55).

QUANTUM DOTS AS PHOTOSENSITIZERS

The investigation of the photophysical properties of semiconductor QDs has been a major research area for over two decades (1–10). Following their initial preparation in 1982 by Brus *et al.* (9), significant progress has been achieved both experimentally and theoretically towards the understanding of the unique properties of these nanosized materials. Weller was the first to present the quantum confinement properties of semiconductor QDs in a review article (8), which introduced the concept of a nanocrystal and outlined the new opportunities afforded through their use. Alivisatos (3) and Bawendi (4) were among the pioneers in exploring the optoelectronic properties of these novel semiconductor nanoscale materials, and Murray *et al.* were the first to develop a novel and highly reproducible method for the synthesis of monodisperse QDs with outstanding optical quality (10). Since then, several groups have exploited the photophysical properties of QDs for biological imaging and diagnostic applications (29,30, 46–48).

In addition, QDs can participate in fluorescence resonance energy transfer (FRET) based assays (56,57). Compared to their molecular fluorophore counterparts, QDs exhibit long term photostability and highly tunable emission properties making them ideal donor species in FRET studies. Bawendi *et al.* were among the first groups to report on the energy transfer process in semiconductor QDs (58,59). Their findings demonstrate a resonance transfer of electronic excitation occurring between close packed CdSe QDs consisting of two distinct sizes, which results in the quenching of the luminescence of the small dots at 3.85 nm accompanied by the enhancement of the luminescence of the larger dots at 6.2 nm (58,59). Since then various protein and DNA assays utilizing QD-based FRET have been explored by several groups (60–65).

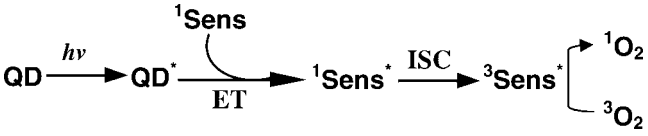

Figure 2. Schematic diagram of the energy transfer photoprocess between a QD and a conventional molecular photosensitizer (Sens), resulting in an indirect generation of reactive oxygen species ($^1O_2$).

Recently, we discovered the potential of QDs as possible therapeutic agents for PDT applications (21). QDs posses several characteristics that make them potentially good photosensitizers for PDT. Most importantly, they can serve as energy donors to conventional photosensitizers through FRET or interact directly with molecular oxygen via energy transfer (ET) mechanisms, to generate reactive $^1O_2$ species that can be exploited for PDT (21) (Fig. 2).

Energy transfer to a molecular PDT photosensitizer

Recently, we have linked CdSe QDs to a known silicon Pc photosensitizer (Pc4) that is currently undergoing clinical trials (21). Following excitation of the QD-Pc4 conjugate at 488 nm, we observed the indirect excitation of Pc4 through a FRET mechanism from the QD to Pc4, and the Pc4 emission was observed at 680 nm (Fig. 3a). By monitoring the decrease in emission intensity of the energy donor (QD) a 77% FRET efficiency was determined. The CdSe QD emission at 568 nm was used to activate the Pc4 photosensitizer and the combination of semiconductor QDs and PDT photosensitizers enabled the use of an excitation wavelength where the molecular photosensitizer alone does not absorb (Fig. 3b). To our knowledge, this was the first demonstration of utilizing QD-based FRET to facilitate excitation of a PDT photosensitizer, which is known to generate reactive $^1O_2$ species available for photodynamic cancer therapy. Since the QD exhibits a broad absorption spectrum (Fig. 3b), its conjugation to the Pc4 photosensitizer provides the flexibility of utilizing variable excitation wavelengths to activate the sensitizer molecule. Moreover, the spectral properties of the QDs can be adjusted to match those of any PDT photosensitizer by simply adjusting the size, shape and composition of the QD.

The linking of the photosensitizer to the QD is facilitated by the binding of an axial amine group of the phthalocyanine molecule to the CdSe nanoparticle. We have recently investigated the dynamics of this QD-Pc FRET pair formation process and found that it needs a 24-hour period to reach the maximum energy transfer efficiency between the conjugate pair (Fig. 4).

Furthermore we found an optimum ratio of [Pc]/[QD] equal to 3 for maximum energy transfer efficiency. In addition, we have extended our studies to other phthalocyanine homologues with varying linker chain lengths and terminal groups in order to study the photophysics of the energy transfer process between the QD and the Pc molecule. To date, our studies have focused on Pcs with different linker chain lengths and we observed variable energy transfer efficiencies ranging from 23–77% for the different QD-Pc FRET pairs (Fig. 5). More types of QD-Pc FRET pairs will be investigated in order to assess the distance dependence of the energy transfer process in this system.

The dynamics of the energy transfer from QDs to a molecular PDT sensitizer was also recently investigated in our group via transient absorption pump-probe measurements with a time-resolution of 150 fs. These measurements reveal an energy transfer from the photoexcited QDs to the adsorbed phthalocyanine Pc4 in the picosecond time range (66). The fast dynamics are expected due to the close proximity of the energy donor-acceptor moieties. Shown in figure 6a are the transient absorption spectra and the early bleach at 575 nm from photoexcited CdSe QDs, which decay and transfer energy to an attached energy acceptor molecule Pc4 (66). The excitation of PC4 can be monitored as transient bleach at 675 nm. The involved kinetics is summarized in Fig. 6b (66). The wavelength used for the excitation of the QD-Pc4 pair was 400 nm where the phthalocyanine does not absorb and can only be excited via sensitization through the QD.

Energy transfer to molecular oxygen

In the process of investigating the 2-step energy transfer mechanism in the QD-Pc4-oxygen system, we discovered that semiconductor QDs alone can generate $^1O_2$ without a mediating molecular photosensitizer (21) (Fig. 7a). The radiative relaxation of $^1O_2$ causes emission at 1270 nm (67,68), which is monitored using laser-flash photolysis (Spectra Physics Pro 230 – MOPO 730) and a liquid nitrogen cooled Ge detector (North Coast EO-817P) positioned behind a 1200 nm long-pass filter, and coupled to a digital oscilloscope (LeCroy LT 342). Shown in Fig. 7b are the decay kinetics of the $^1O_2$ emission measured at 1270 nm after 488 nm excitation in toluene (21); in argon-purged solution (solid circles), and in oxygen-saturated solution (open circles). To evaluate the $^1O_2$ quantum yield in toluene, perinaphthenone (Aldrich, $\Phi_\Delta = 1.0$) was used as reference compound. Using CdSe QD with 65% emission quantum yield, the $^1O_2$ quantum yield was found to be

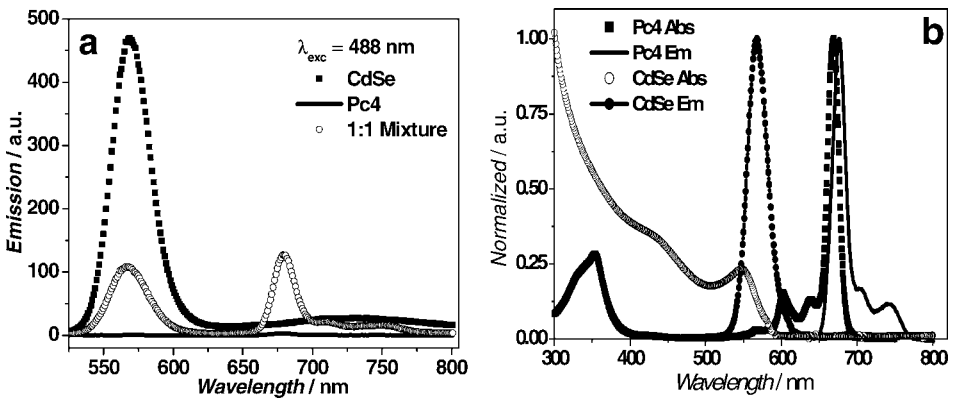

Figure 3. a) Indirect activation of a molecular PDT photosensitizer using 488 nm excitation through CdSe quantum dot assisted FRET. b) Spectral features of the free molecular sensitizer (Pc4) and CdSe quantum dot, respectively. Reprinted with permission from reference (21), Samia *et al., J. Am. Chem. Soc.* (2003), 125, 15736. © 2003, American Chemical Society.

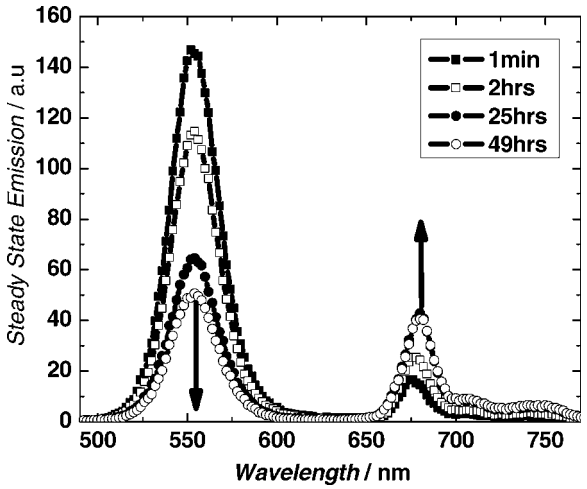

Figure 4. Steady state fluorescence measurement of the CdSe-Pc4 FRET pair at different formation times.

small (~5%) (21) in comparison to the Pc4 photosensitizer that was reported to have a $^1O_2$ efficiency of 43% (69).

In addition to the ability of a compound to efficiently generate $^1O_2$, an ideal photosensitizer must also posses several other important physical and chemical properties (22). A first criterion is that the compound should have a defined chemical composition. Currently used hematophorphyrin-based photosensitizers even in their purified form are a complex mixture of monomers, dimers, and higher oligomers (70). This inherent problem has resulted in large amounts of work devoted to the synthesis of single pure compounds that have well-established structures. A second criterion is that the compound should not posses any dark toxicity, *i.e*. it should be nontoxic in the absence of light. Third, it should be as selective as possible to the targeted tissues to avoid damage of healthy cells. Although new types of molecular photosensitizers have been prepared for selective targeting, their selectivity is not high enough for clinical applications and remains a major challenge in PDT (34). Fourth, it should be easily eliminated from the body to avoid significant side effects. To date, following the treatment with first-generation photosensitizers, the patient needs to remain in low-level lights for several weeks to prevent excessive burning from the photosensitizer remaining in the skin. It is therefore one of the goals of current research to develop a new generation of photosensitizer with low skin photosensitivity (34). A fifth requirement is that the compound should to some degree be water soluble to avoid aggregation. Most photosensitizer molecules are hydrophobic and can aggregate in aqueous media, which in turn results in the decrease of $^1O_2$ quantum yield (44–46). Sixth, the photosensitizer should be photostable and strongly absorbing at the excitation wavelength. The maximum transmittance of skin tissue is in the 700–800 nm region, and the development of a photosensitizer with a maximum absorbance in this region remains a major challenge. Photofrin, which is one of the two FDA-approved sensitizers is usually irradiated at 630 nm, which limits the penetration of light through the tissue to just a few mm (46). Additionally, molecular photosensitizers suffer from photobleaching after long exposure to light.

Interestingly, QDs have the potential to meet most of the criteria for good PDT photosensitizers, as mentioned above. They are species with well-defined size, shape, and composition, and can be synthesized by relatively simple and inexpensive methods. They

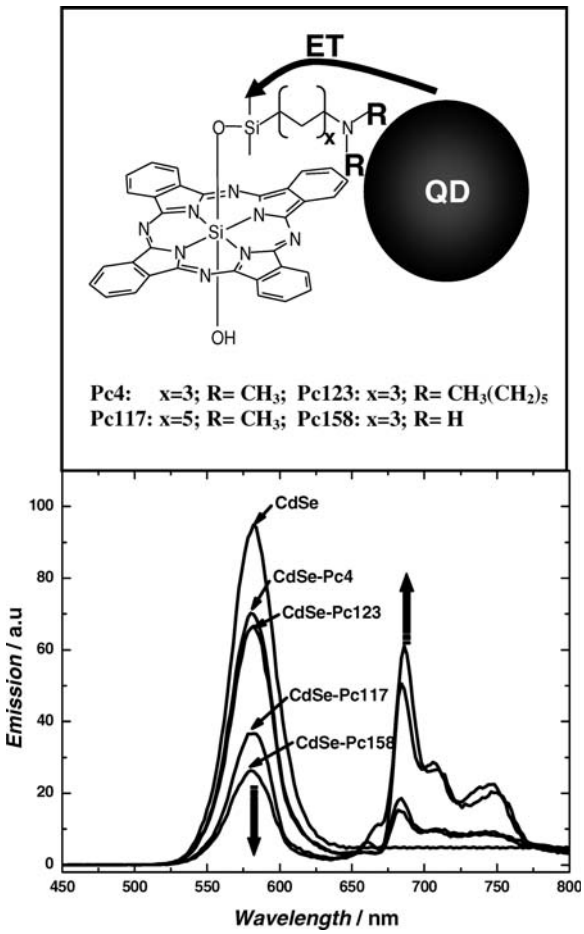

Figure 5. Steady state fluorescence measurement of different CdSe-Pc FRET pairs with varying linker chain lengths.

have been shown to be nontoxic in the absence of light but have the potential to be cytotoxic under irradiation. They have excellent photostability, and tunable and strong absorption, which can be tuned from the UV to the Near-IR spectral range depending upon their composition and size (6). The alloyed nanoparticles of Cd, Se and Te synthesized in our lab based on a method developed by Nie *et al*. (71) show extended absorption and emission wavelengths compared to their binary analogs as shown for one of the composition in Fig. 8. This property may prove to be particularly useful for PDT applications since only very few molecular sensitizers absorb in the therapeutic window of 600–800 nm, where our alloyed nanoparticles conversely exhibit strong absorption.

Furthermore, the surface coating of QDs can be modified to enable them to become water soluble, biocompatible and target-specific. However, despite the many desirable properties of QDs, there still remain several important issues that need to be addressed to fully assess their applicability as photosensitizers in PDT applications. One major issue is the toxicity profile of the QDs inside the cells and their overall photostability once exposed to biological environments. Another important matter that should be carefully investigated is how their surface composition affects the photosensitization process. The knowledge of which should provide valuable insights in improving the $^1O_2$ generation in QDs. So far we have reported a singlet oxygen yield of $\Phi_\Delta \cong 5\%$ for the case of trioctylphosphine oxide (TOPO) capped-CdSe QDs in

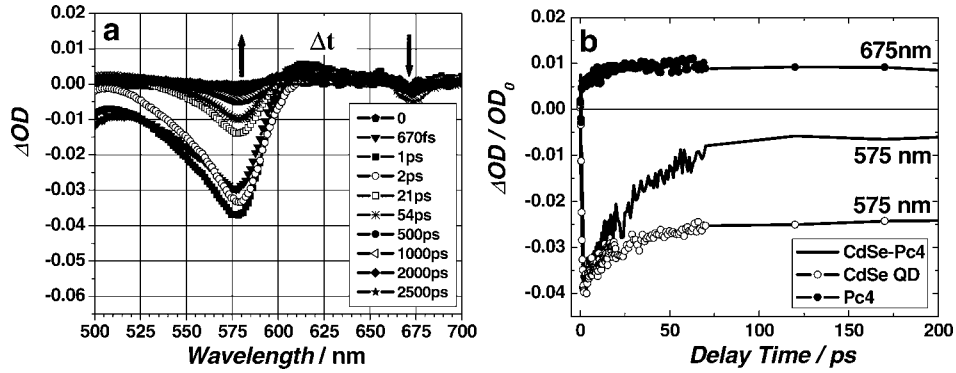

Figure 6. a) Transient absorption spectra of QD-Pc4 conjugates with QD bleach at 575 nm and a Pc4 bleach at 675 nm at different delay times (0–2500 ps). b) Kinetic traces obtained from the same set of measurements. (66).

toluene (21). The optimization of these properties is an ongoing research in our laboratory and in the following we aim to touch on these important aspects.

QUANTUM DOTS IN BIOLOGICAL SYSTEMS

Biocompatibility

QDs are usually synthesized in non-polar organic solvents, making the as-prepared samples insoluble in aqueous buffers. A first step to make them compatible for biological applications is to exchange their capping ligands with materials that will render them both water soluble and accessible for conjugation to target biomolecules. Over the past few years, a wide range of QD solubilization and functionalization strategies have been developed ranging from a simple ligand exchange method with bi-functional thiol molecules (48), dendrons (72), oligomeric phosphines (73) and peptides (15), to the more sophisticated ones involving encapsulation in polymer beads (16), block co-polymers (17), silica shells (11), and phospholipids micelles (47). All of these techniques result in either amine- or carboxy-terminated QDs, which can be cross-linked to biomolecules by means of standard bioconjugation reactions (11,15,48). Another approach utilizes interactions between QDs and proteins or adapter molecules. These functionalization steps can be repeated to add or change bio-functionality. For example, streptavidin-coated QDs can be used in combination with biotinylated proteins and antibodies, which can be designed to target specific cell types and organelles (18). As such, low concentrations of the functionalized QDs were found compatible for live-cell and small-animal labeling experiments, without any noticeable adverse effects in the period of several hours to a few days, within the duration of the experiment (19). However, at this stage, a great number of systematic experiments have yet to be conducted to fully assess QD transport and compatibility *in vivo*.

Delivery and transport

The effective delivery of QDs into cells and tissues will be a major issue for any future *in vivo* biomedical applications. No matter how potent any therapeutic agent is *in vitro*, if it cannot reach its site of action *in vivo*, it is useless. Furthermore, efficient delivery can also allow a reduction in dosage level, avoid non-specific side effects and reduce toxicity risks.

Many different technologies that have been devised to deliver drugs intracellularly can also be applied to QDs. Basically, these methods can be grouped into three major categories: 1. cellular internalization mechanisms; 2. physical or mechanical methods; and 3. biomolecule-assisted transport. They are briefly discussed in the following:

Cellular internalization mechanisms. Although it is possible for some hydrophobic molecules to pass directly through the cell membrane by a process of simple diffusion, most water-soluble

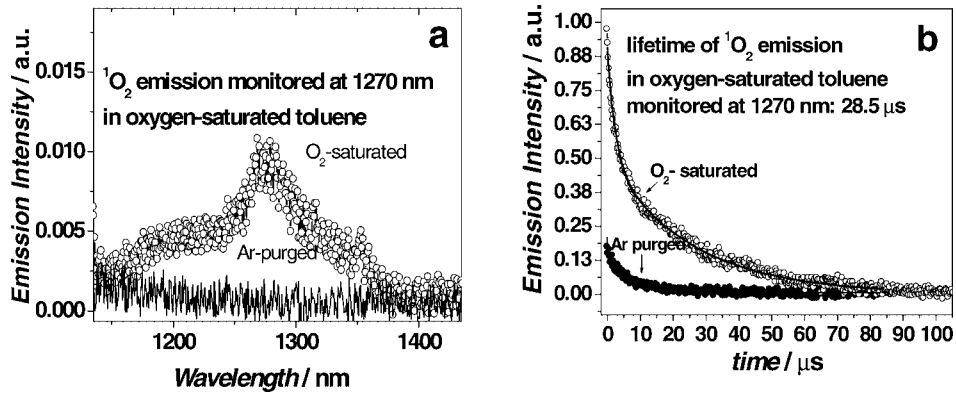

Figure 7. $^1O_2$ dynamics through semiconductor QDs in toluene solution following 488 nm excitation: a) Emission spectra of the $^1O_2$ generated from CdSe QDs; b) Decay kinetics of $^1O_2$ emission observed at 1270 nm. Reprinted with permission from reference (21), Samia *et al., J. Am. Chem. Soc.* (2003), 125, 15736. © 2003, American Chemical Society.

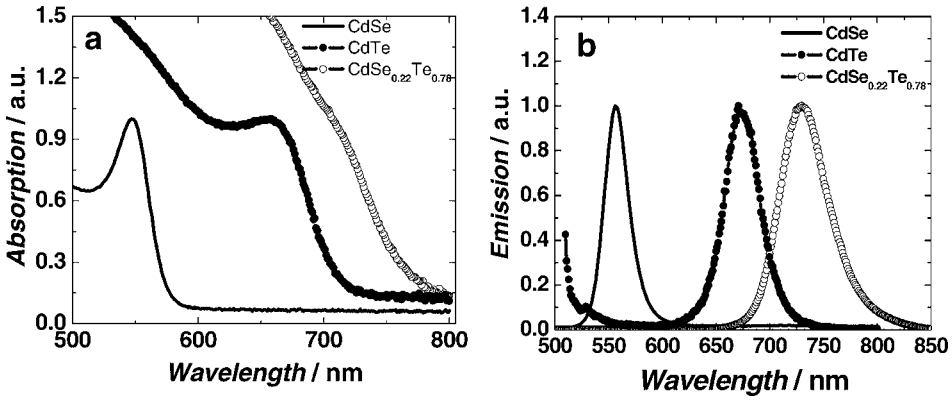

Figure 8. Absorption (a) and emission (b) spectra of CdSe, CdTe and $CdSe_{0.22}Te_{0.78}$ alloy nanoparticles.

substances need a means of facilitating their passage into the cell. Active transport is usually required to pump substances inside cells. This process uses ATP-derived energy to overcome the highly selective barrier of the cell membrane.

One such energy dependent mechanism is endocytosis, which allows cellular entry without passage through the cell membrane. Endocytosis is a process used by many cell types to internalize larger proteins or nutrients. This cell internalization process results in the formation of an intracellular vesicle through the invagination of the plasma membrane and membrane fusion (74). Recent reports have indicated that QDs can in fact be internalized into living cells using specific coatings to induce endocytotic mechanisms (75). Although the internalized QDs were confined to endocytotic vesicles inside the cells (20,75), these methods present one step forward toward the use of QDs for therapeutic use.

Physical or mechanical methods. Drug delivery methods that rely on mechanical force to facilitate passage of the cell membrane could also find application for the transport of QD-based therapeutic agents. For example particle bombardment, which has been shown to facilitate the penetration of DNA-coated microparticles in cell membranes (76), can be adapted for QD delivery. Another mechanical method, electroporation, which uses electric fields to induce temporary permeability of membranes, could also be adapted for QDs. Furthermore, recent studies have demonstrated the potential of the microinjection technique for the delivery of functionalized QDs to the mitochondria or cell nucleus (47,77). However, although relatively efficient, this approach is slow and laborious and would probably not be practical for *in vivo* applications.

In our group, we have explored an alternative strategy, which uses protein microbubbles (78) to transport QDs to the tumor. An ultrasonication pulse at the resonance frequency of the bubble allows it to burst, and ejection of the QDs through the cell membrane becomes feasible. Figure 9 shows some microbubbles doped with QDs developed in our laboratory (79).

Biomolecule-assisted transport. Studies on the cell's own transport mechanisms have enabled a different class of delivery methods, which exploit these routes to transport disguised therapeutics into the cell. These techniques, although not yet thoroughly exploited for QD delivery, could have great potential for the transport of QD-based therapeutics in the future. For example, cell penetrating peptides, *i.e.* short peptide sequences known as protein transduction domains (PTDs), which could cross the plasma membrane (80) could be used to facilitate QD intracellular uptake. The therapeutic peptide delivery appears to be independent of size, PTDs have been shown to internalize covalently linked proteins of greater than 700 kDa and has been used to deliver liposomes of over 200 nm in diameter (81). In addition, PTDs have been utilized to deliver therapeutics in cells both *in vitro* and *in vivo* and have been shown capable of delivering functional protein into a live animal model, using intraperitoneal injection. The advantage of

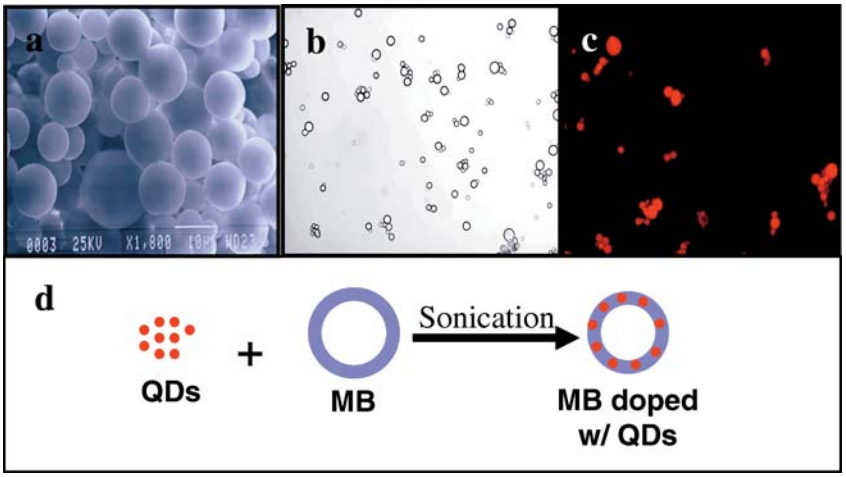

Figure 9. Optical microscope images of luminescent microbubbles doped with quantum dots. Reflectance mode images showing microbubbles before a) and after b) sonication and centrifugation, respectively. c) Fluorescence mode image showing photoluminescence of microbubbles. d) Procedure of doping the microbubbles (MBs) with luminescent quantum dots. (79).

this technique is the inherent specificity of the process, which is ensured by the fact that only species covalently attached to the PTD can access the cells, which may prove critical for *in vivo* applications.

LIMITATIONS OF QDs AS PHOTOSENSITIZERS

Toxicity aspects

To date, while much work has been reported on the potential biological applications of semiconductor QDs (11–13,16–19, 22,47–48,82) comparatively very few articles exist that examine how they might affect the health of people or the environment in the long term (83,84). Studies on ultrafine particle toxicology suggest that particle size can impact toxicity equally if not more so than chemical composition, hinting the complexity of assessing the potential human health effects of QDs. For example, toxicity studies conducted by Oberdörster at the University of Rochester imply that smaller particles react differently from larger ones with exactly the same composition (85). There are indications in the literature that manufactured nanoscale materials may distribute in the body in so far unpredictable ways and certain nanoscale materials have been observed to preferentially accumulate in particular organ (86,87). While the standard way of assessing doses in toxicology studies involves the measurement of the mass of the material, quantity is not the only critical parameter for nanoscale therapeutics. When particles get extremely small, their surface area matters most because the most significant part of the particle is the exposed surface. Coating nanoparticles with different materials can change surface properties. A variety of published solubilization and functionalization protocols reported no adverse effects on cell viability, function, or development over the duration of the experiments (from several hours to several days) at QD concentrations optimized for labeling applications (19,83,84). At higher concentrations, however, effects on embryo development were noticeable (84). However, also the surface chemistry is influenced by the size of the particle. This interaction of surface area and particle composition in eliciting biological response adds an extra dimension of complexity in evaluating effects of exposure to these nanomaterials.

Another issue is the fact that many current QDs have cores, which consist of heavy metal elements such as cadmium that raises a major concern in the medical community. To address this issue a ZnS shell is usually added, which serves to encapsulate the Cadmium-based core material and also improve its optical properties (49). Furthermore, a variety of inorganic and organic materials have been used to passivate QDs to make them more biocompatible (11,14–17,47,48,82–84). Recent studies conducted by Derfus and co-workers have revealed the detrimental effects of uncoated CdSe QDs on cultures of rat-liver cells (84). By coating the QDs, they were able to reduce the toxicity (84). We are currently exploring non-heavy metal based QD analogues that could serve as alternative material for the Cadmium-based QDs being employed in current biological studies. Over the past two years we have been developing Copper-based and other metal-oxide QDs that exhibit optical properties that could become comparable to that of the standard CdSe QDs (88–92).

Overall, there is a great need for research directed towards increasing the fundamental understanding of QD interactions at the biological and cellular level. Before QDs can be applied for real *in vivo* therapy, massive efforts must be conducted to assess their fate, transport, and compatibility in biological systems.

Photoinduced surface chemistry

It is in principle known and published that semiconductor QDs can undergo photocatalytic reactions on the particle surface (90,92–95). Many different reactions were studied and often oxygen is a major player in this photochemistry (95–97). However, this is both good and bad news. The good news is that oxygen is converted into singlet oxygen, which itself has a very specific chemistry, mainly on unsaturated aliphatic chains, as they are plentiful in cell membranes. There is also a possibility of excited state electron transfer to surface adsorbed oxygen, which might be a reason for the lower QDs emission yields in aerated solutions. The formation of peroxy radicals, peroxides and singlet oxygen can cause the death of a targeted cell. However, additional reaction pathways compete with energy transfer and therefore limit the yield of $^1O_2$. On the other hand, the formation of these cytotoxic species is a potential side effect during imaging in cells and tissues. This could lead to significant alteration of the biochemical composition in the vicinity where QD-based imaging is performed—an aspect that has to be at least considered. Furthermore, while QD-coatings can afford them to be water-soluble, biocompatible and target-specific, additional surface layers are critical issues for the energy transfer process between the QD and oxygen. To date, the investigation of singlet oxygen generation by photoexcited semiconductor QDs is still in its beginnings and how the different surface coatings affect the quantum yield for singlet oxygen formation is currently being investigated. On the other hand, by conjugating these unique nanomaterials to molecular photosensitizers, we can create efficient QD-molecular sensitizer FRET pairs that may prove to be beneficial in PDT applications.

CONCLUSIONS

Semiconductor QDs exhibit potential as photosensitizers for PDT applications. They can be used to sensitize other PDT agents or molecular oxygen through an energy transfer process (21). Both mechanisms result in generation of reactive singlet oxygen species that can be used to treat diseased tissues. While QDs offer outstanding optical properties and tunable surface chemistries, there still remain several issues that need to be addressed before they could be of general practical use in the clinical setting. An in depth understanding of how QDs get transported, metabolized, excreted, and their compatibility profile in biological environments is critical before they can be used *in vivo* as photosensitizers for therapeutic applications.

Acknowledgements—The authors acknowledge the financial support of NSF Chemistry Division (#0239688) and ACS-PRF (#39881-G5M). We also gratefully appreciate the many fruitful discussions with our close collaborators Prof. N. L. Oleinick and Prof. M. E. Kenney, and we acknowledge Prof. Kenney and his group for providing the phthalocyanine compounds.

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