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Review Article

Impacts of quantum dots in molecular detection and bioimaging of cancer

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Abstract

Introduction: A number of assays have so far been exploited for detection of cancer biomarkers in various malignancies. However, the expression of cancer biomarker(s) appears to be extremely low, therefore accurate detection demands sensitive optical imaging probes. While optical detection using conventional fluorophores often fail due to photobleaching problems, quantum dots (QDs) offer stable optical imaging *in vitro* and *in vivo*.

Methods: In this review, we briefly overview the impacts of QDs in biology and its applications in bioimaging of malignancies. We will also delineate the existing obstacles for early detection of cancer and the intensifying use of QDs in advancement of diagnostic devices.

Results: Of the QDs, unlike the II-VI type QDs (e.g., cadmium (Cd), selenium (Se) or tellurium (Te)) that possess inherent cytotoxicity, the I-III-VI 2 type QDs (e.g., $AgInS_2$, $CuInS_2$, ZnS-$AgInS_2$) appear to be less toxic bioimaging agents with better control of band-gap energies. As highly-sensitive bioimaging probes, advanced hybrid QDs (e.g., QD-QD, fluorochrome-QD conjugates used for sensing through fluorescence resonance energy transfer (FRET), quenching, and barcoding techniques) have also been harnessed for the detection of biomarkers and the monitoring of delivery of drugs/genes to the target sites. Antibody-QD (Ab-QD) and aptamer-QD (Ap-QD) bioconjugates, once target the relevant biomarker, can provide highly stable photoluminescence (PL) at the target sites. In addition to their potential as nanobiosensors, the bioconjugates of QDs with homing devices have successfully been used for the development of smart nanosystems (NSs) providing targeted bioimaging and photodynamic therapy (PDT).

Conclusion: Having possessed great deal of photonic characteristics, QDs can be used for development of seamless multifunctional nanomedicines, theranostics and nanobiosensors.

Introduction

To date, malignancies have been categorized as one of the leading life-threatening diseases worldwide. This is mainly due to lack of imaging devices for detection of cancers at its early stage of development. As a golden rule, the earlier the diagnosis of the cancer, the higher the chance for the patient's survival. While over 200 diverse forms of cancers (e.g., breast, lung, prostate and ovarian cancers) are becoming a real menace worldwide,[1] currently used cancer diagnosis treatment modalities often fail to provide significant improvements. Thus, emergence of novel effective and specific strategies for cancer detection and therapy continue to become an inevitable need and indispensable scope of oncologists and cancer field researchers, for which implementation of new technologies are required to advance the early detection and management of cancers.[2]

Among different treatment strategies, use of multifunctional NSs such as polymer-/lipid-based nanoparticles (NPs), gene-based nanomedicines, Ab/Ap bioconjugates with drugs/toxins, monoclonal antibodies (mAbs), Ab scaffolds, Ab fragments (e.g., Fab, scFv), as advanced classes of pharmaceuticals, may provide much more effective means for the targeted therapy of cancer. Nevertheless, the fast growing fields of targeted therapy of cancer using multifunctional NSs and theranostics have yet to attain the final objectives as cancer therapy modalities. In fact, conjugation of homing and imaging devices with therapeutic agents is deemed to significantly improve the efficacy of these NSs.[3] Of the conjugation steps, decoration of NSs with optical imaging devices is an important pace because it provides great possibility for concurrent imaging and therapy, however in this process the conjugation moieties (e.g., types of fluorophores

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and conjugating linkers such as homobifunctional and heterobifuctional linking agents) needs to be carefully selected.[4] While the conventional fluorophores suffer from photobleaching, the QDs nanocrystals display stable optical properties necessary for targeted molecular imaging of cancer. Conjugation of organic/inorganic fluorophores to advanced NSs has resulted in emergence of a new class of seamless NSs called theranostics with simultaneous imaging and immunotherapy competencies. From biophotonic viewpoints, QDs are heterogeneous NPs and show unique optical characteristics such as broad absorption and distinct emission bands, upon which they have been nominated for various potential applications ranging from medicine to energy.[5] In fact, their multimodal photonic characteristics make them very attractive agents for molecular photoacoustic (MPA) imaging.[6] QDs with a size range between 2 and 10 nm in diameter used for bioimaging/biosensing show mobility of charge carriers (e.g. electrons and holes), which are constrained within the nanoscale dimensions. Once conjugated with homing devices such as Abs and aptamers (Aps), the bioconjugated QDs are capable of tracking different targets at molecular and cellular dimensions. Thus, QD-based monitoring of cancer metastasis and cancer development is achievable through monitoring the relocation of cancer cells.

In comparison with other normal organic dyes, QDs have a wide excitation spectrum along with symmetric and narrow emission spectra. Above all, the dissimilar QDs can concurrently emit different fluorescence under a similar excitement. Thus, simultaneous monitoring/tracking several biomarkers may provide a promising platform for more precise diagnosis through such multicolor QD probes.[7] Further, at equal excitation photon flux, QDs are capable of taking up 10-50 times more photons than organic dyes. This can grant much brighter fluorescence, while the QDs possess greater tenability than organic dyes for an accurate wavelengths from ultraviolet (UV) to near infrared (NIR).[8] Typically several manufactured QDs are able to emit in the range of 700-900 nm (Fig. 1), while NIR QDs display great potential to be exploited for *in vivo* fluorescence monitoring as well as quantifying a panel of biomarkers on intact cancer cells.[9] It should be enunciated that the simultaneous detection of various antigens by means of different emission properties of QDs will be very beneficial in cancer biology, in particular when detection of colocalized biomarkers are required. In fact, molecular diagnostics can become an important element when some procedures for diagnosing are achieved at the point-of-care, in particular for developing of personalized medicines. The QDs immunoconjugates appear to provide a highly stable fluorescence with simple excitation and instrumentation.

In this review, we provide some important insights on structural and physicochemical properties as well as surface modifications and impacts of QDs for *in vitro* and *in vivo* imaging and sensing in various tumors.

Structural and optical properties of QDs

QDs semiconductors, as one of the most studies nanocrystals, have been used as imaging agents for formulation of anticancer nanomedicines and theranostics because of possessing superior fluorescent properties.[10] Once excited by a laser beam, the QDs can emit fluorescent light based on their size, while the band

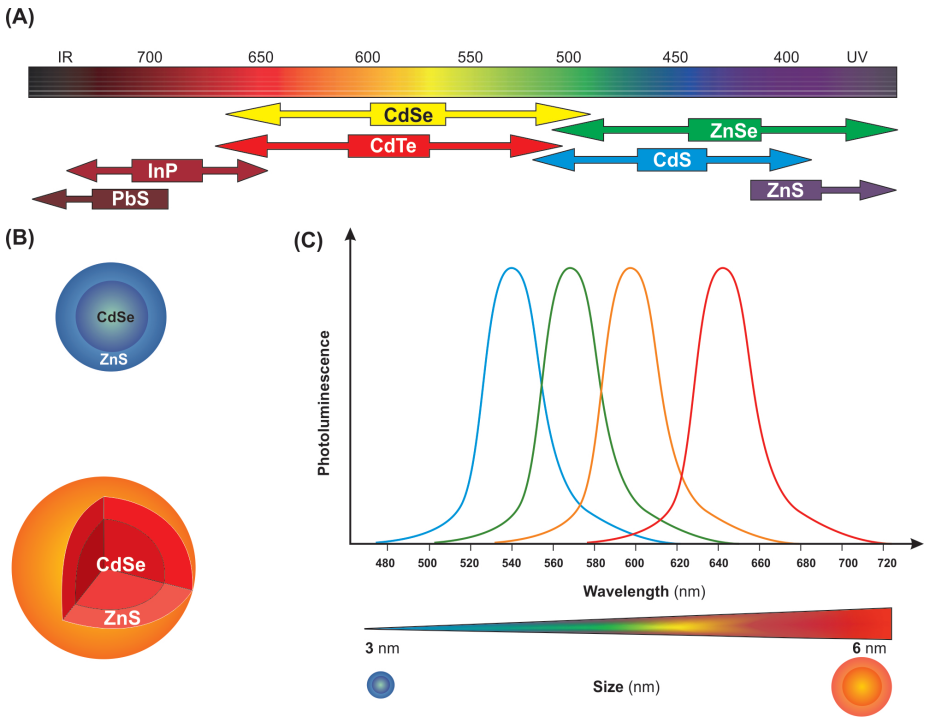

Fig. 1. Schematic representation of various quantum dots (QDs). A) Different types of QDs and thier coresponding emissions. B) Anatomy of QDs. C) Size-dependent emission of QDs. Image was adapted with permission from a study published by Barar and Omidi.[4] Note: not drawn to scale.

gap energy determines the energy and therefore the color of a particular QD. It should be pointed out that the QDs fluorescent light is inversely proportional to the size of the QD – the smaller the size, the bluer the emission and the larger the size, the redder the emission. The optical spectra of various QDs appear to be different (Fig. 1A), so is the architecture of QD nanocrystals (Fig. 1B). Fig. 1 (panel C) schematically shows the emission spectra of the monodispersed CdSe/ZnS QDs with diameters from 3 to 6 nm. Such potentiality makes them very attractive for detection of various markers using multimodal NSs. They are typically composed of atoms from groups II-VI (e.g., CdSe, CdS, CdTe, ZnSe), III-V (InP and InAs) and IV-VI (PbSe), for more details reader is directed to see a study conducted by Michalet and coworkers.[11]

For the production of bulk quantities of these semiconductor nanocrystals, the common method used is the colloidal suspension synthesis under high-temperature conditions in organic solvent with nucleation of semiconductor materials.[12] In this method, briefly, an organic solvent (e.g., octadecene) is stirred at constant rate and heated up to over 300°C, and then solutions containing the semiconductor metals are injected. The metals first decompose under high heat, then recombine to form alloys that contain particulated seeds, where a single QD nanocrystal construction contains approximately 200-10,000 atoms.[13]

Many distinctive characteristics of QDs make them very attractive imaging agents for biomedical applications since they possess:

- high photoluminescence (PL) quantum yield,
- markedly high molar extinction coefficient values in comparision with tradionally used organic dyes,
- broad absorption with narrow and symmetric emission spectra spanning the UV to NIR,
- large distinction between the excitation and emission spectra.[14]

Unlike organic dyes, these inorganic hydrophobic nanostructures are exceedingly stable showing repeatability on cycles of excitation or fluorescence for a long period.[11] Optically, QDs are able to emit light with a decay time roughly between the 30–100 nanoseconds, which is remarkably slower than the autofluorescence background decay.[15]

Specific optical properties of the QDs (e.g., size-tunable and wide absorption, sharp and symmetrical photoluminescence spectra, large two-photon absorption cross-section, and brilliant photostability) make QDs as perfect imaging agent for biomedical approaches.[16] QDs are photoactivated through one- or multi-photon excitation by the large two-photon absorption cross-section. Further, in the band-edge state, contribution of the sharp photoluminescence bands of QDs has transpired through carrier recombination.[9] Of note, the photoluminescence spectra can be adjusted from UV to NIR regions through fluctuation of the core/shell materials (Fig. 1). Accordingly, based upon the core size as well as core material, the photoluminescence of QDs is utilized for bioimaging and PDT. For instance, some QDs (e.g., CdS, CdSe, InP, CdTe, PbS and PbTe) with 2.5 nm in diameter show near visible to NIR band-edge absorption. However, like all other QDs, *in vivo* applications of NIR QDs are limited due to their toxicity resultant from the heavy metals.[17-19] The broad absorption bands in QDs are advantageous in two/multi-photon excitation, and arbitrarily selecting NIR wavelength and wavelength of two-photon excitation is resolved based on the main energy gap of a QD.[20]

In sp^3-hybridized semiconductors (e.g., InP, GaAs, CdSe), a single electron created by exitment moves very quickly in response to an applied energy. As a result, the excited states decay radiatively in a defect-free direct-gap semiconductor such as CdSe.[21] For example, in CdSe nanocrystals, the lowest unoccupied band encompasses Cd 5s orbitals, while the highest occupied band comprises Se 4p orbitals. The energy states of QDs have already been delineated through implementation of particles in a sphere model (e.g. lowest hole-state is signified as $1S_{3/2}$ for CsSe QDs), and the electron/emitting state is depicted by the total angular momentum $F=L\pm1/2$ [21]. Larson *et al.* (2003) determined the two-photon absorption cross-section value as Goeppert–Mayer unit.[22]

Thus far, several evidences demonstrated that QDs are suitable and efficient probes for bioimaging and PDT. Table 1 lists the optical properties of general QDs.

Once conjugated to certain homing devices/targeting moieties, they can be recruited for detection and sensing of (a) cell surface receptors, (b) biomarkers of a wide range of diseases (e.g., malignancies), and (c) molecular markers of biological fluids. For example, the QD-based western blotting technique is able to detect bio-macromolecules (e.g., proteins) as low as 20 pg per lane).[28]

Synthesis and preparation of QDs

Huge endeavors have been devoted to achieve safe QDs with strong and stable photo luminescence distributed in the visible and NIR region towards imaging of cancer cells. So far, CdSe and CdTe core-only QDs and CdSe/ZnS, CdSe/ZnCdS and CdTe/CdSe core/shell QDs have wildly been used for cancer research, nevertheless the safety of these nanocrystals has been a big concern for *in vivo* uses despite great achievements for *in vitro* cell based applications.[29, 30]

QDs are primarily prepared by means of organometallic and aqueous synthesis (the-so-called orQDs and aqQDs, respctively). Methodologically, the colloidal synthesis technique using organic solvents/phase has been reported to be the most used technique for preparation of high quality core and core/shell QDs.[31] Having capitalized on this technique, CdSe QDs were synthesized in classic reaction at 230–300 °C using dimethyl cadmium ($CdMe_2$) dissolved in trioctylphosphine (TOP) and TOPSe.[32] Because of toxic and pyrophoric impacts of $CdMe_2$, somewhat safer cadmium precursors such as cadmium oxide and cadmium acetate have been used in the structure of QDs. Further, CdTe QDs has directly been produced in

Table 1. Optical properties of ordinary QDs

Core composition	Excitation wavelength (nm)	Emission wavelength (nm)	Quantum yield	References
Si	320–450	480–650	0.1–0.25	[23]
CdS	350–470	370–500	0.34	[24]
CdSe	450–640	470–660	0.85	[25]
InP	550–650	620–1100	0.30–0.60	[26]
PbSe	900–4000	>1000	0.12–0.81	[27]

the aqueous phase with quantum yield as high as 80%.[33] The CdTe QDs modified with thioglycolic acid (TGA) have been used for *in vitro* detection of cancer cells as reported for the GSH-TGA co-capped CdTe QDs-antibody probe to label the colorectal cancer cells, CCL187, *in vitro*.[34] Regardless of all the advantages of CdSe and CdTe QDs in the biological science as size-tunable absorption and photoluminescence, unfortunately *in vivo* applications of these nanocrystals can impose intrinsic toxicity.[29, 35] To resolve the toxicity concerns, based on band gaps, various nanocrystals based have been synthesized. As a result, it was found that the indium phosphide (InP) QDs can be used for *in vivo* imaging with less toxicity impacts as compared with Cd-based QDs,[14] even though aspiration of InP was shown to produce pleural fibrosis.[36] In 1997, Dabbousi *et al.* prepared the ZnS shells on CdSe core to create CdSe/ZnS QDs. In practice, a solvent mixture which has composed of tri-n-octylphosphine
oxide (TOPO) and TOP was organized by heating TOPO at 190 °C and then cooling to 60 °C and adding TOP.[37] Furthermore, hexane was used as a required factor for construction a CdSe QD suspension. The mentioned suspension then relocated into the solvent mixture for purifying hexane. CdSe suspension was added to a solution of hexamethyldisilathiane and diethyl zinc in the TOP and ZnS were grown at 140–220 °C as a shell for CdSe QDs.[38] Thereupon, after achieving the required width of ZnS shells, the core/shell QDs were separated by of 1-butanol and methanol in the room temperature. Of various synthesized QDs, CdTe/CdSe, CdSe/ZnS and CdSe/CdS QDs that are able to emit photoluminescence in the NIR range are deemed to be appropriate probes for *in vivo* imaging and PDT,[39] even though they also show toxic effects to some extent. Compared to the organometallic methods, aqueous synthetic approaches appear to be easier, environmental friendly and cost-effective.[33, 40] The aqQDs are water-dispersed nanosystems that need no further post-modifications due to presence of hydrophilic ligand molecules on their surfaces. The aqQDs appear to possess profoundly smaller hydrodynamic diameter (typically < 5.0 nm) in comparison with orQDs, nevertheless these nanocrystals may display poor optical properties.[29]

Surface functionalization and decoration of QDs

Surface modification and stabilization of QDs appears to be the most essential steps for the regulation of biological functions (e.g., cytotoxicity) and successful biomedical uses.[41] The surface of QDs need to be decorated because (a) most of the synthesized QDs are water-insoluble and must be modified to become hydrophilic, (b) uncoated QDs are very reactive and may inadvertently interact with nonspecific biomacromolecules, (c) QDs are mainly made from heavy metal and impose undesired toxic effects when applied in cell/animal models.[42] Fig. 2 shows general approaches for surface modification of QDs.

Under some circumstances (e.g., exposure to UV), QDs may be oxidized and hence liberate heavy metals (e.g., cadmium ions) into the biological environment.[41] This may exacerbate its undesired intrinsic biological impacts on nucleic acids, enzymes and other biomolecules through generation of reactive oxygen species (ROS), while an appropriate coating method seems to efficiently reduce such inadvertent impacts.[43]

In general, for solubilization of QDs, they can be decorated with hydrophilic polymers such as polyethylene glycol polymer (PEG), an approach so-called PEGylation. In addition, dihydrolipoic acid (DHLA), dendrimers and multidentate phosphine polymers can be attached to the surface of QDs, at which they become more hydrophilic NSs with functionalized groups.[44] Other amphiphilic polymers, triblock copolymer and acrylic acid can also

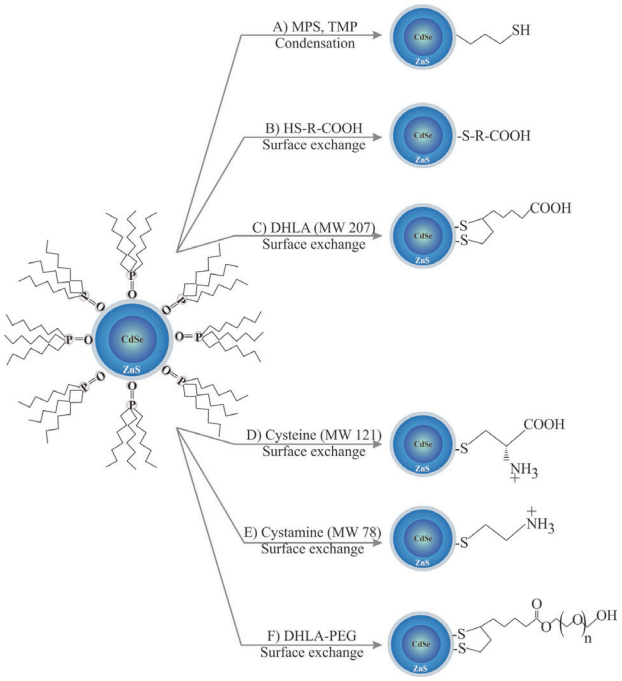

Fig. 2. A schematic examples for surface decoration of QDs. Note: not drawn to scale. DHLA: dihydrolipid acid.

be used for solubilization/stabilizing of QDs even though these coating protocols may inevitably enlarge the overall size of these nano-assemblies and consequently interfere with the end point aims.[44] While amphiphilic phospholipids, calixarenes and cyclodextrins have been used for coating of QDs,[45] the microemulsion strategy with silica-coating method was reported to provide uniform sizes of QDs. Such methodologies can be also used for functionalization of QDs through conjugation with diverse biofunctional molecules.[46] Generally speaking, to become a functionalized NS, QDs need to be cross-linked with desired small molecules or ligand of biomolecules (e.g., Ab, Ap) by means of conjugating linkers such as SPDP.[4] Such functionalization can be initiated through various functionalized carboxylic, thiol and amine groups. For example, swapping a thiol with molecules containing a sulfhydryl group or proteins with cysteine residue can be used for functionalization.[47] Streptavidin modified QDs is deemed to be a specific strategy for linking QDs to biotin-tagged biomolecules (e.g., peptides, Aps, Abs and small molecules), which can be utilized for engineering nano-scaled theranostics, cancer diagnosis probes or even QDs functionalized with cell-penetrating entity as specific ligand for the intracellular delivery of cargos.[48] Table 2 represents some selected applications of functionalized QDs.

To accomplish the anticipated optical properties of affinity-conjugated QDs, surface modification(s) of QDs as a key step seems to be necessary for controlling the undesired aggregation and non-specific binding.[56] Having capitalized on such strategies, QDs-based assays such as Ab-QD and Ap-QD bioconjugates have been designed and used, as cost effective and more stable platform, for specific detection of biomarkers involved in various diseases,[57,58] in particular different types of malignancies.[59-61] Of various methods used in detection of biomarkers, detection of nucleic acids through hybridization methodology using QDs conjugated-oligonucleotides seems to be one of the most promising approaches.[62,63] For example, the Ab-QD conjugates have successfully been used for the detection of insulin-like growth factor receptor in human breast cancer MCF7 cells,[64] and targeted imaging of BxPC3 human pancreatic cancer cells using near-IR CdTeSe/CdS QDs armed with single-domain antibody (sdAb) 2A3.[65]

QDs bioconjugates

Conjugation of QDs with other macromolecules/ small molecules can be accomplished using different conjugation methods such as streptavidin-biotin complex,[66] and 1-ethyl-3-(3-dimethylamino- propyl) carbodiimide hydrochloride (EDC) together with N-Hydroxysuccinimide (NHS) chemistry.[67] Further, to use the bisarsenical affinity probes (e.g., FlAsH, ReAsH,5 and AsCy3) as tool for assessing protein location/function, smart nanohybrids have recently been developed through conjugation of CrAsH (a FlAsH analogue) to hydro-soluble and biocompatible QDs, which was used to target proteins by selectively binding to cystein-tagged proteins [68]. These researchers used amino polyethylene glycol (PEG) phospholipids in the micelle QD for covalent linkage of CrAsH to the QD by EDC-mediated coupling method. The resultant nano-hybrids showed efficient and selective binding potential to 4Cys-tagged proteins with high resistance to photobleaching.

QDs are considered as attractive tools for detection of various antigens mainly due to their wide emission properties.[69, 70] Such characteristics have been exploited for the in situ hybridization (FISH) assay with higher detection sensitivity and also QD-FISH technique. The latter approach has successfully been used for identifying the mRNA expression of neurons in the midbrain region of mouse.[69] Perhaps, the best example for their clinical application is their NIR emission properties, which has successfully been implemented for the sentinel lymph node (SLN) mapping in lung cancer,[71] and *in vivo* imaging of oral squamous cell carcinoma through targeting epidermal growth factor receptor (EGFR).[72]

To be conjugated with desired bioelements, the surface properties of QDs need to be modified towards better aqueous solubility.[7] To this end, QDs have been functionalized using bifunctional linkers such as N-succinimidyl S-acetylthioacetate (SATA) and N-Succinimidyl 3-(2-pyridyldithio)propionate (SPDP).[73] Besides, amphiphilic molecules (e.g., octylamine-modified poly-acrylic acid) can be used for modifying the hydrophobic surface of QDs,[74] which seem to pose no/

Table 2. Well-known targeting molecules used for QDs modification in cancer monitoring

Type of QD	Target molecule	Conjugation/modification method	Cell type	References
CdSe/ZnCdS (cysteine)	GPI/cRGD	NHS-EDC reaction	Prostate cancer cells	[49]
CdSe/CdS/ZnS (N-(2-aminoethyl)-6, 8-dimercaptooctanamide, amine-DHLA)	Hyaluronic acid	Electrostatic interaction	HeLa cells	[50]
CdTe/CdS (-COOH)	Carbohydrate	NHS-EDC reaction	HeLa (intracellular)	[51]
QDs 605,655,705 (-COOH)	MUC-1, AS1411, TTA1	EDC reaction	PC-3, NPA, HeLa cells	[52]
CdSe (-COOH)	Lectin	NHS-EDC reaction	Leukemia cells	[53]
ITK QD 525/655 (NH2-PEG)	Dendrotoxin-1 (DTX-1)	N-succinimidyl iodoacetate and 2-iminothiolane reaction	C6 glioma cells	[54]
CdSe/ZnSe (-COOH)	β-CD-L-Arg	Electrostatic interaction	ECV304 cells	[55]

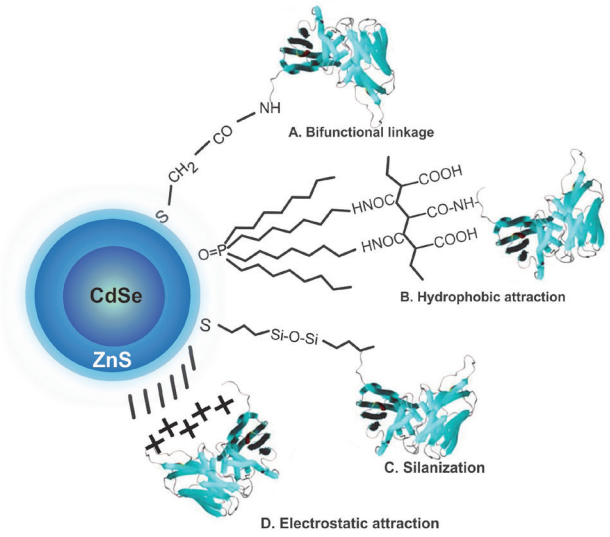

Fig. 3. Schematic representation of a general methodology for the conjugation of QDs to various biomolecules such as mAbs, siRNAs and/or small molecules. Seme selected paradigms of QD bioconjugations are shown as A, B, C and D approaches. Note: not drawn to scale.

less interference with the structure/nature of the surface and optical properties of QDs. Fig. 3 represents schematic illustration of a general methodology for conjugation of QDs to various biomolecules such as mAbs, siRNAs and/or small molecules.

It should be also stated that surface oxidation and pH are two important factors which are able to influence QDs surface modification strategies.[75] Using EDC/NHs chemistry, the JT95 IgM Ab, specific to thyroid carcinoma associated antigen, was conjugated to the CdSe carboxyl QDs to form QD-JT95 NSs that have successfully been used for immunoblot and immunoquantitive assays.[76] Recently, a site-specific covalent conjugation of QDs with target proteins *in vivo* has been reported using an intein-based method, which possessed key steps including (a) fusion of Pleckstrin-homology (PH) domains with the N-terminus half of a split intein (I_N), (b) conjugation of the C-terminal (I_C) intein-derived peptide to streptavidin-coated QDs *in vitro*, and (c) *in vivo* expression of PH–I_N following microinjection of PH–I_N RNA and I_C –QDs into Xenopus embryos.[77] These QD–PH based NSs provided a real time monitoring possibility within live embryos, in which NIR-emitting QDs allowed monitoring of the QD conjugates within depths where the enhanced green fluorescent protein (EGFP) was not detectable. Anti-HER2 monoclonal antibody (mAb) armed CdTe QDs decorated with RNase A (HER2-RQDs) were harnessed in gastric cancer nude mouse models. These HER2-RQDs nanoprobes were found to be able to selectively target the gastric cancer MGC803 cells and inhibit the growth of the gastric cancer tissues, resulting in extended survival time of the tumor bearing mice.[78] For engineering such nanoprobes, the ribonuclease-A-conjugated CdTe QD Clusters (RQDs) were first synthesized and then the N-succinimidyl iodoacetate (SIA) molecules were used for coupling of RNase A with the amine group and grafting of the thiolated HER2 mAb with the iodoacetyl group.

Taken together, QDs are characterized with high levels of brightness and photostability (e.g., in drug delivery and *in vitro* imaging), broad absorption spectra, size- and composition-tunable (e.g., in multicolor imaging) and narrow fluorescence emission (e.g., *in vivo*/ *in vitro* diagnosis).[79] Fig. 4 epitomizes functionalization of QDs by heterobifunctional cross-linkers such as N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) or N-succinimidyl S-acetylthioacetate (SATA), reader is directed to see our recent review in surface modified multifunctional nanomedicines.[4]

Toxicity of QDs

Most of the orQDs have been reported to impose cytotoxicity to some extent.[80] Such cytotoxicity appear to be largely dependent upon various factors such as size, capping materials, dose of QDs, surface chemistry, coating bioactivity and route of exposure,[81] while the residual organic molecules can also induce toxic impacts in the target cells/tissue.[80] For example, in a study, male Wistar rats were head-nose exposed to 0.52 mg Cd/m^3 for 5 days (6 h/day). Histological examination, clinical factors in blood, bronchoalveolar lavage fluid and lung tissue were examined 3 days after the last exposure. It was found that the Cd-based QDs were able to cause local neutrophil inflammation in the lungs, while no CNS toxicity was reported.[82]

Su *et al.* (2011) studied the short- and long-term *in vivo* biodistribution, pharmacokinetics, and toxicity of the aqQDs in mice. These researchers showed that the aqQDs

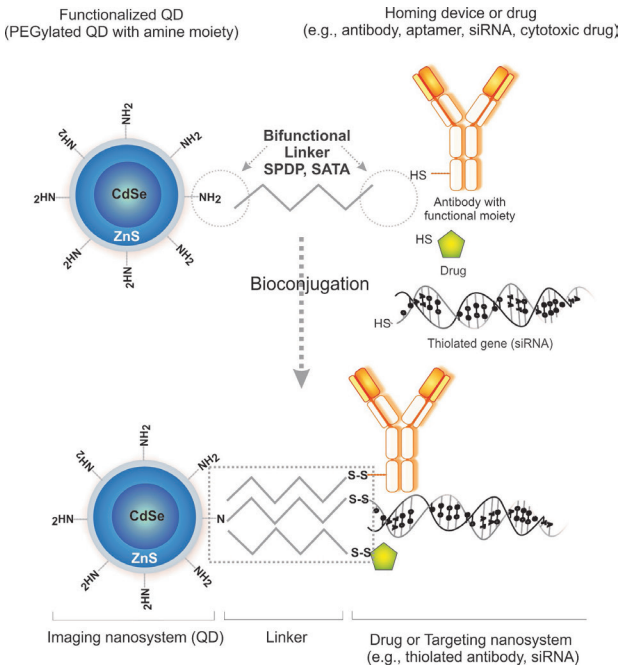

Fig. 4. Functionalization of QDs by heterobifunctional cross-linkers. SPDP: N-succinimidyl 3-(2-pyridyldithio) propionate. SATA: N-succinimidyl S-acetylthioacetate. Note: not drawn to scale.

could initially accumulate in the liver after 0.5-4 h post-injection, then in kidney and blood circulation in long term (15-80 days). They also reported the size-dependency of the biodistribution of aqQDs, in which accumulation of larger aqQDs was observed in the spleen. Their findings highlighted that aqQDs impose negligible toxicity in mice even at long-term exposure.[83] These researchers have previously reported that use of a series of water-dispersed CdTe QDs, CdTe/CdS core–shell QDs, and CdTe/CdS/ZnS core–shell–shell QDs can be well tolerated even at very high concentration and incubation for long-time by various cell lines, perhaps due to the protective impacts of the ZnS shell impeding the release of Cd ions from the inner side.[80] To study the genotoxicity impacts of QDs *in vivo*, the long-term toxicity of CdSe-ZnS QDs with different surface coatings was investigated in Drosophila melanogaster.[84] The results revealed that all differently coated QDs could significantly affect the lifespan of treated group, inducing a significant escalation in ROS levels and enhanced genotoxicity with increased rate of apoptosis in haemocytes. These researchers concluded that the *in vivo* degradation of QDs with consequent release of Cd^{2+} ions may be the main reason for such toxic effects as the coated QDs displayed decreased overall toxicity.

Mechanistically, desorption of Cd and creation of free radical may result in inadvertent interaction with intracellular components. It has been reported that surface oxidation of QDs can lead to the formation of reduced Cd that can be released from QDs causing cell death within primary hepatocytes isolated from rats, which was largely dependent upon processing conditions and QDs dose.[85] Derfus *et al.* (2004) showed that, capitalizing on standard conditions of synthesis with solvent TOPO under an inert atmosphere and water-solubilization with mercaptoacetic acid (MAA), the CdSe QDs were not cytotoxic (Fig. 5A). However, once exposed to air for 30 min, TOPO-capped CdSe QDs can become oxidized and very toxic to cells (Fig. 5B) in dose-dependent manner. Such toxic impacts appear to be from generation of free radicals.[86] Hepatocytes co-cultured with non-parenchymal 3T3 fibroblasts cells to support *in vitro* liver-specific functions were exposed to the EGF-coated red QDs and examined

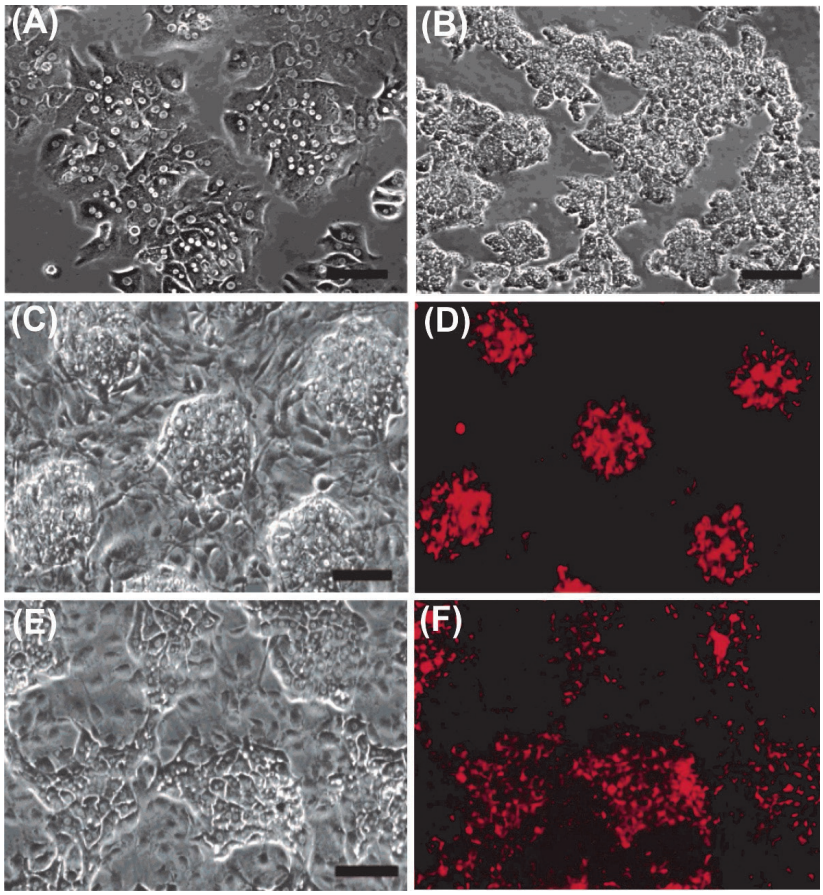

Fig. 5. Cytotoxicity of CdSe quantum dots (QDs) *in vitro*. Toxicity of CdSe QDs in liver culture model is dependent on the processing conditions and the dose of QDs. Panels A and B respectively represent the phase contrast microscopies of the control hepatocyte cultures with well-defined intercellular boundaries and nuclei (A), and nonviable cultures exposed to cytotoxic QDs with granular cytoplasm and undefined intercellular boundaries and nuclei (B). C) Phase contrast image on day 1 showing co-culture of hepatocyte colonies surrounded by fibroblasts. D) Fluorescence image of QD-labeled hepatocytes. E) Phase contrast micrograph of reorganization of hepatocyte colonies. F) Fluorescence image of QD-labeled hepatocytes after 7 days of co-culture. Data were adapted with permission from a study reported by Derfus *et al.*[85]

by "micropatterning" techniques (Figs. 5C, D, E and F). As examined through cell viability, migration, and differentiated function for up to 2 weeks in culture, it was found that organically coated, ZnS-capped CdSe QDs could be considered as biocompatible NSs with hepatic tissue.[85]

To further elucidate the mechanisms underlying CdSe-core QD-induced apoptosis, Chan *et al.* studied the mitochondrial membrane potentials and mitochondrial release of cytochrome c in human neuroblastoma cells. They showed that these nanocrystals are able to elicit loss of mitochondrial membrane potential and mitochondrial release of cytochrome c in a concentration dependent manner.[87] Having looked at signaling pathway, these researchers showed that CdSe-core QDs, but not ZnS-coated CdSe QDs, can induce apoptosis via various signaling pathways such as ROS-, JNK-, caspase-9- and caspase-3-mediated apoptotic pathways in IMR-32 cells, with attendant under-expressed survival signaling molecules including HSP90, Ras, Raf-1 and ERK-1/2.[87]

Further, it should be noted that the commonly used materials for solubilization such as MAA and MPA may also induce cytotoxic effects as previously reported for cysteamine and TOPO causing DNA damage.[19] Nevertheless, PEGylation of QDs appears to reduce cytotoxicity of QDs via slowing the uptake of QDs by the reticuloendothelial system (RES), liver and spleen.[73] In fact the physicochemical properties of QDs (e.g., size, morphology, shell coating and surface characteristics) and their diverse intracellular destinies may determine their endpoint cytotoxicity, therefore approximation of the factual extent of QD cytotoxicity seems to be a difficult issue even though it can be somewhat predicted. In short, among numerous types of QDs, groups III–V QDs have been reported to display lower cytotoxicity than groups II–VI QDs.[88, 89] Biocompatible and less toxic Zn_x S-Ag_y In_{1-y} S_2 (ZAIS) QDs appear to offer greater plausibleness as the optical probes *in vivo* even though synthesis of these QDs may encounter with some shortcomings, including: (a) high reaction temperatures, (b) poorly-controlled growth rates, (c) long-reaction times, (d) difficulties in high throughput synthesis, and (e) requirement for intricate synthetic approaches to engineer QDs with different emissions profiles.[89] Such limitations have been resolved through a novel sonochemical approach for the synthesis of a library of biocompatible ZAIS QDs.[89]

Having highlighted all these studies, it should be pointed out that, to fully understand mechanisms of QDs toxicities in target cells/tissues, high throughput genomics, proteomics and metabolomics studies need to be conducted similar to the investigations previously reported for non-viral gene delivery systems by Omidi and coworkers.[90-100]

QDs-based paradigms for imaging of cancer

By far, QDs have widely been used for *in vitro* biological applications due to their unique characteristics such as broad absorption bands with sharp and symmetrical characteristics, size-tunable absorption and photoluminescence spectra, large two-photon absorption cross-section, photoluminescence spectra and excellent photo-stability.

It should be highlighted that the sharp and size-tunable photoluminescence of QDs favors multiplexed bioimaging. Owing to the tunable absorption and photoluminescence spectra (from UV to NIR regions) by varying the core material, one single laser beam can excite several QDs with different sizes. Further, a designated size of a single QD can display near visible to NIR band-edge absorption and photoluminescence based upon nature of alloy (i.e., CdS, CdSe, InP, CdTe, PbS, PbSe and PbTe QDs). Thus, core size or the core material can bestow multicolor flexibility in terms of absorption spectrum and photoluminescence color for biological imaging and PDT even though blinking of QDs continues to be a limitation for single-molecule imaging. Fundamentally, due to poor tissue penetration and robust tissue autofluorescence, the NIR excitation appears to be a better option over visible excitation for *in vivo* bioimaging and PDT. Besides, the NIR lights (~1000 nm) can induce vibrational excitation in particular within photoacoustic NSs such as QDs in cells, resulting in the generation of heat. This phenomenon is the basis of the photothermal and PDT of cancer using QDs despite their toxicity concerns that have limited *in vivo* applications. Further, during a non-linear process of two-photon absorption, QDs display absorption cross-section values significantly larger than organic dyes, upon which they are considered as attractive probes for two-photon imaging.[101] Of note, the lower toxicity of aqueous QDs *in vivo* has accelerated their translations into clinical applications. For example, it has been reported that encapsulation of QDs in PEGylated phospholipid nanomicelles can result in reduced toxicity of the PbS QDs, which may be used as an imaging tool.[102] In fact, multispectral fluorescence imaging (MSFI) potential of QDs make them very attractive and promising tool for sensitive detection of cancer.

Targeted imaging of cancer

Targeted imaging of cancer as MSFI can revolutionize detection and consecutive therapy of cancer. Recently, Han *et al.* (2010) reported on the improvement of photoluminescence and biocompatibility for the NIR gold-doped CdHgTe (Au:CdHgTe) QDs through an aqueous solution route with L-glutathione and L-cysteine as stabilizers.[103] In this investigation, the Au:CdHgTe QDs were covalently conjugated to the series of targeting molecules such as arginine-glycine-aspartic acid (RGD) peptide, anti-EGFR mAb, and anti-carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM1) mAb. These researchers assessed the cytotoxicity of Au:CdHgTe QDs in both A549 cells and mice and showed IC_{50} of 158.63 μg/mL and 84.169 μg/mL respectively for Au:CdHgTe QDs and CdHgTe QDs in A549 cells, while the LD_{50} values of Au:CdHgTe QDs and CdHgTe QDs were respectively 34.919 mg/kg and 29.928 mg/kg body mass in

mice. The data show a better tolerance of Au:CdHgTe QDs in both *in vitro* and *in vivo* experiments. They successfully implemented these bioconjugates (i.e., QD800-RGD, QD820-anti-CEACAM1, and QD840-anti-EGFR) for *in vivo* targeted MSFI in tumor bearing xenografts. Similarly, cyclic-RGD-peptide-conjugated type II CdTe/CdS QDs have successfully been implemented for recognition of cancer cells in mice xenografted with pancreatic tumor cells.[104]

As ideal targets for imaging and treating markers, overexpressed tumor-specific markers (TSMs) or tumor-associated markers (TAMs) have widely been used for *in vitro* and *in vivo* applications in various cancers. Of note, QDs conjugated with homing agents have commonly been exploited for targeting and sustained fluorescence visualization of cancer cells. Perhaps, one of the best classic examples for the cancer detection using QDs was demonstrated by Gao et al. (2004), whose work on labeling the human prostate cancer cells (C4-2) via conjugation of QDs with Abs specific to the prostate specific membrane antigen (PSMA). The PSMA positive C4-2 cells were efficiently detected by the QD-Ab conjugate, but not the PSMA-negative PC-3 cells.[5]

Likewise, Her2 receptor that is a recognized cancer marker molecule up-regulated in many breast cancers has widely been used for detection and therapy of such malignancies. QD conjugated with Trastuzumab (Herceptin™), an anti-Her2 Ab was used for specific imaging of the breast cancer cells. Wu *et al.* (2003) targeted the human breast cancer cells (SK-BR-3) and mouse mammary tumor sections using these conjugates. These researchers labeled SK-BR-3 cells by means of QD-streptavidin conjugate via targeting the cells with a primary humanized anti-Her2 Ab and secondary biotinylated goat anti-human IgG. Later on in 2007, Yezhelyev *et al.* exploited such approach and labeled MCF-7 and BT-474 breast cancer cells selectively with visible/NIR QDs conjugated with Abs specific to Her2, EGFR, estrogen receptor (ER), progesterone receptor (PR) and mammalian target of rapamycin (m-TOR).[105] Similarly, they harnessed such QDs for multiplexed and quantitative immunohistochemistry.[106]

In 2010, Kawashima *et al.* exploited CdSe/ZnS QDs conjugated with EGF and anti EGFR Ab to target the EGFR-overexpressing human epidermoid carcinoma A431 cells.[107] Interestingly, Ren *et al.* used multiplexed single-cell array staining approach, in which QDs were coated with water-soluble thioglycolic aid (TGA) to become biocompatible multi-wavelength bioprobes conjugated with Abs specific to some selected antigens.[108] Likewise, the KPL-4 breast cancer cells were selectively labeled using NIR QDs conjugated with Herceptin™. Labeling cancer cells with QDs was further carried on by Weng *et al.* (2008) who utilized a multimodal method through targeting cancer cells using an Ab, drug delivery using immunoliposomes (ILs), and imaging cells using QDs.[109] They exploited carboxylic acid functionalized CdSe/ZnS QDs to conjugate them to a primary amino group in a liposome using EDC chemistry (Fig. 6A). Nude mice xenografted with MCF-7/HER2 in the lower back after i.v. injection with anti-HER2 QD-ILs revealed

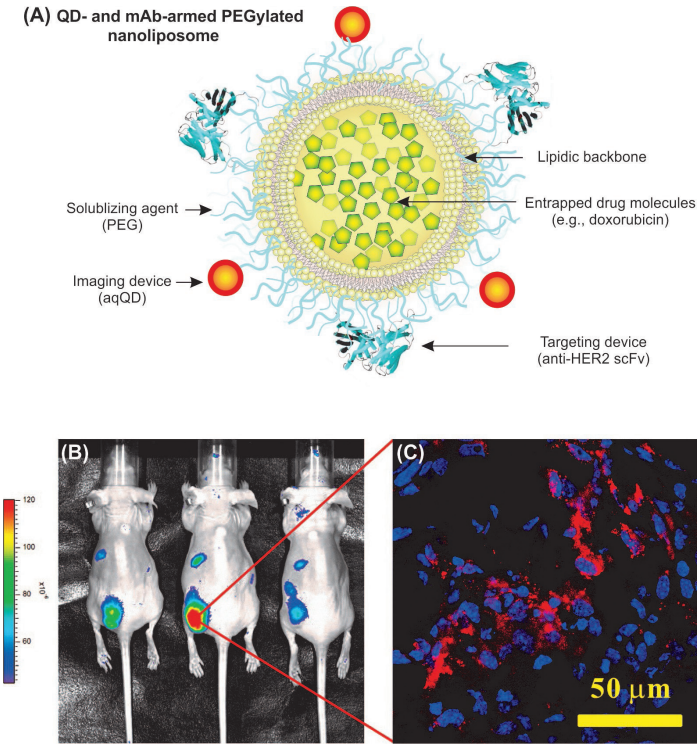

Fig. 6. Impacts of targeted multimodal ILs conjugated with QDs. A) Functionalized CdSe/ZnS QDs conjugated ILs armed with scFv. B) Fluorescence image of nude mice bearing MCF-7/HER2 xenografts 30 h after i.v. injection with anti-HER2 QD-ILs. C) Confocal microscopy analysis of section of HER2-overexpressed tissue (5 μm) harvested at 48 h postinjection analyzed using two-color scanning mode for nuclei stained by DAPI (blue) and QD-ILs (red). Data were adapted with permission from a study published by Weng *et al.*[109] QDs: Quantum dots. ILs: immunoliposomes.

significant accumulation of QD-armed ILS in treated mice (Fig. 6B). Also, confocal microscopy analysis of sections of frozen tumor tissues (5 μm) harvested at 48 h post-injection revealed significant accumulation of ILs on the HER2-overexpressed tissue analyzed in two-color scanning mode for nuclei stained by DAPI and QD-ILs, shown as blue and red colors, respectively (Fig. 6C), for detailed information, reader is directed to see following work.[109]

Benefits of the multifunctional ILs appear to be (a) selective labeling of the target cancerous cells, (b) bioimaging with high-contrast fluorescence, (c) encapsulating anticancer agents such as doxorubicin (DOX), and (d) intracellular drug delivery. In 2009, in a study, Zhang *et al.* proposed QDs conjugated with anti-type 1 insulin-like growth factor receptor (IGF1R) as promising multimodal NS for simultaneous targeting and imaging breast cancer cells. The key idea in their approach appeared to be the detection of up-regulated IGF1 R in MCF-7 breast cancer cells by QD-anti-IGFR1 conjugate.[64] QDs have also been tailored to single wall carbon nanotubes, resulting in a multifunctional hybrid nanoconstruct for cellular imaging and targeted photothermal therapy,[110] even though the safety issues of such approach are unclear. Yong *et al.* (2009) detected human pancreatic cancer cells selectively by means of QDs conjugated with anti-C1audin-4 Ab and anti-prostate stem cell antigen (anti-PSCA). These NSs were shown to be recognized by the membrane proteins C1audin-4 and PSCA over-expressed metastatic pancreatic cancer.[111] Given the successful *in vitro* detection of cancer cells using QDs conjugated with anticancer antibodies, Kaul *et al.* devised an antibody-conjugated internalizing QDs for long-term live imaging of cells.[112] QDs has been used for multiplexed and quantitative immunohistochemistry.[106] Fig. 7 represents multiplexed and quantitative immunohistochemistry of prostate tissue specimens stained with traditional IHC and bioconjugated QDs.

Further, self-assembled nanoscale biosensors have been engineered based on QD FRET donors.[113] FRET comprises the transfer of fluorescence energy from a donor particle to a targeted acceptor particle but occasionally it is known as the Forster radius, if the distance between them is smaller than a critical radius.[113] FRET causes donor's emission reduction and increasing in acceptor's emission intensity. It is suitable for measurement of protein conformational/interaction changes and enzyme activity assay. For example, QD-FRET has been utilized for monitoring protein interactions in the Holliday Junction, immunoassay and play a role as an intermediate in the in the recombination of DNA.[114] Detection of DNA arm motion is promising by varying in emission of QD585 as a donor on one arm of the DNA, and Cy5 on a perpendicular arm as an acceptor. In a recent research, maltose binding protein (MBP) has

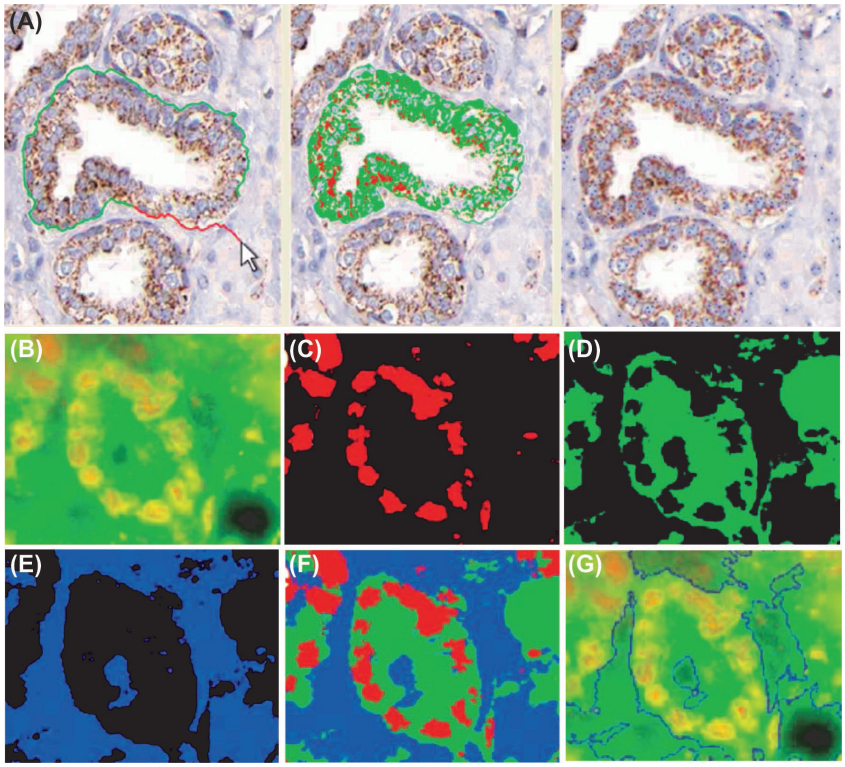

Fig. 7. Multiplexed and quantitative IHC using bioconjugated QDs. A) Prostate tissue specimens stained with traditional IHC and bioconjugated QDs. K-means clustering to segment QD-stained tissue image is highlighted by light green and light red colors. Panels B to G represent multiplexed QD-based IHC of the formalin fixed, paraffin embedded (FFPE) prostate tissue samples, and quantitative analysis of cancer biomarkers p53 and EGR-1. The blue color shows the tissue background. B) Original multicolor image. C) p53 protein stained red with QD655. D) EGR-1 protein stained green with QD565. E) Tissue background. F) Superimposed map of dominant markers and background. G) Automated boundary segmentation using level-set algorithms. IHC: immunohistochemistry. Data were adapted with permission from a study published by Xing *et al.*[106]

been conjugated to QDs and concentration-dependent upsurge in luminescence was detected, owing to binding affinity similarity of the quenching molecule which readily replaced on addition of maltose.[113] For QD-FRET application in imaging activity of proteases, QD-probe via a peptide sequence is bound to a quencher probe that is familiar to a protease. Consequently Emission is resulted after cleavage of the two molecules by protease.[115, 116] Following studies in bioimaging filed, have evinced the potential of QD-FRET to detect activity of caspase-1, thrombin, trypsin and b-lactamase and discrimination between normal and cancerous breast cells are conceivable by means of the QD-FRET assay of collagenase.[47, 115]

Different environmental factors such as pH and ionic strength of the solution and condition have an efficacious effect on FRET variations.[117] In ovarian tumors for identification of Kras oncogene point mutations, a specific nanosensor with producing of FRET was developed and target DNA was acknowledged by a biotinylated capture oligonucleotide and reporter oligonucleotide which labeled with Cy5 fluorophore. Thereupon, through the connection between the QD-streptavidin and biotin, FRET would then transpire and Cy5 fluorescence would be detected because QD and Cy5 were in adjacent proximity. It should be noted that by a mismatch in base pairing fluorescence has not been produced and Cy5 was incapable to accept energy from the QD.[118] Fig. 8 shows

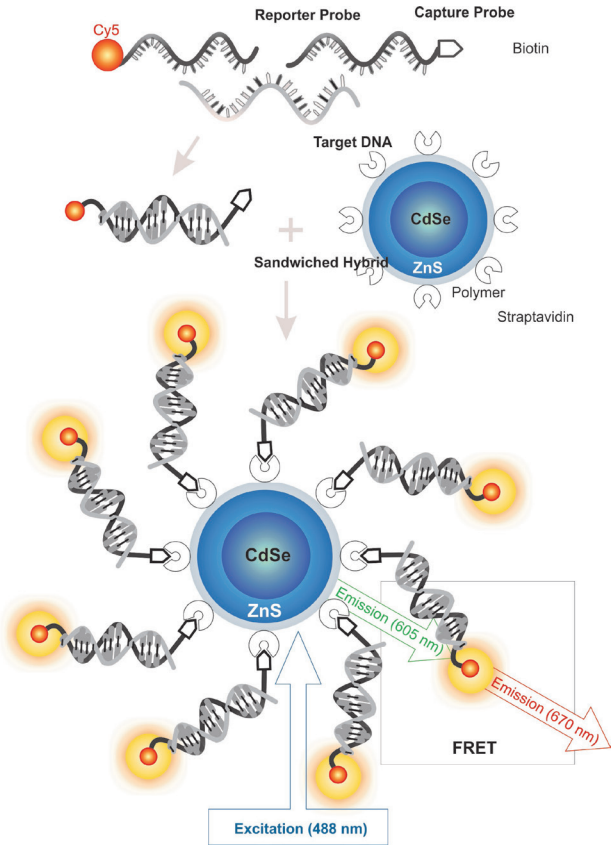

Fig. 8. Schematic representation of fluorescence resonance energy transfer (FRET). Image was adapted with permission from a study published by Barar and Omidi.[4] Note: not drawn to scale.

schematic representation of FRET.

Methylation-specific QD fluorescence resonance energy transfer" (MS-qFRET) technique has been advanced to quantify the extent of DNA methylation that can be altered in malignancies and hence can be used as an epigenetic biomarker sensing tool.[119] This interesting approach has successfully exploited for determination of methylation in CDKN2A, PYCARD and CDKN2B tumor suppressor genes.[119] Cheng *et al.* devised a FRET-based method to target the glycoprotein mucin 1 (MUC1) in epithelial adenocarcinoma biomarkers using anti-MUC1 aptamer, in which the aptamers fold up and avoid involvement with the DNA conjugated to the QD in the absence of MUC1 and DNA hybridization occurs to make a QD-DNA resulting in placement of the quencher strand in close proximity to the QD.[61] This unique bioassay encompassed a dynamic range between 0.25 to 2 μmol/L with the limit of detection (LOD) of 250 nmol/L. In a protease assay using QDs, a peptide sequence for a particular protease tethers a QD to a specific fluorescent dye/protein or quencher dye and gold NPs, and expanding of the QD fluorescence emission was shown by the cleavage of the peptide layer via protease.[120]

Cellular tracking and fluorescent labelling

Intracellular delivery of QDs are difficult topic, so many methods have been designed for delivering of QDs to the cytoplasm. Labeling with QDs in xenopus and zebrafish embryos have been approved by microinjection techniques.[121] It has successfully been used for multispectral fluorescence imaging.[122] Fig. 9 represents five-color spectrally unmixed QDs for detection of lymphatic system anatomy.

It has been shown that QDs can be taken up through endocytic/ non-endocytic pathways. For example, it has been reported that CdSe/ZnS carboxylic-coated QDs (COOH-QDs) are able to enter fibroblast cells through lipid raft/caveolin-mediated endocytosis accumulating in the multivesicular bodies, but not in the lysosomes. In the later phase, the lipid raft/caveolin-dependent endocytosis is inhibited resulting in prevention of intracellular uptake of new COOH-QDs, while the platelet-derived growth factor (PDGF) conjugated QDs could enter fibroblasts through the clathrin-mediated endocytosis accumulating in lysosomes. This clearly means that the intracellular trafficking and the final biological fate and activities of QDs are largely dependent on the surface coating of QDs with the biologically active entities.[123] For example, it has also been reported that Tat peptide-conjugated QDs (Tat-QDs) can be internalized through macropinocytosis as a fluid-phase endocytosis process, which are then appear to be trapped in cytoplasmic organelles and actively transported by molecular machineries (e.g., dyneins) along microtubule tracks towards the microtubule organizing center.[124]

Monodisperse hybrid nanoparticles (38 nm in diameter) of QDs were engineered through mixing with nanogels of cholesterol-bearing pullulan (CHP) modified with

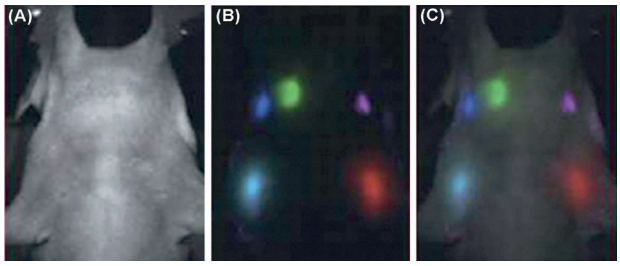

Fig. 9. Detection of lymphatic system using five-color spectrally unmixed QDs. A) Autofluorescence image of mouse. B) Fluorescence image of draining lymph nodes after spectral unmixing. C) Superimposed image of autofluorescence and fluorescence images. Data were adapted with permission from a study published by Zhou and El-Deiry.[122]

amino groups (CHPNH2), which were able to efficiently internalize into various human cells.[125] Labelling individual isolated biotinylated F-actin fibres by streptavidin-coated QDs has been processed, in which smaller percentage of labelled filaments were motile in comparison with an organic fluorophore such as Alexa488.[126] QDs have also been used in tumor biology for labeling of P-glycoprotein (P-gp) molecules, by which three-dimensional imaging revealed localization of P-gp with QDs allowing consecutive z-sections.[127] In other study, concurrent labelling with QDs of nuclear structures/mitochondria and actin filaments have been conducted and successfully produced red labelling of the nucleus and green labelling for the mitochondria.[87] Further, QDs played a role in tyramide signal amplification (TSA) in order to expeditor Ab binding. Labeling of multiple targets such as cAMP response element binding protein (CREB) is achievable by merging of QDs with electron microscopy techniques. By means of this methodology, seeking the dynamics of cell surface receptors which has participated in cellular signaling will be possible.[128, 129] QDs are competent to visualize the movements of many receptors such as TrkA, GABAc and Glycine.[130]

QDs are potent imaging agents to visualize/diagnosis several pathogens (Table 3). In addition, in developmental studies, QDs could also be used for the monitoring of microorganisms populations to explore the starvation effects on *Dictyostelium discoideum,* and they could be tracked with no visible fluorescence destruction.[131]

QDs-based detection technology for in vivo imaging of cancer

QDs conjugated with primary/secondary Abs for over-expressed receptors which are perfect targets for imaging cancers, were extensively used. On the basis of such method, Gao *et al.* labeled human prostate cancer cells or C4-2, utilizing a conjugate of QDs and an Ab intended for PSMA.[5] Prostate cancer cells positive for PSMA were labeled efficiently. Well-known cancer markers that over-expressed in many breast cancers such as Her2 receptor has widely been investigated in detection and therapy. Trastuzumab has been used for conjugation to QDs and anti-Her2 Ab was used for selectively labeling.[56] A unique QD nanoprobe has been manufactured for bio-sensing of glioma cells on the basis of the tenascin-C (extracellular matrix glycoprotein) over-expression which is involved in tissue remodeling mechanism and engaged in assaulting of glioblastoma into the surrounding tissue.[137, 138]

Furthe, Tenascin-C was targeted by the CdSe/ZnS QDs by means of the single-stranded DNA aptamer which was formerly selected by systematic evolution of ligands by exponential enrichment (SELEX) and fluorescence microscopy exposed that the QDs has labeled glioma cells.[139] Other study revealed that transferrin were conjugated to phospholipid micelle-encapsulated silicon QDs which attach to pancreatic cancer cells.[23] There was 95% cell viability after 24 h and subsequently, concentration of silicon QDs approved to the cells was not toxic, nevertheless higher concentrations caused cell death.[23]

QDs have been used to sense the delivery of chemotherapeutic drugs (e.g., DOX) to different cancerous cell. In a study, DOX interposed the PSMA positive cells through a targeted system using QD-RNA aptamer, and then fluorescent signal was shaped while QDs went through prostate cancer cells and released DOX.[140] In other study, Wu *et al.* has labeled human breast cancer cells (SK-BR-3) with QD-IgG conjugates using Herceptin™ as homing device. They utilized a humanized anti-Her2 Ab intended for targeting the cells via the QD-streptavidin conjugate for labeling the SK-BR-3.[56] Using visible and NIR QDs, MCF-7 and BT-474 breast cancer cells have been labeled with QDs armed with Abs specific to some antigens such as ER, EGFR and m-TOR.[105]

Table 3. Summary of QDs used for diagnosis of infectious pathogens

Pathogen	Recognition	Methodology of detection	Detection limit	References
E. coli O157:H7	Biotinylated antibody	Fluorescence microscopy	2 orders more sensitive than other dyes	132
E. coli O157:H7	Fim-H mannose specific lectin	Fluorometry	10^4 bacteria/ml	133
E. coli, Salmonella	Antibody	Fluorescence spectroscopy	10^4 CFU/ml	134
Cholera toxin	Antibody	Fluroimmunoassay	In ng/ml quantity	134
RSV	Antibody	Color change	Single step/short time	135
HBV, HCV, HIV	Antibody	Fluorescence	100 µl sample/50 times more sensitive	136

Furthermore, EGFR single-molecules in human ovarian epidermoid carcinoma cells have been targeted by CdSe/ZnS QDs conjugated with EGF Ab.[141]

Recently, non-Cd-based QDs has been exploited, as highly efficient and nontoxic bioimaging agent, for targeting and live visualizing of pancreatic cancer cells. In this approach, InP/ZnS QDs were functionalized with mercaptosuccinic acid and further conjugated with pancreatic cancer specific mAbs, and then were successfully used for *in vitro* and *in vivo* targeted bioimaging.[142]

Encapsulation of CdSe/ZnS QDs in Fluorine-18 labeled phospholipids micelle resulted in bimodal imaging probes in combined *in vivo* fibered confocal fluorescence microscopy and positron emission tomography (PET).[143] Overall, biomedical imaging in cancer biology or pathology is supposed to receive a new dimension and impulsion by means of manufacturing appropriate and efficient probes based on QDs.

Concluding remarks

To date, fluorescence bioimaging has significantly changed the face of molecular diagnosis *in vitro* and *in vivo*. Because of non-invasiveness with high temporal resolution and lower cost, it is an interesting alternative to the currently used molecular detection methods. Of fluorescence bioimaging methods, QD-based nanoprobes have been considered as stable optical imaging and/or sensing probes that are competent to improve biological monitoring and have been created a prominent achievement in multimodal nanomedicines. There exist compelling evidence that such multifunctional NSs have capability to revolutionize molecular diagnosis of diseases in particular malignancies. They appear to offer simultaneous imaging and therapy with minimized undesired consequences. For detection of cancer biomarkers, different QDs-based assays have been designed successfully, in which QDs provide stable optical characteristics beneficial for multicolor bioassays. In addition, they can be used for development of nanobiosensors which can open several prospects towards identification of a large number of diseases' molecular markers as well as chemical/biochemical entities. However, despite providing great fluorescence potential, the group II-VI QDs contain toxic heavy metals that often limit their *in vivo* applications. Therefore, various surface modification approaches have been conducted to reduce their undesired toxicity. Further, some safer QDs have been developed. For example, silicon (Si), shows desirable optic properties with great biocompatibility, upon which Si QDs have been exploited for safe real time monitoring, imaging and targeting of tumors as a multicolor NIR bioimaging tool *in vivo*. Si QDs provides unique surface functionalization and bioconjugation, producing stable luminescence nanospheres with long (>40 h) tumor accumulation time *in vivo*.[144] We envision that QD-based multicolor arrays will be used for advancing the optical bioimaging techniques with greater stability and less photo-bleaching. And, in near future, we should largely capitalize on implementation of QDs for development of next-generation high throughput sensing and imaging techniques which would benefit early detection and on-demand monitoring and therapy of malignancies.

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Ethical issues

The authors declare no ethical issues.

Competing interests

The authors announce no conflict of interests.

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