# Core-shell semiconductor nanocrystals: effect of composition, size, surface coatings on their optical properties, toxicity, and pharmacokinetics

**ABSTRACT**

Quantum dots are semiconducting nanocrystals that exhibit extraordinary optical properties. QD have shown higher photostability compared to standard organic dye type probes. Therefore, they have been heavily explored in the biomedical field. This review will discuss the different approaches to synthesis, solubilise and functionalise QD. Their main biomedical applications in imaging and photodynamic therapy will be highlighted. Finally, QD biodistribution profile and *in vivo* toxicity will be discussed.

## 1. QD history and chemical composition

In 1973, Leo Esaki, a Japanese physicist won the Nobel Prize in physics for the development of semiconductor devices, and for his early concept of ‘‘artificial solids’’ [1]. The development of different types of semiconductor systems (quantum wells, quantum wires and quantum dots) was tremendously expanded in the 1980s. Semiconductor QD are 1-10 nm nanoparticles consisting mainly of a semiconductor core with or without an inorganic passivation shell usually made of zinc-sulfide (ZnS). The shell is generally used to protect the QD core from oxidation and photolysis [2;3] and minimise the associated-toxicity related to the release of $Cd^{+2}$ ions [4].

QD were initially prepared in 1982 for use as probes to investigate the surface kinetics [5]. It was found that the cadmium-sulfide (CdS) QD fluorescence and quantum yield were sensitive to different surface species. Oxidisable species were poor fluorescence quenchers, while reducible species were excellent quenchers. Species without an appropriate redox capacity did not affect the QD fluorescence properties. Much progress has also been made in the synthesis and characterisation of QD [6]. The first highly crystalline and monodispersed cadmium-selenide (CdSe) QD published by the Bawendi group, were synthesised in a hot coordinating solvent [7]. This was followed by improving the QD photostability by passivating their surface with different semiconducting materials [3]. During all these developments, organic QD were mainly used in physics to design transistors, solar cells and light-emitting diode (LEDs) [8;9]. In 1998, the first synthetic approaches to water soluble QD were published [10;11], highlighting the potential use of QD in the biomedical field.

Currently QD are not only composed of CdS or CdSe but of many different semiconducting materials derived from the II -VI elemental groups (e.g. zinc-sulfide [ZnS], zinc-selenide [ZnSe], and cadmium-telluride [CdTe]); or III-V elemental groups (indium phosphate [InP], indium arsenate [InAs], gallium arsenate [GaAs] and gallium nitride [GaN]), or IV-VI elemental groups (e.g. lead-selenide [PbSe], lead-sulfide [PbS]) [12-15]. In addition to the huge advances in semiconductor synthesis, novel QD such as CdTe/CdSe (core/shell), CdSe/ZnTe (core/shell) [16] and cadmium-free QD, Mn doped ZnSe [17;18] have also been developed.

## 2. QD fluorescence characteristics

QD are fluorescent nanoparticles that offer distinct spectrofluorometric advantages over traditional fluorescent organic molecules (e.g. fluorescein, Nile red) [10;12]. Traditional organic dyes exhibit narrow excitation peaks, where a narrow range of wavelengths can be used for excitation. Moreover, they have asymmetric emission spectra with red-tailing. On the other hand, QD reveal broad excitation spectra, allowing excitation over a wide range of wavelengths. They also exhibit symmetric and narrow emission peaks. Therefore, multiple QD with different sizes can be excited using a single wavelength shorter than their emission wavelengths. Since the emission spectrum is narrow, fluorescence signals can be separated and detected simultaneously, to achieve multiplexing imaging [19-21]. Apart from absorption and emission characteristics, inorganic semiconductor QD have shown high quantum yield, higher resistance to photobleaching and longer fluorescence lifetime compared to organic QD, which makes them suitable for continuous tracking over a prolonged period [22]. The most striking property of QD is the massive changes in the QD optical characteristics as a function of size [8]. As the QD size increases, the emission shifts towards longer wavelengths. Such phenomenon is attributed to the ‘‘quantum confinement’’ effect, which is observed with the optical characteristics of semiconductors smaller than 10-20 nm, and from which QD were named [8].

## 3. Solubilisation and ligand conjugation

QD can either be prepared by water-based synthetic methods [23;24] or in non-polar organic solvents [7;25]. The latter approach produces monodispersed QD with a range of emission colour ranging from ultraviolet to infrared, compared to QD prepared immediately in aqueous solution [26-28]. However, QD synthesised in organic solvents contain organic shells that compromise their water solubility and consequently their compatibility with the biological milieu. Many strategies are being developed to overcome this limitation. Several hydrophilic ligands have been utilised to exchange the hydrophobic trioctylphosphine oxide (TOPO) coat on the QD surface with hydrophilic moieties **(Figure 1, left)**. including; (i) thiol-containing molecules, such as mercaptoacetic acid (MAA), dihydrolipoic acid (DHLA) [29] and mercaptopropyltris (methoxy) silane (MPS) [30], (ii) peptides [31], (iii) dendron [32;33] and (iv) oligomeric phosphine [34]. In spite of maintaining small sizes, this method tends to cause QD aggregation and decreases the fluorescence efficiency [35-40]. Also, as a labile ligand detaches from QD surface, QD-induced toxicity will increase correspondingly due to exposure to the QD core [41;42].

**Figure 1**

The other alternative approach is driven by hydrophobic interactions between the organic QD surface and the hydrophobic domain of amphiphilic molecules **(Figure 1, right)**. Embedding or encapsulating organic QD into phospholipid micelles [22;43], and amphiphilic diblock or triblock copolymers [39;44] was found to increase QD diameter significantly, but more importantly to preserve QD photostability, colloidal stability and enable QD application to physiological conditions without the release of the toxic $Cd^{+2}$ ions [44].

Bioactive ligands have been attached to QD surfaces to enable QD specific binding and targeting or to design multimodal probes. Several approaches have been investigated including non-covalent, electrostatic adsorption onto the QD surface [29], covalent attachment [45] and streptavidin-biotin linking [46]. So far, QD surface has been decorated with different ligands such as proteins [47], antibodies [44;48;49], peptides [50-52], endosome-disruptive polymers [53], aptamers [54], DNA [22;55-57], cell penetrating peptides [58-63], radionuclides [64;65] and magnetic resonance imaging (MRI) agents [66].

**4. Biomedical applications**

QD are being explored as potential imaging agents primarily in fluorescence-based diagnostic applications [22;45;67]. Unlike organic fluorophores, QD have distinctive broad excitation spectra and narrow emission peak, which can be easily used for multiplexing imaging [19-21]. In addition, QD have outstanding fluorescence properties that resist photobleaching over time, which is suitable to label cells *in vitro* [14;21;22;68] and organs *in vivo* [e.g. RES [67;69], blood vessels [50;70], lymph nodes [71-74] and solid tumours [44;52;75;76]]. Recently, DNA conjugated to the QD surface has been described as a promising tool in fluorescent in situ hybridisation (FISH) where gene abnormalities in cells could be identified [22;55-57;77].

More interestingly, multimodal imaging probes can be engineered by combining QD with magnetic [66] or paramagnetic [78] agents, as well as radioactive isotopes [64;65;79]. This significantly improves the sensitivity and the resolution of the imaging procedures *in vivo* [80]. Contrary to diagnostic agents for MRI and positron emission tomography (PET), QD can provide visual guidance during surgery or diagnosis. In addition, these electron-dense QD

can be easily visualised in the target tissue at a microscopic level under the fluorescence and transmission electron microscopes [14;81]. Moreover, quenching QD fluorescence by conjugating fluorescence quenchers such as gold nanoparticles or nitroxide [82;83] to the QD surface can provide information about the QD environment, where cleavage of the quencher at the target tissue (e.g. low pH, enzymes) restores QD fluorescence [68].

Another approach to construct multifunctional devices has been described by combining the QD optical characteristics with therapeutic agents. Samia *et al.* and others have reported the promising potential of QD in photodynamic therapy (PDT) since QD energy can activate surface-conjugated photosensitisers (PS) [84-90]. In addition, the optical characteristics of QD can be easily manipulated to match the PS excitation wavelength by changing the QD size and composition. All above applications will be discussed below in more details.

***Imaging agent:*** Long-term cell tracking can be very important in studies such as cell lineage and differentiation (e.g. embryogenesis, stem cells, transplanted cells) [19;22], also, in metastatic cancer [20;21;91]. Fluorescent cell labelling is a promising tool to track cells [45], many approaches have been developed to label cells either by microinjection with organ fluorophores or by transfecting the cells with reported genes that code for fluorescent proteins like GFP [92]. Conventional fluorophores have broad emission spectra which make it difficult to distinguish different probes administered concurrently. Unlike organic fluorophores, QD have distinctive broad excitation spectra and narrow emission peak, which can be easily used for multiplexing imaging [19-21], also, QD have outstanding fluorescence properties that resist photobleaching over time, they are good candidate to label and track cells *in vitro* [14;21;22;68] and in living animal [20;21;91].

For *in vivo* applications, in 2002 Dubertret and his colleagues, firstly introduced the outstanding fluorescence properties of biocompatible PEG-coated QD injected into *Xenopus* embryo [22]. QD have been used also as tool to study embryogenesis in zebrafish embryo and angiogenesis in chick CAM model [81]. In addition, QD have been used for imaging larger animals such as mice and pigs [93]. QD have been explored as an imaging agent for the lymph nodes [71-74], reticuloendothelial systems (RES) [67;69;94]. Moreover, decorating the QD surface with hydrophilic polymers could escape the RES and can be targeted to other organs, such as tumour and angiogenic blood vessels [44;50;52;70;76].

Several groups could target QD to tumours in living animals. This could be achieved by passive and active targeting as well as direct intratumuoral injection. For passive targeting, the nanoparticles should have nanometer size (≤ 100nm) and exhibit long blood circulation to accumulate preferentially in the tumour which is in agreement with QD characteristics [44]. Moreover, Gao and his colleagues targeted tricoplymer-coated QD to solid prostate tumour model in living mice [44], Akerman *et al.* and Cai *et al.* actively targeted peptide-coated QD to the tumour vasculatures and lymphatic vessels [50;52]. QD also have been targeted to hepatoma xenograft in nude mice [76]. Balluo *et al* . could label M21 humanmelanoma and MH-15 mouse teratocacinoma solid xenograft and sentinel lymph nodes in living mice by direct intratumoural injection [95].

Organic fluorophores have been widely used for optical imaging, despite rapid photobleaching, the excitation wavelength is mainly in the visible light region, where light absorption by the tissue is still problematic for imaging thick sections and whole living animals. Two-photon excitation microscopy has been used heavily in tumour detection studies as it exhibits lower sample scattering and stronger sample penetration than one-photon excitation [20;21;70;75] where thick sections can be visualised with high resolution. QD are a promising tool for optical imaging as they are good labels for multiphoton microscopy [75]. *Larson et al.* could successfully image the blood vessels in living mice into the after QD intravenous administration using multiphoton excitation microscopy, showing that higher contrast and imaging depth can be obtained at a lower excitation power compared to conventional organic dyes [70]. More interestingly, Voura *et al.* could track QD-labelled B16 melanoma metastatic cell extravasation in the lung after systemic administration in C57Bl6 mice using the same technique [21].

Near infrared (NIR) QD are a new class of QD used as imaging agents. They have shown better deep tissue imaging as minimum tissue light absorption occurs in this region [81]. In the last few years, Frangioni group has studied NIR QD extensively as imaging agent for sentinel lymph nodes (SLN) mapping in small animals and large animals. Kim *et al.* were the first to observe the rapid QD accumulation in the axillary lymph nodes after intradermal injection [93]. So far, successive researches have been carried out to mapped SLN of the pleural space [71], oesophagus [72], lung [73] and gastrointestinal tract [74]. Interestingly, similar results were obtained by Ballou and his colleagues following intraumoural injection of QD with different surface charges [95]. NIR QD were found superior to vital blue dye and $^{99m}$Tc colloids [71] since 15-20nm QD were uptaken within 5 minutes by SLN post injection,

and with detectable fluorescence signals up to few centimetres due to low tissue background in the NIR region, also, they are more selective to the first draining lymph node compared to other diagnostics that migrate to distant nodes [71;93]. Therefore, QD can be a promising tool for real-time intraoperative optical imaging which helps in correctly locating the sentinel lymph nodes or small metastatic solid tumour, to remove any tissue affiliated with the tumour [96]. The latter demonstration suggests that NIR imaging of QD can significantly improve tumour removal surgery and reduce the tumour recurrence in human.

Self-illuminating QD for *in vivo* imaging have been successfully described [97]. This new class of QD, based on bioluminescence resonance energy transfer (BRET), where the fluorescence emission of luciferase-QD conjugate occurs only in the presence of luciferase substrate (coelenterazine). Compared to existing QD, self-illuminating QD showed higher sensitivity in both superficial and deep tissue in living animals since tissue autofluorescence is minimal in the absence of external excitation source.

***Dual-modality contrast agent:*** Combining the optical imaging with existing magnetic resonance imaging (MRI) diagnostic agents can significantly improve the sensitivity and the resolution for *in vivo* imaging [80]. Nanocomposite made up of iron oxide nanoparticle and CdSe/ZnS QD have shown dual magneto-optical properties that can be detected by both MRI and fluorescence microscopy [66;98]. Mulder and his colleagues reported conjugation of gadolinium to PEG- QD surface which improved the microscopic resolution of MRI [66;99]. For multimodal detection, the surface of these nanoparticles was functionalised with different bioactive molecules such as RGD peptide to actively target the blood vessels or with Annexin A5 protein to detect apoptotic cells *in vivo* at both microscopic and macroscopic levels. Interestingly, QD- iron oxide nanoparticle distribution inside the body was evaluated by MRI. Moreover, quenching QD fluorescence by conjugating fluorescent quencher, such as gold nanoparticles or nitroxide [82;83] to the QD surface can be useful in providing information about their environment, where cleavage of the surface ligands at the target tissue (e.g. low pH, enzymes) will restore QD fluorescence.

Morgan *et al.* reported a novel CdMnTe/Hg QD having dual electron paramagnetic resonance (EPR) and fluorescence imaging properties as $Mn^{+2}$ has strong EPR signals [78]. These QD were successfully used as fluorescent and angiogenic (blood vessels and heart) contrast agent in living mice after systemic administration. Michalet *et al.* described previously $^{64}Cu$ radioactive- QD hybrids where QD targeting and biodistribution can be easily tracked by

fluorescence imaging and can be quantified in living mice using micro-positron emission tomography (microPET) [67]. Since QD are electron dense they can also be easily visualised in the target tissue using transition electron microscopy.

***Photodynamic therapy:*** QD have a wide surface area where different diagnostics and therapeutics such as photosensitiser (PS) can be conjugated to the surface. Samia *et al.* and others have reported using QD in photodynamic therapy (PDT) [84;85;88], since their energy can be transferred to the PS. QD have tunable emission spectra which is ranging from UV to near infra-red which exhibits better tissue penetration, in contrast to visible emission for most conventional PS (e.g. Photofrin®). Moreover, QD optical characteristics can be manipulated to match the PS excitation wavelength by changing the QD size and composition.

Samia and her colleagues reported for the first time, the excitation of poorly soluble phthalocyanine PS conjugated to QD surface by fluorescence resonance energy transfer (FRET). Thereafter, Tsay *et al.* successfully conjugated Rose Bengal and Chlorin e6 to peptide- coated QD [100]. These conjugates were hydrophilic in contrast to previous studies [84;85]. Furthermore, QD-Dopamine conjugates were found to reduce the cell viability of the cancer cells upon exposure to UV source, as singlet oxygen species were generated [89].

Bakalova *et al.* observed that anti-CD antibodies functionalised QD incubated with leukaemia cells and conventional PS (trifluoperazine and sulfonated aluminium phthalocyanine) sensitised the tumour cells after UV radiations and potentiated the cytotoxicity of PS [90]. Bakalova and his colleagues suggested different mechanisms for QD as PS that collectively will induce tumour cell apoptosis and death [86]. Despite low QD ability for singlet oxygen formation compared to classical PS [84], the high photostability can allow repetitive exposures for the cancer cells to the excitation source to improve the QD cytotoxicity.

## 5. QD biodistribution and pharmacokinetics *in vivo*

The effect of surface coating on QD blood circulation and organ biodistribution was first studied by Ballou *et al*. [69]. Polyacrylic acid-coated QD (PAA-QD) conjugated to low molecular weight PEG (750 Da) and intravenously injected into nude mice exhibited short blood circulation half-life ($t_{1/2}$ < 12 min) with predominant uptake by the liver, spleen, lymph nodes, and bone marrow. Decorating the same QD with $PEG_{5000}$ significantly increased the blood $t_{1/2}$ to 3 hrs with less liver, spleen and lymph node uptake [69]. Similar studies by other groups showed that 15-20 nm QD coated with $PEG_{5000}$ exhibited long $t_{1/2}$ of 5-8 hrs

[44;52;101;102]. In all these reports, the QD biodistribution was qualitatively determined based either on QD fluorescence in tissue sections using fluorescence and confocal microscopy or whole body fluorescence imaging of living animals. Fischer *et al.* described the first quantitative biodistribution study of QD by detecting the Cd atoms in the blood and organs of rats injected intravenously with QD [94]. **Table 1** summarises the *in vivo* studies performed with QD, several of which have been published using radiolabelled QD [65;79;103].

QD have also been explored for tumour imaging by applying a surface coating that maintained QD small size and extended their blood circulation. QD were able to accumulate in tumour sites following systemic administration; though this accumulation was increased by attaching targeting ligands to the QD surface [44;50]. Akerman *et al*. previously showed that F3 peptide-coated QD and LyP-1 peptide-coated QD specifically bound to the blood and the lymphatic vasculatures of MDA-MB-435 human breast carcinoma xenograft following intravenous administration [52]. Gao *et al.* targeted C4-2 human prostate cancer xenograft using prostate-specific membrane antigen-QD (PSMA-QD) [44]. Similarly, Cai *et al.* imaged the tumour vasculature of U87MG human glioblastoma xenografts implanted subcutaneously in mice using RGD-QD [50]. In order to quantify the targeting efficiency of QD, tumour accumulation was evaluated using a dual-function PET/near infrared (NIR) fluorescence probe obtained by conjugating $^{64}$Cu isotope to NIR QD. Tumour accumulation of the RGD-QD was 4% of injected dose per gram tissue (ID/g) compared to less than 1% ID/g with non-targeted QD [64]. Diagaradjane *et al.* demonstrated three different phases of tumour accumulation for epidermal growth factor-QD (EGF-QD) using a colorectal HCT116 xenograft model, which highly expresses EGF receptor (EGFR) [51]. Immediately after systemic administration, both EGF-QD and non-targeted QD (~3 min post injection) influxed to the tumour. This phase was followed by the clearance phase, where the two types of QD were cleared from the tumour interstitium between 3-60 min post-injection. Next, a steady increase in EGF-QD fluorescence in the tumour was observed between 1-6 hrs, reflecting receptor-specific binding and internalisation. In contrast, QD without the EGF peptide did not accumulate in the tumour tissue during this period. These observations suggest that the increase in tumour fluorescence over time was due to EGFR-specific binding and internalisation of the EGF-QD.

There has been a growing concern regarding QD elimination from the body, since QD accumulation can potentiate increased toxicity. Ballou *et al.* previously reported retention of QD in the liver, lymph nodes, and bone marrow in mice up to several months [69]. In the same study, relocalisation of QD from the liver to the intestine content was described based on fluorescence imaging of dead animals, suggesting QD faecal excretion. Contrary to this report, others showed slow degradation of inorganic QD in mice and rats with no urine or faecal elimination up to 28 days post-injection [94;104]. Frangioni and colleagues correlated the QD size with their degree of elimination [103;105]. QD with an average diameter of 5-6 nm, which is below the renal filtration threshold, were excreted via urine 4 hrs post-injection. QD of larger diameters undesirably remained in the liver, which may increase the potential toxicity of these nanoparticles on the long-term.

The fate of QD following the different route of administrations has also been studied. Polymer-coated QD with an average diameter of 15-20 nm were found to migrate rapidly to the sentinel lymph nodes (SLN) after subcutaneous, intradermal or intraparenchymal injection in living animals [71-74;93]. QD migration to the lymph nodes occurred within 1-5 min post-injection and was found selective to the first lymph node. Similar behaviour was observed after injecting QD of different size and surface charge properties in M21 human melanoma and MH-15 mouse teratocarcinoma xenograft models implanted subcutaneously in nude mice [95]. This observation can be advantageous in the diagnosis of cancer metastasis by identifying SLN residing nodules. Overall, it can be seen that QD can reside in different organs in living animals depending on QD characteristics (size, surface charge, and coating) and the route of administration. Furthermore, these studies have shown that QD can accumulate in the body for extensive periods of time which requires further investigation to identify the long-term toxicity of QD before embarking on any clinical use of QD.

**Table 1: *In vivo* studies of *f*-QD.**

| QD characteristic | QD colour | QD dose | Animal/ Route of administration | Aim of the study | Methods of detection | Main findings | Reference |
|---|---|---|---|---|---|---|---|
| **Biodistribution** | | | | | | | |
| $PEG_{750}$, $PEG_{3400}$,$PEG_{5000}$-PAA-CdSe/ZnS QD | red | 360 pmol | athymic nude mice ( i.v) | Evaluate the effect of surface coating on QD biodistribution | Fluorescence imaging of living animal | $PEG_{5000}$ increased QD blood circulation and reduced liver and spleen uptake | [69] |
| LM-QD, BSA-QD | red | 5 nmol | Sprague-Dawley rats ( i.v) | Quantify QD tissue distribution | ICP-AES | - QD blood half-life was 39-59 min.<br>- liver uptake 40-90%ID after 90 min | [94] |
| $PEG_{5000}$ -CdTe/ ZnS QD | NIR | 40 pmol | Mice ( i.v) | Assess QD fate in mice following i.v. injection | ICP-MS | QD were retained in liver & spleen with no urine or faecal excretion up to 28 days | [104] |
| Cysteine-coated CdSe/ZnS QD, $^{99m}$Tc-labelled cysteine- QD | Green-red | rats: 3 nmol<br>mice: 300 pmol | Sprague-Dawley rats<br>CD-1 mice ( i.v) | Evaluate QD elimination from the body | Gamma counting | QD with a final diameter < 5.5 nm were excreted rapidly in the urine | [103] |
| MAA-coated Cd $^{125m}$Te/ZnS QD+mAb 201B | Not specified | 5 μg QD | Balb/ c mice ( i.v) | Quantify *in vivo* targeting efficiency of QD-antibody | Micro SPECT/ CT, gamma counting | QD-antibody revealed high lung targeting | [79] |
| $^{64}$Cu-DOTA ±$PEG_{2000}$ -QD | Green & NIR | 25 pmol | Nude mice ( i.v) | Evaluate the biodistribution of commercially available QD | Micro PET, gamma counting | Rapid liver and spleen uptake regardless of the size or presence of surface $PEG_{2000}$ | [65] |
| **Tumour targeting & imaging** | | | | | | | |
| F3 Peptide- or , LyP-1 Peptide - $PEG_{5000}$ CdSe/ZnS QD | Green & red | 100-200 μg | Balb/c *nu/nu* mice ( i.v) | Explore the feasibility of *in vivo* targeting using QD | Confocal & epifluorescence microscopy | Peptide-coated QD specifically bound to the MDA-MB-435 human breast carcinoma vasculatures | [52] |
| PSMA-$PEG_{5000}$-CdSe/ZnS QD | red | 0.4 nmol, 6 nmol | Balb/c nude mice ( i.v) | Combine QD tumour targeting and imaging in living animals | Fluorescence imaging of living animal, epifluorescence microscopy | PSMA-QD showed faster and efficient accumulation in human prostate cancer (C4-2) xenograft than non-targeted QD | [44] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| RGD-$PEG_{2000}$ -CdTe QD | NIR | 200 pmol | Athymic nude mice ( i.v) | Non-invasive imaging of tumour vasculature in living animals using peptide-coated QD | Fluorescence imaging of living animal | Only RGD-QD accumulated in the U87MG human glioblastoma xenograft 6 hrs post-injection | [50] |
| RGD-$PEG_{2000}$-CdTe QD, $^{64}$Cu-DOTA- RGD-$PEG_{2000}$-QD | NIR | 20 pmol | Athymic nude mice ( i.v) | Quantify the tumour-targeting efficiency of QD | Small-animal PET, *ex vivo* NIR fluorescence imaging | RGD peptide increased QD tumour accumulation from <1% ID /g to 4% ID/g | [64] |
| EGF- $NH_2$ -CdTe QD | NIR | 10 pmol | Swiss *nu/nu* mice | *In vivo* imaging of epidermal growth factor (EGF) receptor (EGFR) overexpressed on tumours | *In vivo* and *ex vivo* NIR fluorescence imaging | -Identified the phases of QD accumulation in colorectal HCT116 tumour<br>- Only EGF-QD accumulated in the tumour 1-6 hrs post-injection | [51] |
| mPEG-CdSe/ZnS QD<br>COOH- PEG QD<br>$NH_2$ –PEG QD | Red & NIR | 25-100 pmol | Athymic nude mice ( i.t) | Evaluate the behaviour of QD injected directly into solid tumours | Fluorescence imaging of living animal, necropsy | All QD injected into tumour models migrated rapidly to SLN | [95] |
| **Lymph node mapping** | | | | | | | |
| Oligomeric phosphine- coated CdTe QD | NIR | 400 pmol<br>10 pmol | Yorkshire pigs<br>SKH1 mice (i.d) | Explore the feasibility of using QD in lymph node mapping | NIR fluorescence imaging | QD Localisation in SLN occurred within 3-4 min post-injection | [93] |
| COOH-polymer CdTe/ZnS QD | NIR | 8-16 pmol | Athymic mice ( i.c, s.c.) | Map lymphatic drainage from two different basins into same lymph node | NIR fluorescence imaging | Non-invasive and simultaneous visualisation of two separate lymphatic flows into the axillary lymph node | [106] |
| Oligomeric phosphine- coated CdTe QD | NIR | 200- 400 pmol | Yorkshire pigs ( i.p) | Identify SLN following direct injection in the organ | Intraoperative NIR fluorescence imaging | Mapping the SLN of the lung, oesophagus, pleural space and GIT | [71-74] |

PAA: polyacrylic acid; QD-LM: QD coated with mercaptoundecanoic acid crosslinked with lysine; QD-BSA: QD-LM conjugated to bovine serum albumin; ICP-AES: inductively coupled plasma atomic emission spectroscopy; ICP-MS: inductively coupled plasma mass spectroscopy; $^{99m}$Tc: Technetium-99m; $^{125m}$Te: Tellurium -125m; MAA: mercaptoacetic acid; mAb 201B: mouse lung thrombomodulin antibody; SPECT: single photon emission computed tomography; CT: computerised tomography; PET positron emission tomography; F3: peptide preferentially binds to blood vessels and tumour cells; Lyp-1: peptide recognises lymphatic vessels and tumour cells; PSMA: prostate-specific membrane antigen; RGD: arginine-glycine-aspartic acid; EGF: epidermal growth factor; mPEG: methoxy polyethylene glycol; i.t: intratumoural; i.d: intradermal; i.c: intracutaneous; s.c: subcutaneous; i.p: intraparenchymal; GIT: gastrointestinal tract.

## 6. QD toxicity

QD have been applied to molecular biology due to their brighter fluorescence and higher photostability, however potential concerns about the QD toxicity have risen, caused by the well-known toxicity of cadmium and selenium, the physicochemical properties of the surface coat, and QD small size that provides large surface area that would interact with different biological molecules [100;107-109]. Potential routes for QD exposure are environmental, workshops, therapeutics, and diagnostics. Few studies reported that QD nanoparticles could be inhaled and deposited in the respiratory passages depending on their sizes [107;110]. Also, it has been shown that QD are capable of penetrating the skin barrier [111-113] and can cause inflammation and irritation in epidermal keratinocytes *in vitro* after long-term exposure (48hours) [114]. These preliminary studies suggest extra care to be taken, as systemic QD toxicity can happen via inhalation and direct skin contact, especially during the QD production step.

**QD toxicity *in vitro*:** The toxicity of QD is mainly derived from their intrinsic core composition such as CdSe and CdTe. $Cd^{+2}$ ions have been shown to be toxic upon their release from the QD core due to photolysis and/or oxidation [4]. Other mechanisms contributing to QD-induced cytotoxicity (Figure 2) have also been identified including the formation of reactive oxygen species (ROS) tha§t induce cell damage [41]; the interaction of QD nanoparticles with the individual cell components (e.g DNA or proteins) or with the cell membrane [115]. QD-induced cell dysfunction is accompanied by apoptotic and necrotic biochemical changes including: morphological alteration in the plasma membrane; mitochondria and nucleus damage [41]; lysosome enlargement [116]; reduction in cytochrome C concentration [41;116;117]; loss of mitochondrial membrane potential [117]; and upregulation of peroxidised lipids [118].

The correlation between cytotoxicity and free $Cd^{2+}$ ions has been established [4;115;119] with the occurrence of significant cell death in the range of 100 μM to 400 μM $Cd^{2+}$ ions [4]. The blue-shift in QD fluorescence spectrum was observed as a sign of QD size reduction and $Cd^{2+}$ release [4;120]. In addition, QD-induced cytotoxicity dramatically increased in the case of QD exposure to oxygen or ultraviolet (UV) light [4;41]. The concept of QD phototoxicity has been exploited in photodynamic therapy as previously mentioned [84;86;88-90]. Several

attempts to reduce QD toxicity have also been described. For instance, ZnS coating protects the QD core from oxidation, which minimises $Cd^{+2}$ leakage and reduces the QD-induced cytotoxicity [109;116;119]. In addition, the use of antioxidants, such as N-acetylcysteine (NAC) has been shown to be effective in reducing QD cytotoxicity.

In general, most cytotoxicity studies used QD solubilised by direct exchange of the organic coat (TOPO) with hydrophilic ligands, such as mercaptopropionic acid (MPA-QD) [41;42;109;121], mercaptoacetic acid (MAA-QD) [4], mercaptoundecanoic acid (MUA-QD) [42], cysteamine (QD-$NH_2$) [109;116] and thioglycerol (QD-OH) [109]. However, ligand detachment from the QD surface may occur due to the weak interaction between the QD surface and the ligands [38;122], especially under unfavourable conditions like those in the endosomal compartment [10]. Several studies have indicated that cell incubation with QD solubilised by surface ligand exchange was mostly associated with severe cell death with increasing QD concentrations, similar to that induced by $Cd^{2+}$ ions [4;41;115;116;121], which indiciets the need for more stable QD.

Hoshino *et al.* reported that no evidence of $Cd^{2+}$ induced cytotoxicity was identified once ZnS coating was used [109]. Kirchner *et al.* showed that PEG coating greatly improved CdSe/ZnS QD toxicity profiles [115]. PEG-silica coated QD, which were fabricated by embedding the CdSe/ZnS QD in a shell of cross-linked silica molecules and then conjugated with PEG were shown to be non-toxic up to 30 μM in $Cd^{+2}$ surface concentration [119]. Similar to this, Pellegrino *et al.* demonstrated that silica-coated QD (silanised) were highly resistant to chemical and metabolic degradation [123]. Furthermore, conjugating peptides to silanised CdSe/ZnS QD showed low cytotoxicity even once translocated to the cell nucleus [124]. Zhang *et al.* investigated the genotoxicity of PEG-silica coated QD in human skin fibroblast (HSF-42) cells exposed to QD dose between 8 nM and 80 nM, verifying that PEG-silica coated QD were non-toxic even at the gene level [125]. Overall, the cytotoxicity studies carried out so far have shown that the key determinants of QD toxicity are the composition and functionalisation. However, other factors including cell type [42], QD size [121], and QD exposure to oxygen and UV light [4] were also found to influence QD cytotoxicity.

**QD toxicity *in vivo*:** The concern about the potential toxicity of QD *in vivo* is growing due to the well-established toxicological profile of cadmium, the physicochemical characteristics

of certain QD surface coatings, and the size of QD. In general, the small size of nanoparticles provides a large surface for interaction with different biological molecules [107;109]. Moreover, nanoparticles of few nanometers in size can enter vital organs such as heart, lung, and brain following intravenous administration. Therefore, studies that will help determine the QD toxicity *in vivo* are highly required.

Information about QD toxicity were initially obtained by alternative models of more complex organisms. *Xenopus* embryos [22] and zebrafish embryos [19] are some of the most sensitive models in which QD toxicity has been tested. Zebrafish embryos and *Xenopus* embryos microinjected with $1\times10^8$ QD/cell and $2\times10^9$QD/cell, respectively, did not exhibit any sign of toxicity. However, both Zebrafish and *Xenopus* embryos exhibited abnormalities as the doses were increased to $2\times10^8$ QD/cell and $5\times10^9$QD/cell, respectively. This is thought to be due to either the intrinsic toxicity of QD or the osmotic equilibrium changes [22].

Several groups have injected QD in animals for targeting and imaging purposes. However, very few studies reported QD toxicity in living animals. QD injected systemically (via tail vein or jugular vein) in mice and rats (pmol-nmol range), showed no apparent toxicity several months post-injection [20;94;95]. Other studies have shown that 200-400 pmol of near-infrared (NIR) QD were injected locally to map the sentinel lymph nodes (SLN) into Yorkshire pigs, no changes in the heart rate, blood pressure, and oxygen level were observed during the experimental procedure and after several hours [71-74;93]. The low toxicity observed in these latter studies was expected since the QD were injected locally and in most cases the injected site (tumour and SLN) was removed by the end of the surgical procedure.

## 5. CONCLUSION

Semiconductor nanocrystals are tiny light-emitting nanoparticles with a mean diameter of 1 to 20 nm. They are generally composed of a semiconductor core covered with a shell of a second semiconductor material, mainly ZnS. QDs offer many advantages in comparison to conventional organic dyes such as bright photoluminescence, narrow symmetric emission, broad excitation spectrum, and high photostability. The diversity of QD composition, size and surface functionalisation results in their versatile applications. They can be made very

effective for cellular and *in vivo* imaging by coupling with targeting ligands such as, proteins, peptides or antibodies. The photogeneration of free radicals by QDs is experimentally used in photodynamic therapy. Interestingly, the *in vivo* behaviour is be easily manipulated by modulating the particle size and surface coating, which impact QD blood circulation, organ distribution, excretion and toxicity.

Reference List


1. Esaki L. The evolution of nanoscale quantum effects in semiconductor physics. Nanostruct Mater 1999; 12(1-4):1-8.

2. Dabbousi BO, RodriguezViejo J, Mikulec FV, Heine JR, Mattoussi H, Ober R et al. (CdSe)ZnS core-shell quantum dots: Synthesis and characterization of a size series of highly luminescent nanocrystallites. J Phys Chem B 1997; 101(46):9463-9475.

3. Hines MA, Guyot-Sionnest P. Synthesis and characterization of strongly luminescing ZnS-Capped CdSe nanocrystals. J Phys Chem 1996; 100(2):468-471.

4. Derfus AM, Chan WC, Bhatia SN. Probing the cytotoxicity of semiconductor quantum dots. Nano Lett 2003; 4(1):11-18.

5. Rossetti R, Brus L. Electron-hole recombination emission as a probe of surface-chemistry in aqueous Cds colloids. J Phys Chem 1982; 86(23):4470-4472.

6. Hezinger AFE, Tessmar J, Gopferich A. Polymer coating of quantum dots - A powerful tool toward diagnostics and sensorics. Eur J Pharm Biopharm 2008; 68:138-152.

7. Murray CB, Norris DJ, Bawendi MG. Synthesis and characterization of nearly monodisperse Cde (e = S, Se, Te) semiconductor nanocrystallites. J Am Chem Soc 1993; 115(19):8706-8715.

8. Alivisatos AP. Semiconductor clusters, nanocrystals, and quantum dots. Science 1996; 271:933-937.

9. Schaller RD, Klimov VI. High efficiency carrier multiplication in PbSe nanocrystals: Implications for solar energy conversion. Phys Rev Lett 2004; 92(18).

10. Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos AP. Semiconductor nanocrystals as fluorescent biological labels. Science 1998; 281(5385):2013-2016.

11. Chan WC, Nie S. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science 1998; 281(5385):2016-2018.

12. Gao X, Yang L, Petros JA, Marshall FF, Simons JW, Nie S. In vivo molecular and cellular imaging with quantum dots. Curr Opin Biotechnol 2005; 16(1):63-72.

13. Costa-Fernandez JM, Pereiro R, Sanz-Medel A. The use of luminescent quantum dots for optical sensing. TrAC, Trends Anal Chem 2006; 25(3):207-218.

14. Alivisatos AP, Gu W, Larabell C. Quantum dots as cellular probes. Annu Rev Biomed Eng 2005; 7:55-76.

15. Maysinger D, Lovric J, Eisenberg A, Savic R. Fate of micelles and quantum dots in cells. Eur J Pharm Biopharm 2007; 65(3):270-281.

16. Kim S, Fisher B, Eisler HJ, Bawendi M. Type-II quantum dots: CdTe/CdSe(core/shell) and CdSe/ZnTe(core/shell) heterostructures. J Am Chem Soc 2003; 125(38):11466-11467.

17. Pradhan N, Goorskey D, Thessing J, Peng XG. An alternative of CdSe nanocrystal emitters: Pure and tunable impurity emissions in ZnSe nanocrystals. J Am Chem Soc 2005; 127(50):17586-17587.

18. Pradhan N, Battaglia DM, Liu YC, Peng XG. Efficient, stable, small, and water-soluble doped ZnSe nanocrystal emitters as non-cadmium biomedical labels. Nano Lett 2007; 7:312-317.

19. Rieger S, Kulkarni RP, Darcy D, Fraser SE, Koster RW. Quantum dots are powerful multipurpose vital labeling agents in zebrafish embryos. Dev Dyn 2005; 234(3):670-681.

20. Stroh M, Zimmer JP, Duda DG, Levchenko TS, Cohen KS, Brown EB et al. Quantum dots spectrally distinguish multiple species within the tumor milieu in vivo. Nat Med 2005; 11(6):678-682.

21. Voura EB, Jaiswal JK, Mattoussi H, Simon SM. Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy. Nat Med 2004; 10(9):993-998.

22. Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A. In vivo imaging of quantum dots encapsulated in phospholipid micelles. Science 2002; 298(5599):1759-1762.

23. Ma J, Chen YJ, Guo J, Wang CC, Yang WL, Xu L et al. Photostability of thiol-capped CdTe quantum dots in living cells: the effect of photo-oxidation. Nanotechnology 2006; 17:2083-2089.

24. Kho R, Torres-Martinez CL, Mehra RK. A simple colloidal synthesis for gram-quantity production of water-soluble ZnS nanocrystal powders. J Colloid Interface Sci 2000; 227(2):561-566.

25. Yu WW, Qu L, Guo W, Peng X. Experimental determination of the extinction coefficient of CdTe, CdSe, and CdS nanocrystals. Chem Mater 2003; 15(14):2854-2860.

26. Park J, An K, Hwang Y, Park JG, Noh HJ, Kim JY et al. Ultra-large-scale syntheses of monodisperse nanocrystals. Nat Mater 4 A.D.; 3(12):891-895.

27. Nirmal M, Murray CB, Norris DJ, Bawendi MG. Size-dependent spectroscopy and photodynamics of some Ii-Vi semiconductor nanocrystallites (quantum dots). Ultrafast Pulse Generation and Spectroscopy 1993; 1861:280-284.

28. Yu WW, Wang YA, Peng XG. Formation and stability of size-, shape-, and structure-controlled CdTe nanocrystals: Ligand effects on monomers and nanocrystals. Chem Mater 2003; 15(22):4300-4308.

29. Jaiswal JK, Mattoussi H, Mauro JM, Simon SM. Long-term multiple color imaging of live cells using quantum dot bioconjugates. Nat Biotechnol 2003; 21(1):47-51.

30. Gerion D, Pinaud F, Williams SC, Parak WJ, Zanchet D, Weiss S et al. Synthesis and properties of biocompatible water-soluble silica-coated CdSe/ZnS semiconductor quantum dots. J Phys Chem B 2001; 105(37):8861-8871.

31. Pinaud F, King D, Moore HP, Weiss S. Bioactivation and cell targeting of semiconductor CdSe/ZnS nanocrystals with phytochelatin-related peptides. J Am Chem Soc 2004; 126(19):6115-6123.

32. Huang BH, Tomalia DA. Dendronization of gold and CdSe/cdS (core-shell) quantum functionalized dendrons dots with tomalia type, thiol core, poly(amidoamine) (PAMAM) dendrons. J Lumin 2005; 111(4):215-223.

33. Wang YA, Li JJ, Chen H, Peng X. Stabilization of inorganic nanocrystals by organic dendrons. J Am Chem Soc 2002; 124(10):2293-2298.

34. Kim S, Bawendi MG. Oligomeric Ligands for luminescent and stable nanocrystal quantum dots. J Am Chem Soc 2003; 125(48):14652-14653.

35. Kalyuzhny G, Murray RW. Ligand effects on optical properties of CdSe nanocrystals. J Phys Chem B 2005; 109(15):7012-7021.

36. Kuno M, Lee JK, Dabbousi BO, Mikulec FV, Bawendi MG. The band edge luminescence of surface modified CdSe nanocrystallites: Probing the luminescing state. J Chem Phys 1997; 106(23):9869-9882.

37. Mekis I, Talapin DV, Kornowski A, Haase M, Weller H. One-pot synthesis of highly luminescent CdSe/CdS core-shell nanocrystals via organometallic and "greener" chemical approaches. J Phys Chem B 2003; 107(30):7454-7462.

38. Dollefeld H, Hoppe K, Kolny J, Schilling K, Weller H, Eychmuller A. Investigations on the stability of thiol stabilized semiconductor nanoparticles. Phys Chem Chem Phys 2002; 4(19):4747-4753.

39. Smith AM, Duan H, Rhyner MN, Ruan G, Nie S. A systematic examination of surface coatings on the optical and chemical properties of semiconductor quantum dots. Phys Chem Chem Phys 2006; **8**:3895-3903.

40. Breus VV, Heyes CD, Nienhaus GU. Quenching of CdSe-ZnS core-shell quantum dot luminescence by water-soluble thiolated ligands. J Phys Chem C 2007; 111:18589-18594.

41. Lovric J, Cho SJ, Winnik FM, Maysinger D. Unmodified cadmium telluride quantum dots induce reactive oxygen species formation leading to multiple organelle damage and cell death. Chem Biol 2005; 12(11):1227-1234.

42. Shiohara A, Hoshino A, Hanaki K, Suzuki K, Yamamoto K. On the cyto-toxicity caused by quantum dots. Microbiol Immunol 2004; 48(9):669-675.

43. Carion O, Mahler B, Pons T, Dubertret B. Synthesis, encapsulation, purification and coupling of single quantum dots in phospholipid micelles for their use in cellular and in vivo imaging. Nat Protoc 2007; 2:2383-2390.

44. Gao X, Cui Y, Levenson RM, Chung LW, Nie S. In vivo cancer targeting and imaging with semiconductor quantum dots. Nat Biotechnol 2004; 22(8):969-976.

45. Parak WJ, Pellegrino T, Plank C. Labelling of cells with quantum dots. Nanotechnology 2005; 16(2):R9-R25.

46. Goldman ER, Balighian ED, Mattoussi H, Kuno MK, Mauro JM, Tran PT et al. Avidin: a natural bridge for quantum dot-antibody conjugates. J Am Chem Soc 2002; 124(22):6378-6382.

47. Liu W, Howarth M, Greytak AB, Zheng Y, Nocera DG, Ting AY et al. Compact biocompatible quantum dots functionalized for cellular imaging. J Am Chem Soc 2008; 130(4):1274-1284.

48. Jayagopal A, Russ PK, Haselton FR. Surface engineering of quantum dots for in vivo vascular imaging. Bioconjug Chem 2007; 18(5):1424-1433.

49. Tada H, Higuchi H, Wanatabe TM, Ohuchi N. In vivo real-time tracking of single quantum dots conjugated with monoclonal anti-HER2 antibody in tumors of mice. Cancer Res 2007; 67(3):1138-1144.

50. Cai W, Shin DW, Chen K, Gheysens O, Cao Q, Wang SX et al. Peptide-labeled near-infrared quantum dots for imaging tumor vasculature in living subjects. Nano Lett 2006; 6(4):669-676.

51. Diagaradjane P, Orenstein-Cardona JM, Colon-Casasnovas E, Deorukhkar A, Shentu S, Kuno N et al. Imaging epidermal growth factor receptor expression in vivo: pharmacokinetic and biodistribution characterization of a bioconjugated quantum dot nanoprobe. Clin Cancer Res 2008; 14(3):731-741.

52. Akerman ME, Chan WC, Laakkonen P, Bhatia SN, Ruoslahti E. Nanocrystal targeting in vivo. Proc Natl Acad Sci U S A 2002; 99(20):12617-12621.

53. Duan H, Nie S. Cell-penetrating quantum dots based on multivalent and endosome-disrupting surface coatings. J Am Chem Soc 2007; 129(11):3333-3338.

54. Bagalkot V, Zhang L, Levy-Nissenbaum E, Jon S, Kantoff PW, Langer R et al. Quantum dot-aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer. Nano Lett 2007; 7(10):3065-3070.

55. Schroedter A, Weller H, Eritja R, Ford WE, Wessels JM. Biofunctionalization of silica-coated CdTe and gold nanocrystals. Nano Lett 2002; 2(12):1363-1367.

56. Fu A, Micheel CM, Cha J, Chang H, Yang H, Alivisatos AP. Discrete nanostructures of quantum dots/Au with DNA. J Am Chem Soc 2004; 126(35):10832-10833.

57. Pathak S, Choi SK, Arnheim N, Thompson ME. Hydroxylated quantum dots as luminescent probes for in situ hybridization. J Am Chem Soc 2001; 123(17):4103-4104.

58. Lagerholm BC, Wang MM, Ernst LA, Ly DH, Liu HJ, Bruchez MP et al. Multicolor coding of cells with cationic peptide coated quantum dots. Nano Lett 2004; 4(10):2019-2022.

59. Zhang Y, So MK, Rao J. Protease-modulated cellular uptake of quantum dots. Nano Lett 2006; 6(9):1988-1992.

60. Rozenzhak SM, Kadakia MP, Caserta TM, Westbrook TR, Stone MO, Naik RR. Cellular internalization and targeting of semiconductor quantum dots. Chem Commun (Camb ) 2005;(17):2217-2219.

61. Ruan G, Agrawal A, Marcus AI, Nie S. Imaging and tracking of tat peptide-conjugated quantum dots in living cells: new insights into nanoparticle uptake, intracellular transport, and vesicle shedding. J Am Chem Soc 2007; 129(47):14759-14766.

62. Susumu K, Uyeda HT, Medintz IL, Pons T, Delehanty JB, Mattoussi H. Enhancing the stability and biological functionalities of quantum dots via compact multifunctional ligands. J Am Chem Soc 2007; 129(45):13987-13996.

63. Delehanty JB, Medintz IL, Pons T, Brunel FM, Dawson PE, Mattoussi H. Self-assembled quantum dot-peptide bioconjugates for selective intracellular delivery. Bioconjug Chem 2006; 17(4):920-927.

64. Cai W, Chen K, Li ZB, Gambhir SS, Chen X. Dual-function probe for PET and near-infrared fluorescence imaging of tumor vasculature. J Nucl Med 2007; 48(11):1862-1870.

65. Schipper ML, Cheng Z, Lee SW, Bentolila LA, Iyer G, Rao J et al. microPET-Based Biodistribution of Quantum Dots in Living Mice. J Nucl Med 2007.

66. Mulder WJ, Koole R, Brandwijk RJ, Storm G, Chin PT, Strijkers GJ et al. Quantum dots with a paramagnetic coating as a bimodal molecular imaging probe. Nano Lett 2006; 6(1):1-6.

67. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ et al. Quantum dots for live cells, in vivo imaging, and diagnostics. Science 2005; 307(5709):538-544.

68. Parak WJ, Boudreau R, Le Gros M, Gerion D, Zanchet D, Micheel CM et al. Cell motility and metastatic potential studies based on quantum dot imaging of phagokinetic tracks. Adv Mater 2002; 14(12):882-885.

69. Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS. Noninvasive imaging of quantum dots in mice. Bioconjug Chem 2004; 15(1):79-86.

70. Larson DR, Zipfel WR, Williams RM, Clark SW, Bruchez MP, Wise FW et al. Water-soluble quantum dots for multiphoton fluorescence imaging in vivo. Science 2003; 300(5624):1434-1436.

71. Parungo CP, Colson YL, Kim SW, Kim S, Cohn LH, Bawendi MG et al. Sentinel lymph node mapping of the pleural space. Chest 2005; 127(5):1799-1804.

72. Parungo CP, Ohnishi S, Kim SW, Kim S, Laurence RG, Soltesz EG et al. Intraoperative identification of esophageal sentinel lymph nodes with near-infrared fluorescence imaging. J Thorac Cardiovasc Surg 2005; 129(4):844-850.

73. Soltesz EG, Kim S, Laurence RG, DeGrand AM, Parungo CP, Dor DM et al. Intraoperative sentinel lymph node mapping of the lung using near-infrared fluorescent quantum dots. Ann Thorac Surg 2005; 79(1):269-277.

74. Soltesz EG, Kim S, Kim SW, Laurence RG, De Grand AM, Parungo CP et al. Sentinel lymph node mapping of the gastrointestinal tract by using invisible light. Ann Surg Oncol 2006; 13(3):386-396.

75. Yu X, Chen L, Deng Y, Li K, Wang Q, Li Y et al. Fluorescence analysis with quantum dot probes for hepatoma under one- and two-photon excitation. J Fluoresc 2007; 17(2):243-247.

76. Yu X, Chen L, Li K, Li Y, Xiao S, Luo X et al. Immunofluorescence detection with quantum dot bioconjugates for hepatoma in vivo. J Biomed Opt 2007; 12(1):014008.

77. Parak WJ, Gerion D, Zanchet D, Woerz AS, Pellegrino T, Micheel C et al. Conjugation of DNA to silanized colloidal semiconductor nanocrystalline quantum dots. Chem Mater 2002; 14(5):2113-2119.

78. Morgan NY, English S, Chen W, Chernomordik V, Russo A, Smith PD et al. Real time in vivo non-invasive optical imaging using near-infrared fluorescent quantum dots. Acad Radiol 2005; 12(3):313-323.

79. Woodward JD, Kennel SJ, Mirzadeh S, Dai S, Wall JS, Richey T et al. In vivo SPECT/CT imaging and biodistribution using radioactive (CdTe)-Te-125m/ZnS nanoparticles. Nanotechnology 2007; 18(17).

80. Vuu K, Xie J, McDonald MA, Bernardo M, Hunter F, Zhang Y et al. Gadolinium-rhodamine nanoparticles for cell labeling and tracking via magnetic resonance and optical imaging. Bioconjug Chem 2005; 16(4):995-999.

81. Smith JD, Fisher GW, Waggoner AS, Campbell PG. The use of quantum dots for analysis of chick CAM vasculature. Microvasc Res 2007; 73(2):75-83.

82. Maurel V, Laferriere M, Billone P, Godin R, Scaiano JC. Free radical sensor based on CdSe quantum dots with added 4-amino-2,2,6,6-tetramethylpiperidine oxide functionality. J Phys Chem B 2006; 110(33):16353-16358.

83. Nikoobakht B, Burda C, Braun M, Hun M, El-Sayed MA. The quenching of CdSe quantum dots photoluminescence by gold nanoparticles in solution. Photochem Photobiol 2002; 75(6):591-597.

84. Samia AC, Chen X, Burda C. Semiconductor quantum dots for photodynamic therapy. J Am Chem Soc 2003; 125(51):15736-15737.

85. Hsieh JM, Ho ML, Wu PW, Chou PT, Tsai TT, Chi Y. Iridium-complex modified CdSe/ZnS quantum dots; a conceptual design for bi-functionality toward imaging and photosensitization. Chem Commun (Camb ) 2006;(6):615-617.

86. Bakalova R, Ohba H, Zhelev Z, Ishikawa M, Baba Y. Quantum dots as photosensitizers? Nat Biotechnol 2004; 22(11):1360-1361.

87. Tsay JM, Trzoss M, Shi L, Kong X, Selke M, Jung ME et al. Singlet oxygen production by peptide-coated quantum dot-photosensitizer conjugates. J Am Chem Soc 2007; 129(21):6865-6871.

88. Samia AC, Dayal S, Burda C. Quantum dot-based energy transfer: perspectives and potential for applications in photodynamic therapy. Photochem Photobiol 2006; 82(3):617-625.

89. Clarke SJ, Hollmann CA, Zhang Z, Suffern D, Bradforth SE, Dimitrijevic NM et al. Photophysics of dopamine-modified quantum dots and effects on biological systems. Nat Mater 2006; 5(5):409-417.

90. Bakalova R, Ohba H, Zhelev Z, Nagase T, Jose R, Ishikawa M et al. Quantum dot anti-CD conjugates: Are they potential photosensitizers or potentiators of classical photosensitizing agents in photodynamic therapy of cancer? Nano Lett 2004; 4(9):1567-1573.

91. Hoshino A, Hanaki K, Suzuki K, Yamamoto K. Applications of T-lymphoma labeled with fluorescent quantum dots to cell tracing markers in mouse body. Biochem Biophys Res Commun 2004; 314(1):46-53.

92. Tombolini R, Jansson JK. Monitoring of GFP-tagged bacterial cells. Methods Mol Biol 1998; 102:285-298.

93. Kim S, Lim YT, Soltesz EG, De Grand AM, Lee J, Nakayama A et al. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nat Biotechnol 2004; 22(1):93-97.

94. Fischer HC, Liu LC, Pang KS, Chan WCW. Pharmacokinetics of nanoscale quantum dots: In vivo distribution, sequestration, and clearance in the rat. Adv Funct Mater 2006; 16(10):1299-1305.

95. Ballou B, Ernst LA, Andreko S, Harper T, Fitzpatrick JA, Waggoner AS et al. Sentinel lymph node imaging using quantum dots in mouse tumor models. Bioconjug Chem 2007; 18(2):389-396.

96. Cabanas RM. An approach for the treatment of penile carcinoma. Cancer 1977; 39(2):456-466.

97. So MK, Xu C, Loening AM, Gambhir SS, Rao J. Self-illuminating quantum dot conjugates for in vivo imaging. Nat Biotechnol 2006; 24(3):339-343.

98. Wang DS, He JB, Rosenzweig N, Rosenzweig Z. Superparamagnetic Fe2O3 Beads-CdSe/ZnS quantum dots core-shell nanocomposite particles for cell separation. Nano Lett 2004; 4(3):409-413.

99. van Tilborg GA, Mulder WJ, Chin PT, Storm G, Reutelingsperger CP, Nicolay K et al. Annexin A5-conjugated quantum dots with a paramagnetic lipidic coating for the multimodal detection of apoptotic cells. Bioconjug Chem 2006; 17(4):865-868.

100. Tsay JM, Michalet X. New light on quantum dot cytotoxicity. Chemistry & Biology 2005; 12(11):1159-1161.

101. Al-Jamal WT, Al-Jamal KT, Tian B, Cakebread A, Halket JM, Kostarelos K. Tumor targeting of functionalized quantum dot-liposome hybrids by intravenous administration. Mol Pharm 2009; 6(2):520-530.

102. Al-Jamal WT, Al-Jamal KT, Cakebread A, Halket JM, Kostarelos K. Blood circulation and tissue biodistribution of lipid--quantum dot (L-QD) hybrid vesicles intravenously administered in mice. Bioconjug Chem 2009; 20(9):1696-1702.

103. Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Ipe BI et al. Renal clearance of quantum dots. Nat Biotechnol 2007; 25(10):1165-1170.

104. Yang RS, Chang LW, Wu JP, Tsai MH, Wang HJ, Kuo YC et al. Persistent tissue kinetics and redistribution of nanoparticles, quantum dot 705, in mice: ICP-MS quantitative assessment. Environ Health Perspect 2007; 115(9):1339-1343.

105. Liu W, Choi HS, Zimmer JP, Tanaka E, Frangioni JV, Bawendi M. Compact cysteine-coated CdSe(ZnCdS) quantum dots for in vivo applications. J Am Chem Soc 2007; 129(47):14530-14531.

106. Hama Y, Koyama Y, Urano Y, Choyke PL, Kobayashi H. Simultaneous two-color spectral fluorescence lymphangiography with near infrared quantum dots to map two lymphatic flows from the breast and the upper extremity. Breast Cancer Res Treat 2006; 103(1):23-28.

107. Hardman R. A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. Environ Health Perspect 2006; 114(2):165-172.

108. Chang E, Thekkek N, Yu WW, Colvin VL, Drezek R. Evaluation of quantum dot cytotoxicity based on intracellular uptake. Small 2006; 2(12):1412-1417.

109. Hoshino A, Fujioka K, Oku T, Suga M, Sasaki YF, Ohta T et al. Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification. Nano Lett 2004; 4(11):2163-2169.

110. Oberdorster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K et al. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol 2005; 2:8.

111. Paliwal S, Menon GK, Mitragotri S. Low-frequency sonophoresis: ultrastructural basis for stratum corneum permeability assessed using quantum dots. J Invest Dermatol 2006; 126(5):1095-1101.

112. Ryman-Rasmussen JP, Riviere JE, Monteiro-Riviere NA. Penetration of intact skin by quantum dots with diverse physicochemical properties. Toxicol Sci 2006; 91(1):159-165.

113. Upadhyay P. Enhanced transdermal-immunization with diptheria-toxoid using local hyperthermia. Vaccine 2006; 24(27-28):5593-5598.

114. Ryman-Rasmussen JP, Riviere JE, Monteiro-Riviere NA. Surface coatings determine cytotoxicity and irritation potential of quantum dot nanoparticles in epidermal keratinocytes. J Invest Dermatol 2007; 127(1):143-153.

115. Kirchner C, Liedl T, Kudera S, Pellegrino T, Munoz Javier A, Gaub HE et al. Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. Nano Lett 2005; 5(2):331-338.

116. Cho SJ, Maysinger D, Jain M, Roder B, Hackbarth S, Winnik FM. Long-term exposure to CdTe quantum dots causes functional impairments in live cells. Langmuir 2007; 23(4):1974-1980.

117. Chan WH, Shiao NH, Lu PZ. CdSe quantum dots induce apoptosis in human neuroblastoma cells via mitochondrial-dependent pathways and inhibition of survival signals. Toxicol Lett 2006; 167(3):191-200.

118. Choi AO, Cho SJ, Desbarats J, Lovric J, Maysinger D. Quantum dot-induced cell death involves Fas upregulation and lipid peroxidation in human neuroblastoma cells. J Nanobiotechnology 2007; 5:1.

119. Kirchner C, Javier AM, Susha AS, Rogach AL, Kreft O, Sukhorukov GB et al. Cytotoxicity of nanoparticle-loaded polymer capsules. Talanta 2005; 67(3):486-491.

120. Chang E, Thekkek N, Yu WW, Colvin VL, Drezek R. Evaluation of quantum dot cytotoxicity based on intracellular uptake. Small 2006; 2(12):1412-1417.

121. Lovric J, Bazzi HS, Cuie Y, Fortin GR, Winnik FM, Maysinger D. Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. J Mol Med 2005; 83(5):377-385.

122. Boldt K, Bruns OT, Gaponik N, Eychmuller A. Comparative examination of the stability of semiconductor quantum dots in various biochemical buffers. J Phys Chem B 2006; 110(5):1959-1963.

123. Pellegrino T, Parak WJ, Boudreau R, Le Gros MA, Gerion D, Alivisatos AP et al. Quantum dot-based cell motility assay. Differentiation 2003; 71(9-10):542-548.

124. Chen FQ, Gerion D. Fluorescent CdSe/ZnS nanocrystal-peptide conjugates for long-term, nontoxic imaging and nuclear targeting in living cells. Nano Lett 2004; 4(10):1827-1832.

125. Zhang T, Stilwell JL, Gerion D, Ding L, Elboudwarej O, Cooke PA et al. Cellular effect of high doses of silica-coated quantum dot profiled with high throughput gene expression analysis and high content cellomics measurements. Nano Lett 2006; 6(4):800-808.