Review Article

Clinical Potential of Quantum Dots

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Advances in nanotechnology have led to the development of novel fluorescent probes called quantum dots. Quantum dots have revolutionized the processes of tagging molecules within research settings and are improving sentinel lymph node mapping and identification in vivo studies. As the unique physical and chemical properties of these fluorescent probes are being unraveled, new potential methods of early cancer detection, rapid spread and therapeutic management, that is, photodynamic therapy are being explored. Encouraging results of optical and real time identification of sentinel lymph nodes and lymph flow using quantum dots in vivo models are emerging. Quantum dots have also superseded many of the limitations of organic fluorophores and are a promising alternative as a research tool. In this review, we examine the promising clinical potential of quantum dots, their hindrances for clinical use and the current progress in abrogating their inherent toxicity.

1. INTRODUCTION

Quantum dots (QDs) are fluorescent semiconductor nanocrystals [1] with diameters of the order of 2–10 nanometers, or roughly 200–10,000 atoms [2]. QDs are made from a variety of different compounds. They are referred to as II–VI, III–V, or IV semiconductor nanocrystals, based on the periodic table groups that these elements are from. Cadmium selenide (CdSe) and Cadmium telluride (CdTe) nanocrystals are examples of QDs which are group II–VI semiconductor nanocrystal. CdSe contains cadmium (Cd) from group II and selenide (Se) from group VI of the periodic table. Their novel optical and physical properties have attracted immense interest in developing them for biological applications that require long-term, multitarget, and highly sensitive imaging. The general structure of QDs comprises an inorganic core, an inorganic shell, and an aqueous organic coating to which biomolecules are conjugated, as shown in Figure 1. Modifications in development can be used to control the size and composition of the nanocrystal core to create specific spectral properties of the QDs.

Developing high-quality QDs cores with a specific wavelength, chemical composition, and size is a prerequisite. Synthesis is achieved by heating appropriate organometallic precursors with stabilizers in high boiling solvents to produce QDs, which can then be dissolved in nonpolar organic solvents to form transparent colloidal QDs dispersions. Organic solvents such as trioctylphosphine oxide (TOPO) and hexadecylamine are commonly used and contain alkyl chains which extend from the QDs surface, rendering the QDs sterically stable as colloids [3]. Altering the size of the QDs core during synthesis helps in tuning the color of emission [4]. The inherent toxicity of the individual ions (Cd^{2+}, Se^{2-}, and Te^{2-}) within the cores has been circumvented by growing an inorganic shell, that is, zinc sulphide (ZnS) on top of the CdSe or CdTe nanoparticles [5]. The ZnS shell serves as a barrier whereby the CdSe cannot come in contact with the surrounding solvent and thus dissolve through ionization. Secondly, it improves the quantum yield by passivating the surface nonradiative recombination sites. QDs have been rendered water soluble [6] by providing a shell of functionalized silica, phospholipids micelles [7], or linkers, such as mercaptoacetic acid [5], dihydrolipoic acid [8], or amphiphilic polymers, that is, modified polyacrylic acid [9, 10]. In general, stabilization in aqueous solution is achieved by

coating the QDs in amphiphilic polymers or by ligand exchange.

In order to use QDs in biological applications, QDs have been integrated with biomolecules. QDs are modified with bifunctional or amphiphilic molecules with one end binding or interacting with the QDs surface and the other polar end protruding from the surface [11]. QDs are conjugated electrostatically either directly, between QDs and proteins engineered to incorporate charged domains or via a bridge. Covalent coupling has also been harnessed whereby QDs bioconjugates are bound through carboxylic acids and biomolecules. Stabilization of QDs in aqueous solutions provides some QDs with coats which possess reactive functional groups such as amines, carboxylic acids, alcohols, and thiols [6, 8, 12]. Through these functional groups, covalent conjugation with a variety of biological molecules can be achieved. Once a biological interface has been provided, QDs can effectively and specifically target different biomakers at cellular, tissue, tumor, and organ levels. The above developmental process has been summarized in Figure 2 and an overview of the current techniques of QDs—conjugate synthesis, biofunctionalization, and bioconjugation has been summarized in Table 1.

2. CLINICAL POTENTIAL (IN VIVO APPLICATIONS)

The unique properties of QDs can be put to use in a wide variety of biological applications. A key feature is that they can be modified with a large number of molecules and linkers to optimize their functionality for particular applications. QDs have been used to selectively tag molecules, proteins, and cells [8, 9, 13, 14] of interest. QDs have great potential for use in sentinel lymph node (SLN) mapping which is the mapping of the first tumor draining lymph node, [15–19], diagnostic tools (e.g., imaging), for therapeutic purposes (e.g., drug delivery and cancer treatment) [14], live cell labelling, and tracking over long periods of time [20]. Multicolor in vivo imaging has enabled noninvasive surgeries to be carried out in a way that has not been feasible without QD.

3. GUIDING CANCER SURGERY

3.1. Type II QDs

When tissues absorb light, there is a possibility that fluorescent light will be emitted. This causes tissue "autofluorescence" which can severely limit signal to background ratio. Some organs have increased green autofluorescent, for example, skin, small intestine, gall, and urinary bladder when excited with blue light. Exciting the gall and urinary bladder with green light will reduce their autofluorescence [21]; however, the use of a near-infrared (NIR) light reduces fluorescence background immensely. Type II QDs emit light within the NIR spectrum and have been used for cancer-guided surgery. Type I QDs structures are composed of CdSe/ZnS or CdTe/ZnS (core/shell) structure whereas Type II QDs structures are composed of CdTe/CdSe (core/shell) or CdSe/ZnTe (core/shell) heterostructures. Type II structures can allow ac-
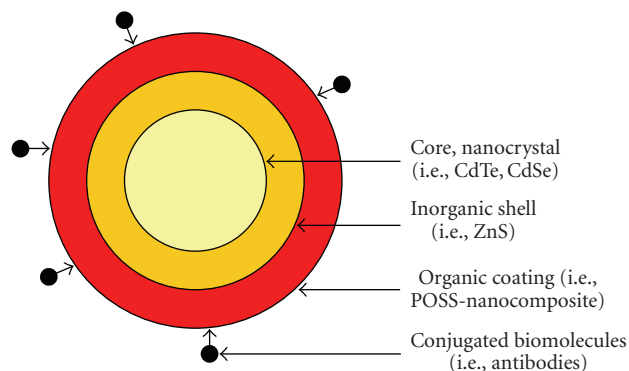

FIGURE 1: General structure of a QDs-modified from [21].

Core, nanocrystal (i.e., CdTe, CdSe)

Inorganic shell (i.e., ZnS)

Organic coating (i.e., POSS-nanocomposite)

Conjugated biomolecules (i.e., antibodies)

Core synthesis

Inorganic shell coating

Stabilization in aqueous solution
- Ligand exchange
- Polymer coating

Bioconjugation for biomedical application
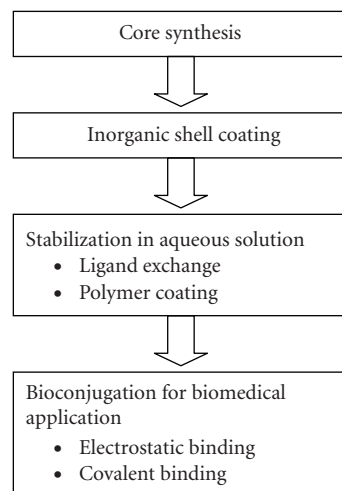- Electrostatic binding
- Covalent binding

FIGURE 2: Overall development of QDs for biological application.

cess to wavelengths that would otherwise not be available with a single material [32].

3.2. Sentinel lymph node mapping

SLN identification and lymphatic mapping are one of the most revolutionary advances in surgical oncology in recent years [33]. Mapping and removal of the SLN provides accurate staging and therapeutic planning, determining the need for adjuvant oncological management [34]. In the USA, lymphatic mapping and SLN biopsy have become the standard of care for melanoma, with increasing acceptance in breast cancer and a growing acceptance in tumors of the gastrointestinal tract [35]. Identification of the sentinel node can be performed by the use of a radioisotope and intraoperative handheld gamma probe, a vital blue dye, or a combination of the two [34]. Current techniques of SLN mapping are limited by unpredictable drainage patterns, high background signal, and the inability to image lymphatic tracers relative to surgical anatomy in real time [15]. Novel fluorescent probes (QDs) have been developed to provide real-time image-guided localization using an NIR fluorescence system which facilitates the resection of the SLN [15–19].

TABLE 1: Overview of current techniques for synthesis, biofunctionalization, bioconjugation of QDs, and their conjugates.

First author	Synthesis	Biofunctionalization	Bioconjugation	Bioapplication
Zhou [22]	ZnS capped	Amphiphilic polymer shell, carboxylic acid, and amine functionalized	Peptide ligand	Selective cell surface and single-molecule imaging
Tortiglione [23]	ZnS capped	Amphiphilic polymer shell, diamino PEG functionalization	Glutathione (GSH)	Biolabelling Hydra vulgaris a freshwater invertebrate
Selvan [24]	Fe_2O_3-CdSe magnetic QDs (no ZnS capping)	Silicanization with surface amine groups	Oleyl-O-poly(ethylene glycol)succinyl-N-hydroxysuccinimidyl ester	Cell membrane targeting for biolabelling and imaging of live cells
Kampani [25]	ZnS capped	PEG layer	Biotin-streptavidin complex	Quantitative analysis of viral binding and attachment of human T cell leukemia virus type I
Medintz [26]	ZnS capped	Hexahistidine peptide linker with thiol	DNA oligonucleotides	Genetic analysis
Albuquerque de farias [27]	CdS/Cd(OH)$_2$(no ZnS capping)	Glutaraldehyde cross-linking	Anti-A monoclonal antibody	Biolabelling of human erythrocytes
Gao [28]	ZnS capped	Mercaptoacetic acid with bovine serum albumin coating to increase quantum yield and to provide amine and thiol functional groups	None	Potential for molecular sensing
Wolcott [6]	Silica capped	PEG and thiol-terminated biolinkers	Immunoglobulin G proteins	Biolabelling and imaging
Tan [29]	ZnS capped	Chitosan	Human epidermal growth factor receptor 2 antibody	Tracking of RNA delivery
Parungo [19]	CdSe inorganic shell	Oligomeric phosphines	None	Pleural space SLN mapping
Van Tilborg [30]	ZnS shell	Paramagnetic lipid Gd-DTPA-bis(stearylamide)	Human annexin A5 protein molecules	Multimodal detection of apoptotic cells
Jaiswal [8]	Zns shell	Dihydrolipoic acid (DHLA)	Avidin or antiP-glycoprotein antibody	Long-term imaging of live cells
Dubertret [7]	ZnS capped	Phospholipids	Deoxyribonucleic acid (DNA)	Labelling of *Xenopus* embryos
Mulder [31]	ZnS shell	Pegylated phospholipids and a Paramagnetic lipid Gd-DTPA-bis(stearylamide)	Arginine-glycine-aspartic acid (RGD) peptides	In viro targeting of human endothelial cells
Bharali [5]	ZnS shell	Mercaptoacetic acid	Folic acid	Imaging of live cells
Wu [9]	ZnS shell	Amphiphilic polymer	Streptavidin or anti-Her2 antibody	Labelling of cancer marker Her2

Tumor staging and treatment planning is improved if assessment of the primary lymph node draining a tumor site is accurate. Gastrointestinal (esophageal, gastric, jejuna, and colonic) and pulmonary SLN mapping have been carried out in in vivo models with a real-time NIR fluorescence imaging system. NIR fluorescent QDs have been used for intraoperative mapping of lymphatic drainage of various organs and for guiding excision of the primary draining node on a patient-specific basis [15, 18].

3.3. Gastrointestinal SLN mapping

In gastrointestinal SLN mapping, injection of 200 pmol of NIR fluorescent QDs into various intra-abdominal organs
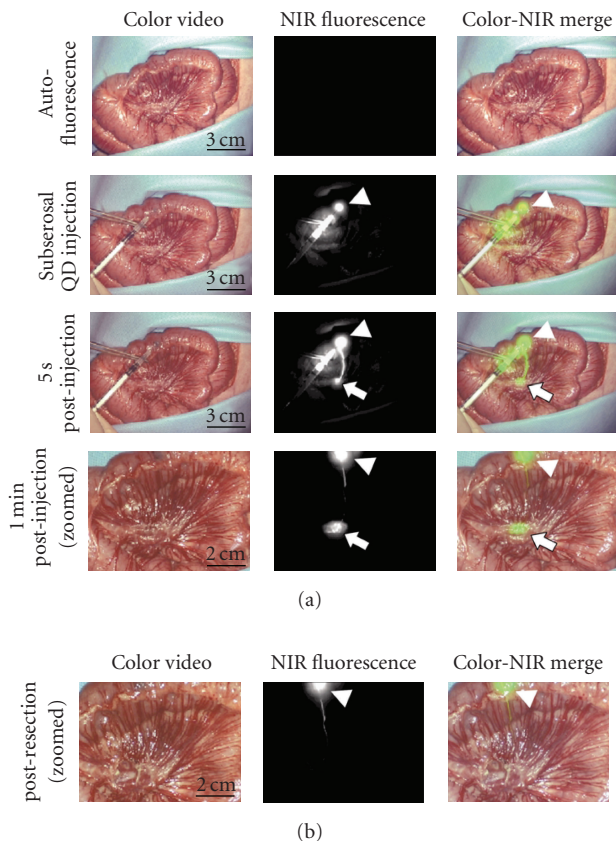
Figure 3: NIR fluorescent SLN mapping of the porcine colon. (a) NIR fluorescent images of the surgical field pre-, during, and postinjection of NIR quantum dots. (b) Same image in "(a)" reveals no QDs fluorescence in the area of the excised SLN [15].

identified the SLN in less than 60 seconds and the afferent lymphatics in 100% of the cases [15]. QDs may be engineered to precise sizes which enable localization in the SLN unlike blue dye which contains particles <5 nm that can pass through multiple nodes therefore leading to false-positive results [15]. Examination of the operative site after surgery can be done to ensure successful removal of the lymph nodes. This has been demonstrated in Figure 3 where images after SLN excision in a porcine colon reveal no fluorescence in the area of the excised SLN. However, SLN analysis by the pathologist is facilitated as the QDs help identify the specific part of the SLN that is most likely to contain malignant cells [16].

3.4. Pulmonary SLN mapping

In pulmonary SLN mapping, injected QDs identified the SLN within 1 minute, whereas isosulfan blue (the gold standard visible lymphatic tracer) could be visualized in the same node within 4 minutes. In contrast to QDs, isosulfan blue reduces clarity due to extravasation. NIR QDs do not interfere with the visualization of the surgical field as they are invisible to the human eye [18]. NIR fluorescence imaging with QDs in two species demonstrated that the highest superior mediastinal lymph nodes are the SLNs of the pleural

space [19]. Advancement of the NIR fluorescence imaging with QDs indicate a promising future in clinical lymphatic mapping. Table 2 shows various studies using NIR fluorescent QDs in SLN mapping.

4. CANCER IMAGING

4.1. Strong potential for sensitivity in cancer diagnosis

QDs have been shown to specifically and effectively label molecular targets at cellular level and they have been used as a diagnostic tool for cancer in in vivo studies [14, 36]. In vivo targeting studies of human prostate cancer developed in nude mice showed that QDs probes accumulated in tumors by both the enhanced permeability and retention at tumor sites through antibodies binding to cancer-specific cell surface biomarkers [14]. For active tumor targeting, antibody-conjugated QDs were used to target a prostate-specific membrane antigen (PSMA). Previous research has identified PSMA as a cell surface maker for both prostate epithelial cells and neovascular endothelial cells [37]. Results obtained from QD-PSMA antibody probes injected into the tail vein of a tumor-bearing mouse showed that nanoparticles were delivered and retained by the tumor xenograft [14]. Comparison with other surface modifications of the QDs probe: carboxylic (COOH) group, polyethylene glycol (PEG) groups, and PEG plus PSMA antibody showed, no tumor signals were detected with the COOH probe, only weak tumor signals were observed with the PEG probe (passive targeting) and intense signals were detected in PEG-PSMA antibody-conjugated probe [14]. The above results present new opportunities for ultrasensitive imaging of biomarkers involved in cancer invasion and metastases, as a result alerting clinician to early intervention.

Using QDs, precancerous biomarkers have been investigated in cervical cancers [13]. It is widely accepted among immunohistochemistry studies in the cervix that epidermal growth factor receptor (EGFR) levels demonstrate a statistically significant increase when a lesion progresses from a dysplastic to an invasive lesion [38]. SiHa cervical cancer cells were targeted with QDs conjugated to anti-EGFR antibodies [13]. This showed specific labelling of EGF receptors. Using optical imaging technologies, they postulated that QDs can help visualize changes in the cervical cancer at the molecular level hence the need for early intervention. The ability to image molecular changes will directly affect patient care by allowing earlier detection of disease and identification of specific molecular targets for treatment [39].

5. THERAPEUTIC

5.1. Photodynamic therapy in cancer treatment

Photodynamic therapy (PDT) has been developed as a novel management technique for a diverse variety of cancers [40]. In conjunction with surgical treatment, PDT has been used successfully in lung cancer and is increasingly being used on gastrointestinal malignancies. This modality is already an established treatment entity in ophthalmology. During PDT,

TABLE 2: Studies using NIR fluorescent QDs (Type II NIR QDs) in SLN mapping, using emission of 840–860 nm wavelength [15–19].

NIR fluorescent Lymph tracer	Model	Tissues/organs	Outcome
Soltesz [15]	Pig	GI tract (gastric, jejunal, colonic)	Identified SLN in less than 1 minute in 100% of pigs
Soltesz [18]	Pig	Lungs	Identified SLN within 5 minutes in 100% of pigs
Parungo [19]	Rat and pig	Pleural space	Demonstrated that station 1 lymph nodes are the SLN of the pleural space in rats and pigs
Kim [16]	Mouse and pig	Limbs (lymphatic flow to axilla and groin)	Identified SLN in 3–4 minutes (percentage not mentioned)
Parungo [17]	Pig	Esophagus	A single SLN was identified within 5 minutes in 100% of pigs

singlet oxygen is generated in the diseased cells by a simple and controllable light-activated process. This process involves a photosensitizer that is capable of absorbing light of an appropriate wavelength and utilizing that energy to excite oxygen to its singlet state which initiates apoptosis of cancer cells [41]. Selectivity is significant in cancer treatment and has been utilized in PDT. Only cells which are simultaneously in contact with the photosensitizer, light and in the presence of oxygen are subjected to the cytotoxic reactions [42]. In the work of Samia et al. [43], CdSe QDs were linked to a silicon phthalocyanine (Pc4) photosensitizer through an alkyl group, and used as a primary energy donor. Excitation of CdSe QDs activated emission of Pc4 photosensitizer at 680 nm, which enabled the use of an excitation wavelength that is not absorbed by the sensitizer. Through the fluorescence resonance energy transfer mechanism from QDs to the silicon Pc4 photosensitizer, oxygen reactive species were generated for photodynamic cancer therapy. Furthermore, the semiconductor nanocrystals alone were found to generate oxygen reactive species without a mediating photosensitizer. In view of their flexible spectral characteristics, QDs can be engineered in size and composition to match those of any PDT photosensitizer and be used as energy donors. Figure 4 shows a summarized mechanism of PDT involving QDs.

5.2. Drug delivery

QDs probes have been shown to accumulate in tumors by enhanced permeability and retention at tumor sites or by antibody binding to cancer-specific cell surface biomarkers [14]. Enhanced permeability and retention (EPR) effect is the basis for selective targeting of macromolecular drugs to tumors and the concept is now utilized for selective delivery of many macromolecular anticancer agents [44]. Styrene-maleic acid-doxorubicin micelles utilizing EPR effect have been shown to enhance the therapeutic effects of doxorubicin while reducing toxicity [45]. Polyethylene glycol-liposomes encapsulating doxorubicin were less extensively taken up by the reticuloendothelial system and were able to extravasate through
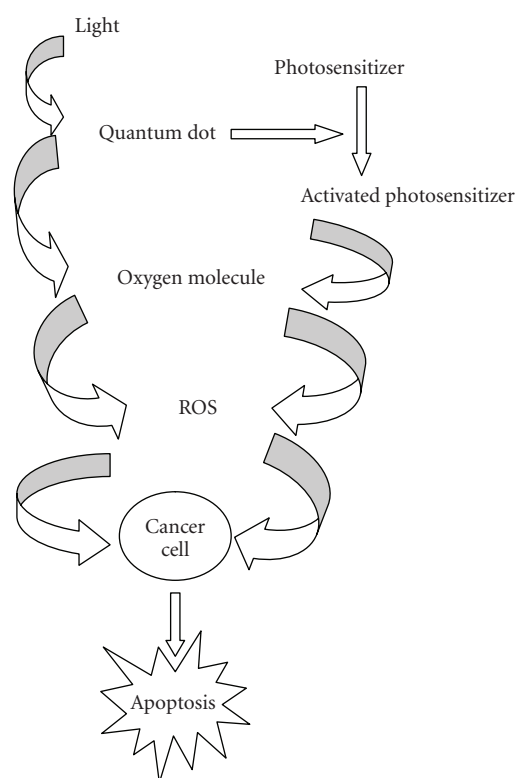

FIGURE 4: Summarized mechanism of PDT involving QDS [43].

"leaky" tumor vasculature resulting into doxorubicin localization in tumor tissue [46]. EPR can deliver therapeutic agents to desired targets while reducing systemic toxicity. Research in drug delivery has benefited from the use of nanotechnology in dendrimers [47] and liposomes [48]. Combining QDs, specifically their ability to bind molecules that recognize cancer cells and a drug, might offer a new strategy for molecular cancer therapy through targeted molecular delivery vehicles.

6. QDs AS A RESEARCH TOOL

6.1. Bio-sensitive

The rate of success of QDs usage in the laboratory has increased immensely. As a biosensitive tool, detection of single bacteria pathogenic Escherichia coli 0157:H7 serotype was made possible with the use of QDs [49]. Under continuous excitation, QDs retained their high fluorescence intensities for hours while organic dyes bleached in seconds, allowing more rapid and accurate identification of E. coli 0157:H7 in single-cell fluorescence-based assays. QDs allowed lower limits of detection, which increased sensitivity, and has important implications in the development of any fluorescent immunoassay for bacteria pathogens. QDs-labeled chromatophores have been used as virus detectors to detect H9 avian influenza virus based on antibody-antigen reaction [50]. Selective determination of free cyanide in water with high sensitivity (detection limit of 1.1×10^{-6} M) has been demonstrated via analyte-induced changes in QDs photoluminescence after photoactivation [51].

6.2. Bioimaging

QDs have been used to stain *Hydra vulgaris* (a fresh water invertebrate) and study its behavior [23], to image in in vivo tumor vasculature in mice [39], and to study vasculogenesis in Zebrafish [52]. Bovine serum albumin-coated QDs have been used as a fluorescent, angiographic contrast agent in the NIR range [39]. Observation of vessels surrounding and penetrating a murine squamous cell carcinoma in a C3H mouse was made possible. Distinction between the superficial vessels associated with the tumor and deep vessels could be made with the deep vessels visible in fluorescence images. Colabelling of Zebra fish embryo's blood vessels with QDs enabled documentation of the embryonic pattern of vasculogenesis as the QDs marked the newly formed vessels [52]. As a result, detailed knowledge about the progression of vascular systems development has been obtained.

6.3. Biolabelling

Different groups have successfully demonstrated that QDs can be tagged and incorporated into cells and drugs without affecting their activation and function [20, 27, 53–55]. Up to 10^8 QDs could be injected in Zebra fish embryos without malformations or developmental problems during embryogenesis [52]. QDs were used to follow labeled cells during their developmental stages to reveal cellular behavior. Semiconductor nanocrystals have been used for in vivo tracking of cancer cells during metastases. Tumor cells labeled with QDs were used in fluorescence microscopy to study extravasation, a part of metastasis formation at high resolution in living animals [20]. QDs-labeled cells survived the selective pressure of the circulation and managed to extravasate into tissues just as effectively as unlabeled cells. The use of QDs provided the opportunity to simultaneously identify and study the interactions of multiple different populations of tumor cells and tissue cells in a natural tissue environment.

7. COMPARISON TO OTHER FLUORESCENT COMPOUNDS

Fluorescent QDs that overcome many of the limitations of organic fluorophores are a promising alternative. Organic fluorophores's excitation and emission wavelengths are dependent on a chemical structure whose tuning to a precise wavelength requires complicated chemistry. The quantum yield of conventional organic fluorophores is usually less than 15% in aqueous environments [21]. QDs have a large absorption cross-section, good quantum yield, and a large saturation intensity that makes them much brighter than fluorescence dyes or fluorescent proteins [56]. QDs have broadband absorbance to the blue of emission which has been exploited for in vivo applications. Studies have demonstrated that tissue scatter and absorbance may sometimes offset increasing QDs absorption at blue light wavelength and counteract this advantage [57]. However, QDs that emit in the NIR region of the electromagnetic spectrum have been developed for live tissue imaging. Within the NIR of the spectrum there is low tissue scattering and absorption, yielding great tissue penetration depth and optical signal [11].

In the work of Gao et al. [14], sensitivity detection and spectral features of QDs and green fluorescent protein (GFP) were compared by linking translocation peptide HIV Tat to QDs and delivering them into living cancer cells. QDs-tagged cells and the GFP-transfected cells were similarly bright in cell cultures, however only the QDs signal was observed in vivo. GFP signals were not discerned at the injection site. Even if results did not provide an absolute intensity comparison between GFP and QD, they provided a qualitative spectral comparison demonstrating that the emission spectra of QDs could be shifted away from the autofluorescence, allowing spectroscopic detection at low signal intensities [14]. The large Stroke's shift which is the difference between the peak absorption and the peak emission wavelengths enables fluorescent signals from QDs to be easily separated from scattered excited light [13].

QDs are more highly photostable than organic fluorophores. Organic dyes are often photobleached and fade by > 90% in less than one minute, whereas QDs are stable for more than 30 minutes under identical experimental conditions [58]. In vivo fluorescence quenching of QDs-micelles and rhodamine green-dextran was compared after 80 minutes of constant illumination at 450 nm under the microscope. The QDs fluorescence intensity remained the same, whereas the dextran had photobleached [7]. While detecting tumor marker CA125 in ovarian carcinoma, Wang et al. [59] compared the photostability of QDs signals and a conventional organic dye fluorescein isothiocyanate (FITC). QDs signals were found to be more specific and brighter than those of FITC, with exceptional photostability during continuous illumination for 1 hour, whereas FITC signals faded very quickly and became undetectable after 24 minutes of illumination. Arguably, the extreme photostability of QDs may have little significance in the context of clinical potential as fluence rates that photobleach organic fluorophores have no place in the clinical settings. The susceptibility of conventional fluorophores to photobleaching limits the fluence

rate that can be applied to a sample and as a result it affects the sensitivity of detection [21]. In order to minimize the potential for toxicity, Kim et al. [16] increased the fluence rate, and proportionally decreased the dose of injected QDs. Their photobleaching data suggested that at least a 100-fold lower dose could be used. Although QDs are photostable and they can achieve high-quantum yields in organic solvents, they underperform organic fluorophores as a function of molecular volume. That is, given their size, QDs are much poorer photoluminescence agents than organic fluorophores. In depth, discussions of various properties of QDs in relation to conventional fluorophores have been published [14, 21, 56, 60].

8. RECENT DEVELOPMENTS

In the burgeoning field of nanotechnology QDs synthesis, biofunctinalization and bioconjugation techniques are advancing rapidly. Conventionally inorganic cores have been capped with inorganic shells, ZnS; while other techniques of capping the core with cadmium sulphide (CdS), Silicon, CdTe or CdSe to form CdTe/CdSe (core/shell), or CdSe/ZnTe (core/shell) [32, 36, 61] are emerging. CdTe/CdSe (core/shell) or CdSe/ZnTe (core/shell) is expected to have many novel properties that are fundamentally different from CdSe/ZnS (core/shell) or CdTe/ZnS (core/shell) because of their valence and conduction band differences [32]. These properties are being exploited in in vivo applications. Water-based synthesis of highly luminescent QDs is a promising alternative to QDs prepared in organic solvents [62–65] in cellular imaging and biolabelling. It offers the advantage of engineering water soluble, stable QDs with smaller hydrodynamic diameters that are easily conjugated to biomolecules. Synthesis of group III–V semiconductor QDs (i.e., InP) as a luminescence probe for imaging in live cells has been reported [5]. Group III–V QDs are potentially less toxic as opposed to II–VI QDs theoretically making them better probes for bioapplications. Drawbacks to utilizing III–V QDs as fluorescent probes are their low quantum yield and laborious synthesis. Future work to harness their full potential will include increasing the quantum yield and minimizing the aqueous size of the quantum dot.

The aqueous size of the QDs should be appropriately matched to the in vivo study of interest [16]. Types 11 QDs with aqueous sizes of 15.8–18.8 nm have been used for SLN mapping [15–19]. Recent work of Zimmer et al. [66] demonstrates the synthesis of a size series of (InAs)ZnSe (core) shell QDs that emit in the NIR and exhibit aqueous size of less than 10 nm. These QDs circulated in the blood before they were able to migrate out of the blood vessels and into the interstitial fluid. It is a significant achievement in the development of QD; hence after intravenous injection, they can possibly penetrate most normal organs or micrometastases.

Bimodal nanoparticles consisting of QDs that are encapsulated in a paramagnetic micelle to enable both optical imaging and magnetic resonance imaging (MRI) are being developed [30, 31]. This has potential to detect pathological processes in in vivo models and tumor angiogenesis with both intravital fluorescence microscopy and MRI [30, 31].

Various strategies of biofunctionalization and bioconjugation have been developed to generate water-soluble QDs as previously discussed however some have encountered problems. QDs solubilization with mercaptoacetic acid [5, 28] have been reported to cause a drop in fluorescence quantum yields after solubilization and desorption of mercaptoacetic acid has led to aggregation and precipitation of solubilized QDs [28]. Bovine serum albumin has been used to improve the fluorescent intensity of the QDs solubilized in mercaptoacetic acid [28]. Techniques of biofunctionalization and bioconjugation have to (i) be reproducible, (ii) maintain the size of the QDs complex to a minimum, (iii) maintain the photoluminescence properties of the QDs, (iv) provide chemical stability of the QDs complex, and (v) be reliable methods for conjugation to biomolecules.

9. DISCUSSION

The future of QDs is promising however there are fundamental questions that still need to be answered. Questions have arisen about their toxicity, long-term in vivo stability and metabolic elimination from the body. Prevention of core atoms, Cd atoms, from being accessible to or potentially released in the surrounding environment has been passivated by shelling the core in extra layers of material. Questions regarding biochemical mechanisms of cytotoxicity are slowly beginning to be answered, mechanisms suggested involve production of reactive oxygen species such as free hydroxyl radicals and singlet oxygen [43, 67, 68]. Treatment with N-acetylcysteine (NAC), an antioxidant, has shown to prevent "naked" QD-induced organelle and cell damage which is mediated by reactive oxygen species (ROS) [68]. In the work of Lovrić et al. [68], NAC improved cell survival by reducing concentration of ROS in cell culture medium. NAC further induced the synthesis of glutathione, an effective cellular antioxidant, and possibly improved QDs surface passivation, leading to less damage to the mitochondrial redox system. Understanding the mechanisms of QDs cytotoxicity is significant in order to make use of their potential.

Studies of bovine serum albumin-QDs conjugates have shown bovine serum albumin to provide protection against QDs-induced cytotoxicity [69]. Albumin reduced or eliminated toxicity through possession of peptides responsible for the extracellular antioxidant defense system. However, work on mercarpto-undecanoic acid QDs (MUA-QDs) in sheep serum albumin (SSA); showed that MUA-QDs caused cell damage even at low concentrations [70]. Cytotoxicity caused by mercapto-undecanoic acid QDs in sheep serum albumin was attributed to the nonchemical bonds between the SSA and the MUA-QDs. There could be a possibility that MUA QDs capping alone without the QDs caused cell damage as effects of MUA alone on vero, hela cells and primary human hepatocytes were not assessed. There is a need to evaluate the strength of bonds between the QDs and their surface coatings. Possible enzymatic, physical and chemical degradation of the semiconductor cores upon injection in live animals could occur. Under what shear stress in vivo circulatory pressures are the surface coatings likely to hold? Arguably, QDs do not have to be in the circulatory system for

clinical application, however, they may gain access to circulation through blood and lymphatic channels therefore their degradation needs to be assessed.

Polydentate phosphine-coated NIR QDs were found to be stable in 100% serum after incubation for twice the amount of time needed for a typical SLN mapping procedure [16]. There was minimal disruption in the optical properties of the NIR QDs, which is promising for biological applications. Biocompatible polymer such as silicon are safer materials to use for encapsulation and are highly unlikely to degrade [14, 70].

QDs surfaces can control serum lifetime and pattern of deposition [10] which has been exploited in many in vivo applications. Deposition in the reticuloendothelial system is significant for detecting SLNs. Polymer encapsulation with surface PEG groups reduced the rate of organ uptake and improved circulation half-life of QDs, leading to accumulation of the nanoparticles in the tumor [10]. The size of the nanoparticles plays a vital role in avoiding filtration by the reticuloendothelial system. In a similar strategy exploited in drug delivery systems, drug-carrying liposomes are believed to have an increased lifespan partly due to their ability to extravasate through the splenic and liver fenestrae [71].

Different reports have been published highlighting the inertness of QDs in vivo studies where physiological function has not been affected, however lots of gaps exist regarding biodegradation and excretion. Encapsulation of QDs prepared in organic solvents is one of the most widely used methods of abrogating QDs toxicity, however new ways of QDs synthesis and negating QDs toxicity need to be devised. Adding extra coating onto the QDs core improves blood halflife which in essence increases the dissolution. Additional QDs surfaces will not enhance the translation of QDs to the clinical setting as they do not eliminate the toxic cores. Surface coatings and surface modification prior to in vivo application may have a big role to play on QDs degradation or elimination. However, the current state and design of the QDs precludes the elimination of QDs from the body. The ability to functionalize QDs with many different chemical groups increases its aqueous size. This presents an enormous predicament since the core, shell, organic coatings, and functional groups will be larger than the pore size of the endothelium and the renal threshold. To the best of our knowledge there are no in vivo studies on the metabolism and excretion of QD.

The engineering of QDs for biological applications is at its infancy. Progress is being made in designing sizable and biocompatible QDs. As optimism in exploiting QDs clinical potential is high, there is need to assess their cytotoxicity, in vivo distribution, and excretion. A much more work needs to be done to combat QDs inherent toxicity before they are applied in the clinical settings.

In summary, the development of QDs for clinical usage will have to circumvent a few more hurdles to gain recognition as a novel fluorescent probe for SLN, early cancer detection, rapid spread, and therapeutic intervention. The idea of engineering QDs for clinical purposes is not far fetched. The ideal QDs for clinical application would possess nontoxic elements, being chemically stable and with tunable size to perfectly negate through the endothelial pores, and would need to be completely eliminated from the body. With improvements in nanotechnology, nanotoxicology, and chemistry, some of the above goals could be achieved; however, it will be difficult to replace the toxic core of the QDs without losing the optical properties, or our efforts have to be redirect to less-toxic elements.

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