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Near-Infrared Quantum Dots as Optical Probes for Tumor Imaging

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Abstract

Molecular imaging plays a key role in personalized medicine, which is the goal and future of patient management. Among the various molecular imaging modalities, optical imaging may be the fastest growing area for bioanalysis, and the major reason is the research on fluorescence semiconductor quantum dots (QDs) and dyes have evolved over the past two decades. The great efforts on the synthesis of QDs with fluorescence emission from UV to near-infrared (NIR) regions speed up the studies of QDs as optical probes for *in vitro* and *in vivo* molecular imaging. For *in vivo* applications, the fluorescent emission wavelength ideally should be in a region of the spectrum where blood and tissue absorb minimally and tissue penetration reach maximally, which is NIR region (typically 700–1000 nm). The goal of this review is to provide readers the basics of NIR-emitting QDs, the bioconjugate chemistry of QDs, and their applications for diagnostic tumor imaging. We will also discuss the benefits, challenges, limitations, perspective, and the future scope of NIR-emitting QDs for tumor imaging applications.

Keywords

Quantum dots; near-infrared; tumor imaging; fluorescence imaging; perspective

1. INTRODUCTION

This review describes the application of quantum dots (QDs) with near-infrared (NIR) emission for *in vivo* tumor imaging. Over the past decades, the research of nanotechnology has grown explosively covering the fields of materials, energy, electronics, biology, and medicine. The integration of nanotechnology with molecular biology and medicine has resulted in active developments of a new emerging research area—nanobiotechnology [1, 2]. Nanobiotechnology is defined as a field that applies the nanoscale principles, materials, and techniques to understand and transform biosystems, and which uses biological principles and materials to create new devices and systems integrated from the nanoscale [1]. This technological innovation, referred to nanomedicine by the National Institutes of Health (NIH), has great potential to offer exciting and abundant opportunities for discovering new materials and tools in biomedicine. One of the most advanced and exciting forefronts of nanobiotechnology is the various applications of QDs in biology and medicine [3]. Compared with the organic dyes and fluorescent proteins, QDs show many unique optical

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properties, such as symmetrical, narrow, and tunable emission spectra, superior photostability, high quantum yields, and the capacity of simultaneous excitation of multiple fluorescence colors. Moreover, there are much more alternatives in QDs with NIR emission for *in vivo* imaging than organic dyes [4]. The QDs emitting at above 700 nm in the NIR region minimize the problems of endogenous fluorescence of tissues and meet the requirements of *in vivo* biological imaging applications.

Cancer is a serious burden for the public health in the world because cancer cells are very aggressive and invasive. In 2007, the NIH estimated an overall cost of $206.3 billion as a result of cancer. There are many traditional medical imaging techniques to detect cancer and monitor the therapeutic effects of cancer intervention, such as computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound. The field of molecular imaging, recently defined by the Society of Nuclear Medicine (SNM) as "the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and other living systems" [5], has flourished over the last decades. Among the various molecular imaging modalities, optical imaging may be the fastest growing area for *in vivo* analysis, [6, 7] mainly because the research on biomedical applications of QDs and other fluorescent materials has evolved over the past two decades. The development of high-sensitivity and high-specificity molecular probes is of considerable interest in many areas of cancer research, ranging from basic tumor biology to *in vivo* imaging and early detection. Non-invasive fluorescent imaging of preclinical animal models *in vivo* is a rapidly developing field with new emerging techniques. QD fluorescent probes with longer emission wavelengths in NIR emission ranges are more amenable to deep-tissue imaging, because both scattering and autofluorescence are reduced as wavelengths are increased [8]. After the surface functionalization using peptides, proteins, and antibodies, QDs have indeed shown great ability to target and detect specific tissues in living subjects by the rapid readout of fluorescence imaging [9–16]. In this review article, we focus on the NIR-fluorescence emitting QDs, from synthesis to modification, from bioconjugation to targeted imaging, from fluorescence imaging to multimodality imaging, and from critical comments to perspective. We hope to arouse readers more interests and attentions on the future scope of NIR-emitting QDs for fluorescence imaging applications.

2. NEAR-INFRARED QUANTUM DOTS

In order to meet the requirements of *in vivo* biological imaging applications, the fluorescent emission wavelengths of the QDs ideally should be in a region of the spectrum where blood and tissue absorb minimally but still detectable by the instruments. Thus, the QDs should emit at approximately 700–1000 nm in the NIR region to minimize the problems of endogenous fluorescence of tissues. As shown in Fig (1), the emission wavelength of the colloidal QDs made of ZnS, CdSe, CdTe, PbS, PbSe, and InAs has covered from the UV to the infrared range. Because of their wide absorption spectra, QDs with different emission wavelengths excited by a single light source can emit various color fluorescence, therefore, QDs have a great potential for multicolor molecular imaging. Considering the toxicity and instability of CdTe and InAs QDs surface, it is extremely important to passivate or cap the CdTe and InAs QDs with a layer of ZnS or ZnSe. This layer protects the QDs against photo-oxidation as well as improves the fluorescence quantum yield of the QDs. The ZnS shell has larger bandgap energy than CdTe, eliminating the core's surface defect states. The strategy of using ZnS or ZnSe shell to cap QDs has become a popular approach. Because of high interest and demand of NIR QDs, the development on the synthesis of NIR QDs based on this approach has progressed rapidly, such as CdTe/ZnS [17], CdTe/CdSe [18], $InAs_xP_{1-x}$/InP/ZnSe [19], CuInSe [20], and Cu-doped InP/ZnSe [21] QDs. However, it is reported that the Cd-containing QDs indeed shows cytotoxicity under extreme conditions [22, 23]. For *in vivo* applications, metabolic clearance of the nanoparticles remains an issue, that is, it is

hard to know about how these nanoparticles would be completely cleared out of the body. To avoid such a dilemma, one strategy is to replace the Cd metal by other more benign elements. For example, $CuInS_2$/ZnS core/shell QDs do not contain any Class A elements (Cd, Pb, and Hg) [24], which may show great potential as biocompatible materials for biomedical applications. Recently, carbon dots (C-dots) as new emerging optical probes have aroused research interests because of their non-toxicity comparing with semiconductor QDs [25].

3. SURFACE MODIFICATION AND BIOCONJUGATION

Most of QDs synthesized in high temperature organic phases are nonpolar coated with organic ligands (e.g., trioctyl phosphine oxide (TOPO) and octadecylamine (ODA)) and insoluble in aqueous solvents. In order to render the QDs water-soluble and prevent the aggregation or precipitation of QDs in aqueous solution, surface modification of the nanoparticles is necessary. As shown in Fig. (2), there are two major strategies for the preparation of the water-soluble QDs [26]. One is chemical exchange of native organic ligands on the QDs with thiolated water-soluble ligands in a water-organic two-phase system (Fig. 2A). The most common thiolated molecules used to stabilize semiconductor QDs in aqueous media are thiolated aliphatic carboxylic acids, such as mercaptoacetic acid (MAA) [27], mercaptopropionic acid (MPA) [28–30], or mercaptoundecanoic acid (MUA). The dihydrolipoic acid (DHLA) ligands provide stable interactions with the QD surfaces owing to the bidentate chelate effect of the dithiol groups [31, 32]. Also, lipoic acid units tethered to polyethylene glycol (PEG) spacers are excellent candidates to modify QDs and further conjugate QDs with biomolecules [32–34]. These modifiers eliminate nonspecific adsorption processes and also provide anchoring sites for the covalent immobilization of the biomolecules. Other thiol-containing materials, such as peptides containing cysteine unit can also replace the organic ligands and produce water-soluble QDs [35, 36]. Recently, using water-soluble dendrons to exchange ODA can stabilize QDs and yield water-soluble QDs with high quantum yields [37]. Another strategy is hydrophobic-hydrophobic interaction between native organic ligands and amphiphilic molecules, such as amphiphilic polymers and phospholipids (Fig. 2B) [38–41]. However, the mean diameter of the resulting QDs is much larger than that of the MPA or cystamine conjugated QDs. Such excessive size may hinder the widespread implementation of QDs for *in vivo* molecular imaging. Moreover, amphiphilic polymers often lead to highly increase the surface charge resulting in nonspecific binding to cell membranes. PEGylation of the polymer coated QDs could reduce the nonspecific binding and adsorption. Nonspecific binding events are minimized in the hydroxy-PEG coated QDs and the carboxy-PEG coated QDs. On the contrary, the amine-PEG coated QDs show the significant enhancement of nonspecific binding, because they exhibit the high positive charges which lead to increase the electrostatic interaction with the cell membrane [42].

After the water solubilization of QDs, the further attachments of biomolecules (e.g., avidin, peptides, proteins, and antibodies) on their surface endow QDs with the biological activity [43], as shown in Fig. (3). The most common methods involve the coupling of primary amines in the biomolecules to the carboxylic acid residues on the encapsulating layer associated with the QDs using 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), for example, DOTA conjugated QDs [44, 45]. Peptides, proteins, and antibodies that containing free exposed thiol groups have the potential to conjugate with QDs that have free amine functionalities in their capping layer using heterobifunctional crosslinkers, such as 4-maleimidobutyric acid N-succinimidyl ester and succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) [46–48]. Because a polyhistidine tag consisting of six histidine residues binds carboxy-functionalized QDs, the assembly of desired biomolecules

on surfaces of QDs can be achieved by adding polyhistidine tagged proteins [42], antibodies [49], short peptides [50], or DNA [51].

4. TUMOR-TARGETED NIR FLUORESCENCE IMAGING

In vivo tumor-targeted imaging with biocompatible QDs has recently become possible in mouse models. Because of the limited tissue penetration and intense scattering of light, optical imaging will be possible in humans only at limited sites, such as tissues and lesions close to the skin surface and tissues accessible by endoscopy and during intraoperative visualization [52, 53]. The NIR fluorescence imaging approach, in which the absorbance spectra for all biomolecules reach minima, thus provides a clear window for *in vivo* optical imaging [54, 55], may have better opportunities for visualizing tumor in both small-animal models and even clinical settings. So NIR QDs after functionalization show great potential as optical probes for *in vivo* molecular imaging. Herein, we will give several examples that using NIR QDs as optical probes for *in vivo* fluorescence imaging and discuss the limitations, challenges, and perspective in their future developments.

4.1. Passive Targeting

Under *in vivo* conditions, there are two modes for the tumor-targeting of entities: passive targeting and active targeting. In the passive mode, macromolecules and nanometer-sized particles accumulate preferentially at tumor sites through an enhanced permeability and retention (EPR) effect because (i) angiogenic tumors produce vascular endothelial growth factors (VEGF) that hyperpermeabilize the tumor-associated neovasculatures and cause leakage of circulating macromolecules and small nanoparticles; and (ii) tumors lack an effective lymphatic drainage system, which leads to accumulation of macromolecules or nanoparticles in tumors [56, 57]. In fact, almost all of the approved targeting systems for anticancer drugs are of passive targeting [58]. For the passive delivery of QDs, the efficiency of targeting highly relies on the inherent physicochemical properties of the QDs (e.g., particles size, charge, and surface properties). Recently, Chen *et al.* reported the high tumor-uptake of ultrasmall NIR non-cadmium QDs due to the EPR effect [29]. One critical issue for the *in vivo* applications of QDs is the hydrodynamic diameter (HD) of nanoparticles. QDs with large size (>20 nm in diameter) suffer from extremely high reticuloendothelial system (RES) uptake, which reduce their efficiency and sensitivity. The ultrasmall water-soluble QDs (<10 nm) have attracted more and more attentions because of their unique properties and the advantages for in vivo applications, that is, the rapid renal clearance [59, 60], low RES uptake, and high possibility of EPR effect [60–62]. MPA coated InAS/InP/ZnSe QDs (QD800-MPA) with emission maximum at about 800 nm showed very small size in HD (<10 nm). As shown in Fig. (4A), ultrasmall QD800-MPA nanoparticles pass through the normal blood vessels, and then extravasate from the vessels when they reach the angiogenic tumor vessels because of the leaky tumor vasculatures, finally, they accumulate preferentially at the tumor sites through the EPR effect because tumors lack an effective lymphatic drainage system. Using 22B and LS174T tumors as the models, in vivo fluorescence imaging showed that QD800-MPA was highly accumulated in the tumor area after 4 h postinjection (p.i.) with good fluorescence contrast from surrounding tissues (Fig. 4B). For the passive targeting of QDs, the circulation half-life should be long enough for the accumulation of QDs in tumors, while too long periods of time in blood circulation may result in the enhancement of toxicity to body.

Sentinel lymph node (SLN) imaging is clinically important since these are the sites where metastatic cancer cells are often found. Frangioni and Bawendi first reported the SLN mapping with NIR QDs in living subjects [18, 19, 63]. After the intradermal injection of NIR CdTe/CdSe QDs (~850 nm emission) into live mice and pigs, these QDs rapidly migrated to local SLNs and imaged virtually background-free (Fig. 5), which allows image-

guided resection of a one centimeter deep lymph node in a pig. After that, they also developed these type-II NIR QDs as noninvasive optical probes to do intraoperative SLN mapping in various locations of the body in adult pigs [64]. SLN mapping using QDs overcomes the limitations of currently available methods and provides highly sensitive, real-time visual feedback for image-guided localization and resection, which may be useful for fluorescence-guided surgery and might eventually permit potential mapping of SLNs and lymphatic flow in patients.

4.2. Peptide Conjugated QDs

Peptides are short polymers formed from the linkage of α-amino acids in a designed order. Because blood vessels express molecular markers that distinguish the vasculature of individual organs, tissues, and tumors, it is a general strategy to search for peptides that recognize tumor-specific vessels other than blood vessels by combining ex vivo and *in vivo* phase display. Ruoslahti *et al.* first reported selective targeting of peptides conjugated QDs *in vitro* and *in vivo* [65]. They described three kinds of peptides: CGFECVRQCPERC (denoted as GFE) which binds to membrane dipeptidase on the endothelial cells, KDEPQRRSARLSAKPAPPKPEPKP KKAPAKK (denoted as F3) which preferentially binds to blood vessels and tumor cells in various tumors, and CGNKRTRGC (denoted as LyP-1) which recognizes lymphatic vessels and tumor cells in certain tumors [66, 67]. These pioneering reports demonstrated the feasibility of specific targeting of QDs *in vivo* and opened up a new avenue to the biomedical applications of QDs, although the visible QDs is not optimal for *in vivo* imaging.

Recently, Chen *et al.* reported *in vivo* targeted imaging of tumor vasculature using peptide-conjugated NIR QDs [46, 47]. Cell adhesion molecule integrin $\alpha_v\beta_3$ is highly expressed on activated endothelial cells and tumor cells but is not readily detectable in resting endothelial cells and most normal organ systems. Integrin $\alpha_v\beta_3$ is a key player in tumor angiogenesis, progression, and spread. Moreover, integrin $\alpha_v\beta_3$ is expressed on both tumor vasculature and tumor cells. The arginine-glycine-aspartic acid (RGD)-containing components specifically bind to integrin $\alpha_v\beta_3$ in interstitial matrix [68]. After the conjugation of QD705 from Invitrogen (emission maximum at 705 nm) with RGD peptide, QD705-RGD exhibited high affinity of integrin $\alpha_v\beta_3$ in cell culture and *in vivo*, as shown in Fig. (6). After the tail vein injection of QD705-RGD into mice bearing subcutaneous integrin $\alpha_v\beta_3$-positive U87MG human glioblastoma tumors, *in vivo* NIR fluorescence imaging indicated tumor intensity reached a maximum at 6 h p.i. with good contrast (tumor-to-background ratio was 4.42 ± 1.88).

Furthermore, Chen *et al.* chose non-cadmium NIR QDs (InAs/InP/ZnSe core/shell/shell nanoparticles with *emission maximum at about 800 nm*) as the low-toxic and efficient fluorescence probe to demonstrate the high specific targeting in the integrin $\alpha_v\beta_3$-positive tumor vasculature after the surface modification of the RGD peptide [69]. After the PEG coating with the amine terminal functional group, it is facile to conjugate QD800-PEG with thiolated peptides (e.g., RGD and RAD). As shown in Fig. (7), the fluorescence imaging (IVIS Imaging System) indicated the tumor uptake of QD800-RGD was much higher than those of QD800-PEG and QD800-RAD. The semi-quantitative analysis of region of interest (ROI) showed high tumor uptake of 10.7 ± 1.5 %ID/g in the mice injected with QD800-RGD, while the tumor uptakes of QD800-PEG and QD800-RAD were 2.9 ± 0.3 %ID/g and 4.0 ± 0.5 %ID/g, respectively, indicating the specific tumor targeting of QD800-RGD.

The size of these QD-RGD (~20 nm in HD) prevented efficient extravasation, thus QD-RGD mainly targeted tumor vasculature instead of tumor cells. Immunofluorescence staining of the tumor vessels indicated that the majority of the QD fluorescence signal in the tumor colocalized with the tumor vessels, which was further confirmed by intravital imaging

of QD800-RGD in real-time [70]. As shown in Fig. (8), QD800-RGD did not extravasate in an SKOV-3 mouse ear tumor model, and they specifically bind their target in tumor neovasculature as aggregates, but no binding happened in control conditions. The high reproducibility of bioconjuction between QDs and RGD peptide and the feasibility of QD-RGD bioconjugates as tumor-targeted fluorescence probes warrant the successful applications of QDs for *in vivo* molecular imaging and diagnosis, which may aid in cancer detection and management including image-guided surgery.

4.3. Antibody Conjugated QDs

Antibodies are gamma globulin proteins that are found in blood or other bodily fluids of vertebrates, and they can identify and neutralize foreign objects (e.g., bacteria and viruses) by the immune system. The most common application of antibodies is to identify and locate intracellular and extracellular proteins that different cell types express. In 2004, Nie *et al.* first reported the prostate cancer targeting and fluorescence imaging *in vivo* using QDs conjugated prostate specific membrane antigen (PSMA)-specific monoclonal antibodies [57]. The fluorescence imaging indicated the high tumor targeting after the injection of QD-PSMA Ab conjugates, although the imaging contrast was moderate in visible window because of low tissue penetration and high autofluorescence. Using NIR QDs as fluorescence probes, Chung *et al.* demonstrated the highly sensitive detection of haman C4-2 and C4-2B prostate tumors in mice after the surface functionalization of the anti-PSMA antibody [71].

Recently, Tada *et al.* reported the tracking of a single particle QD conjugated with the tumor-targeting antibody in tumors of living mice using a dorsal skinfold chamber and a high-speed confocal microscope with a high-sensitivity camera [72]. After the conjugation of QDs and monoclonal anti-HER2 antibody, they injected the QD-antibody into mice with HER2-overexpressing breast cancer to analyze the molecular processes of its mechanisticdelivery to the tumor. They claimed there were six processes of delivery: initially in the circulation within a blood vessel, during extravasation, in the extracelullar region, binding to HER2 on the cell membrane, moving from the cell membrane to the perinuclear region, and in the perinuclear region. The movement of the QD-antibody at each stage was “stop-and-go”. Although it is still unclear whether the “stop and go” pattern is typical for majority of injected QD conjugates or just a small subset of QDs, the image analysis of the delivery processes of single QD *in vivo* will be extremely important on molecular imaging and become a cutting-edge research field. Meanwhile, the research on the conjugation of QDs and antibody is attracting more and more attentions in the application of molecular imaging [9, 48, 49, 73, 74].

5. MULTIMODULITY IMAGING BASED ON NIR QUANTUM DOTS

Combination of multiple modalities can yield complementary information and offer synergistic advantages over any modality alone. Nanoparticles have the advantages in multifunctionality and enormous flexibility, which allow for the integration of therapeutic components, targeting ligands, and multimodality imaging probes into one entity. Quantitative and synergistic imaging of tumor angiogenesis will lead to more robust, reliable, and effective monitoring of personalized molecular cancer therapy. The future of tumor angiogenesis imaging lies in multimodality, and nanoflat-form-based imaging will be one of most important approach in multimodality imaging. Based on the QDs as optical probes, one can integrate MRI contrast agents [75–79], radio-nuclides [44, 80], or therapeutic drugs that combining diagnostics and therapy [81].

Recently, Chen *et al.* described the quantitative tumor targeting efficacy of dual-functional QD-based probes using both PET and NIR fluorescence imaging [44, 45]. Dual-modality

PET/NIR fluorescence imaging probes offer synergistic advantages over the single modality imaging probes by overcoming the low quantitative analysis of fluorescence intensity *in vivo* and ex vivo. Non-invasive PET imaging using radiolabeled QD conjugates can provide a robust and reliable measure of the *in vivo* biodistribution of QDs [80]. As shown in Fig. (9), they conjugated NIR QDs with VEGF protein and DOTA chelator for VEGFR-targeted PET/NIR fluorescence imaging after ^{64}Cu-labeling and evaluated the targeting efficacy *in vitro* and *in vivo* through cell-binding assay, cell staining, *in vivo* optical/PET imaging, *ex vivo* optical/PET imaging, and immunostaining histology analysis [45]. Although the stability and physicochemical properties of QDs after the radionuclides labeling should be concerned, with the further improvement in QD technology, it is expected that the accurate evaluation of *in vivo* tumor targeting efficacy using these dual-modality probes may significantly facilitate applications of QDs in biomedical science as well as clinical benefits.

6. CONCLUSION AND PERSPECTIVE

In this review, we have summarized the recent development of tumor imaging *in vivo* using NIR QDs as fluorescence probes. Nanotechnology indeed has the potential to significantly impact cancer diagnosis and cancer patient management. QDs have already fulfilled some of their promises as a new class of molecular probes for cancer research. However, there are still many considerable challenges and issues remained for the *in vivo* applications of NIR QDs. Besides the purity, dispersity, and stability of QDs in physicological environments, the variable physicochemical properties of each type of QDs may result in the unexpected behavior of QDs *in vivo*. The fundamental investigation of QD physicochemical properties is very important for the systematical analysis in the biodistribution of QDs. Several studies have shown QDs may be systemically distributed in organs and tissues. The absorption, distribution, metabolism, and excretion characteristics are highly variable for QDs because of the wide variation in QD physicochemical properties [82–84]. Moreover, QDs usually suffer from higher RES uptake and poorer extravasation comparing with small molecules or proteins. The majority research of QD is so far limited to vasculature-related disease, although the QDs with smaller size (normally <10 nm in HD) may extravasate from tumor angiogenic vessels because of EPR effect. Until now, it is still far away from QD-based imaging in small animals to scale up to *in vivo* imaging in patients due to the limited optical signal penetration depth. In clinical settings, optical imaging is relevant for tissues close to the surface of skin, tissues accessible by endoscopy, and intraoperative visualization. The major roadblocks for clinical translation of QDs are inefficient delivery, potential toxicity, and lack of quantification.

According to the potential toxicity *in vivo*, QD-based ex vivo protein nanosensors (e.g., FRET and BRET) [85–89] may be better choices for the future applications of QDs in cancer management. As shown in Fig. (10), patients can have their tumors biopsied and blood samples drawn for protein profiling by ex vivo nanosensors to detect and predict the response before and after the treatment without any potential side effects. Ex vivo diagnostics combining with *in vivo* imaging can markedly impact future cancer patient management by providing the synergistic information that neither strategy can provide alone. After further efforts, development, and validation, the major issues for clinical translation of QDs will became clearer and clearer, QD-based approaches (e.g., ex vivo nanosensor, *in vivo* imaging, and multimodality imaging) will eventually have the ability to predict and detect cancer in patients and monitor their response to personalized therapy.

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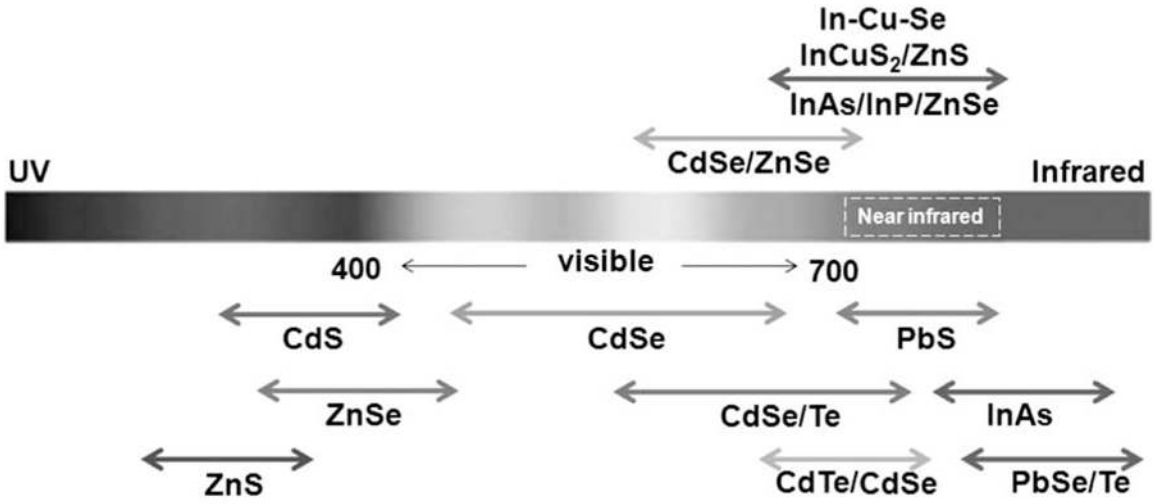

Fig. 1.

Representative QDs with different materials scaled as a function of their emission wavelengths superimposed over the spectrum. There are several kinds of QDs with the emission wavelength in the NIR region.

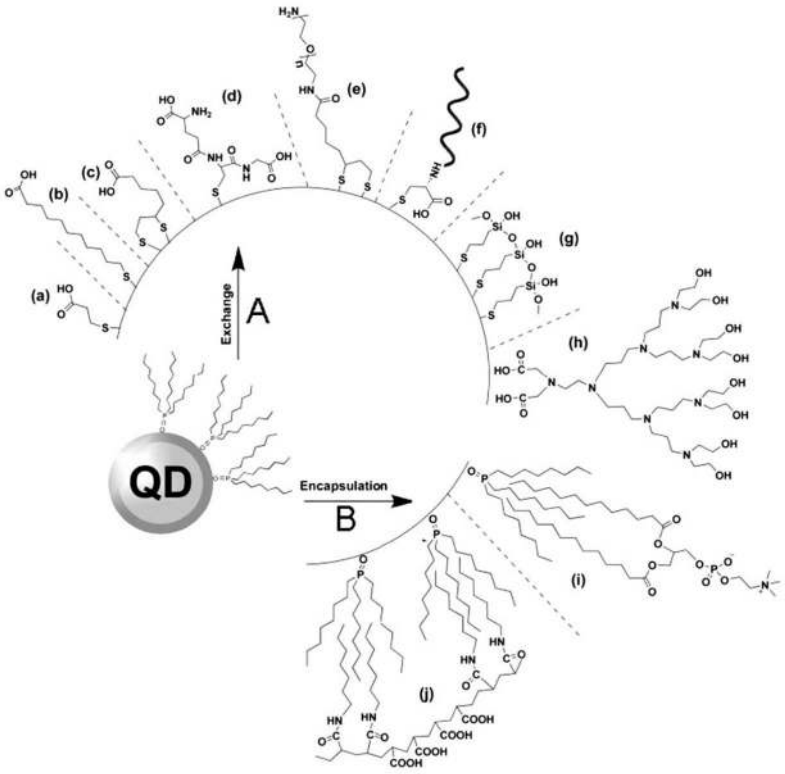

Fig. 2.

Overview of strategies to prepare water-dispersible QDs. (**A**) Exchange of the organic surfactant layer with a water-soluble layer: (**a**) – (**d**) thiolated or dithiolated functional monolayers, (**e**) glutathione (GSH) layer, (**f**) cysteine-terminated peptides, (**g**) thiolated siloxane, and (**h**) carboxylic acid-functionalized dendrone. (**B**) Encapsulation of QDs stabilized with an organic encapsulating layer in functional bilayer films composed of (**i**) a phospholipid encapsulating layer, and (**j**) a diblock copolymer layer. Reprinted with permission from ref. [26]. Copyright 2008, Wiley-VCH.

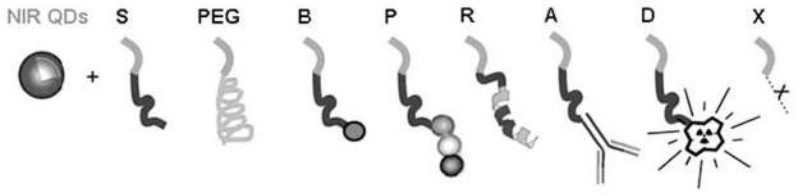

Fig. 3.

The Overview of bioconjugation and functionalization on NIR QDs. S, solubilization sequence; PEG, polyethene glycol; B, biotin; P, peptides; R, protein sequence; A, antibody; D, DOTA; X, any unspecified peptide-encoded function. QDs can be targeted with B, P, R, A, or other chemical moieties. For simultaneous PET and fluorescence imaging, QDs can be rendered radioactive by D chelation of radionuclides; for simultaneous MRI and fluorescence imaging, QDs can be rendered radioactive by D chelation of nuclear spin labels. Reprinted with permission from ref. [43]. Copyright 2005, AAAS.

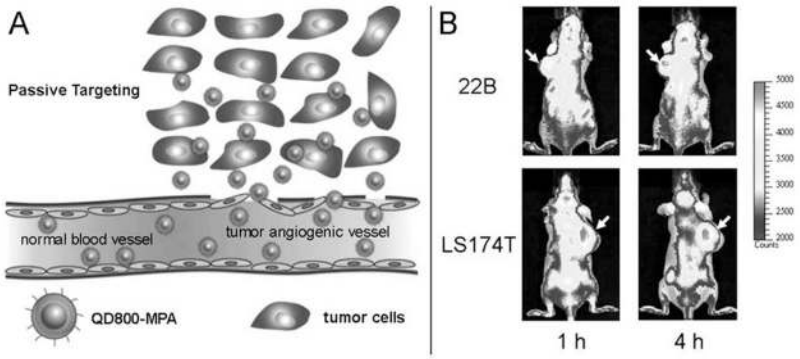

Fig. 4.

Ultrasmall QD800-MPA for *in vivo* tumor imaging. (**A**) The structure of QD800-MPA and the illustration of the passive tumor targeting of QD800-MPA in tumor models. (**B**) *In vivo* NIR fluorescence imaging of 22B or LS174T (arrows) tumor-bearing mice at 1 h and 4 h after the tail vein injection of QD800-MPA. Reprinted with permission from ref. [29]. Copyright 2010, Wiley-VCH.

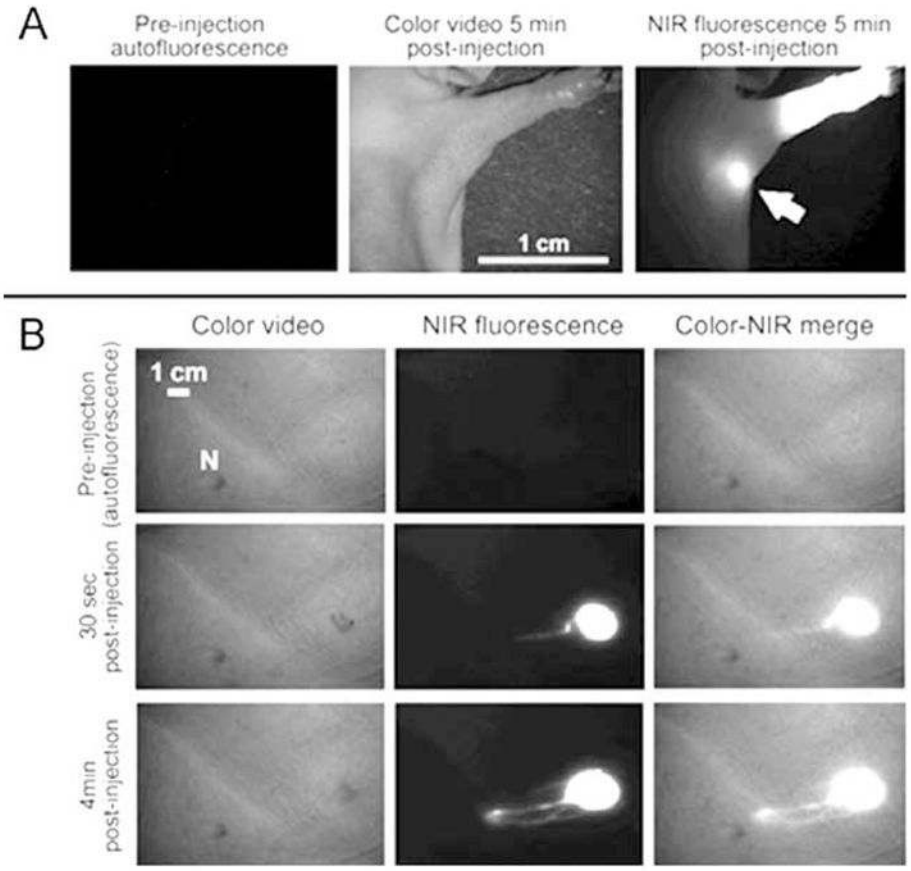

Fig. 5.

Sentinel lymph node mapping in the mouse and pig using NIR QDs. (**A**) Images of mouse injected intradermally with 10 pmol of NIR QDs in the left paw. Left, pre-injection NIR autofluorescence image; middle, 5 min post-injection white light color video image; right, 5 min post-injection NIR fluorescence image. An arrow indicates the putative axillary sentinel lymph node. (**B**) Images of the surgical field in a pig injected intradermally with 400 pmol of NIR QDs in the right groin: before injection (autofluorescence), 30 s after injection, and 4 min after injection. The position of a nipple (N) is indicated. Reprinted with permission from ref. [18]. Copyright 2004, Nature Publishing Group.

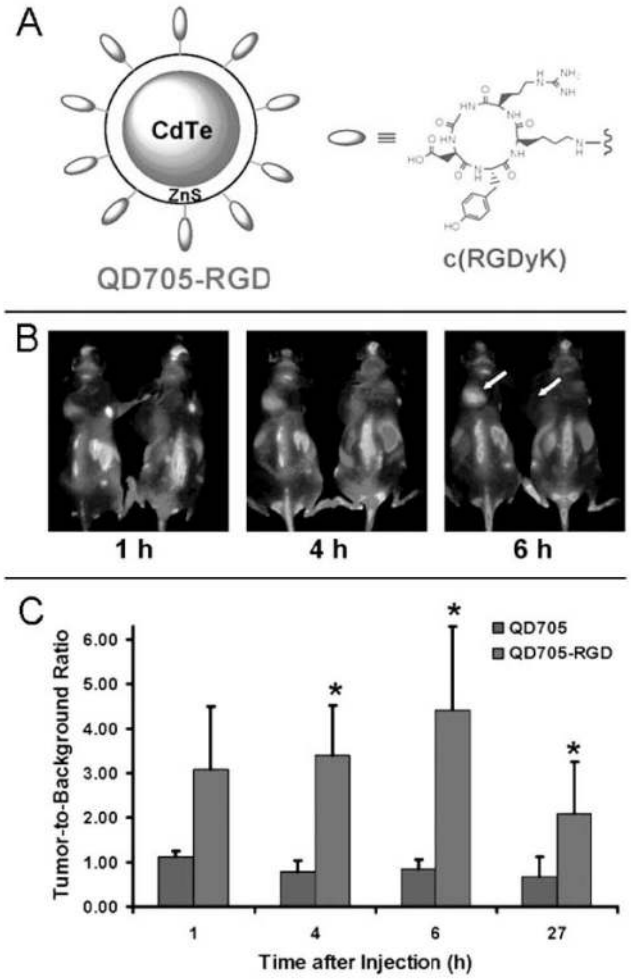

Fig. 6.

RGD peptide-conjugated QD705 for NIR fluorescence imaging of tumor vasculature. (**A**) the schematic illustration of QD705-RGD. (**B**) *In vivo* fluorescence imaging of tumor vasculature in U87MG tumor-bearing mice at 1 h, 4 h, and 6 h after the tail vein injection of QD705-RGD (left) and QD705 (right). (**C**) Tumor-to-background ratios of mice injected with QD705 or QD705-RGD. Reprinted with permission from ref. [46]. Copyright 2006, American Chemical Society.

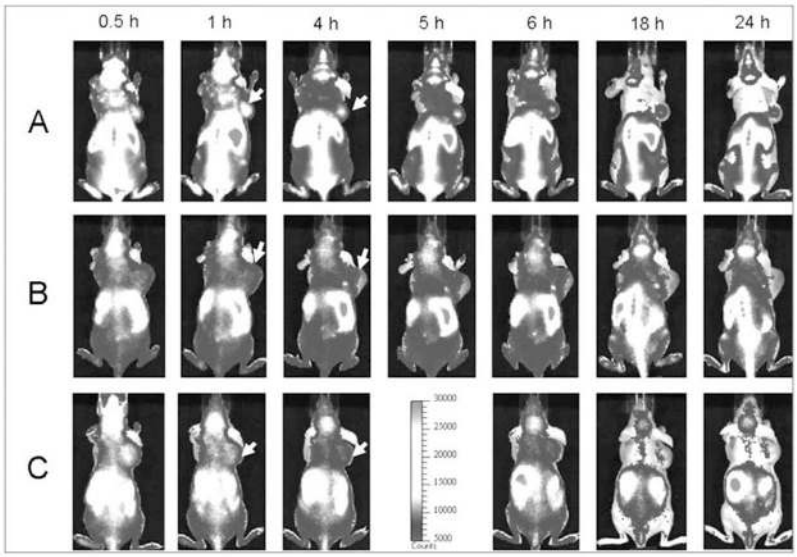

Fig. 7.

Non-cadmium NIR QDs as the low-toxic and efficient fluorescence probe to image the integrin $\alpha_v\beta_3$–positive tumor vasculature after the surface modification with the RGD peptide. *In vivo* fluorescence imaging of U87MG tumor-bearing mice (arrows) injected with (**A**) QD800-RGD, (**B**) QD800-PEG, and (**C**) QD800-RAD, respectively. Reprinted with permission from ref. [69]. Copyright 2010, American Chemical Society.

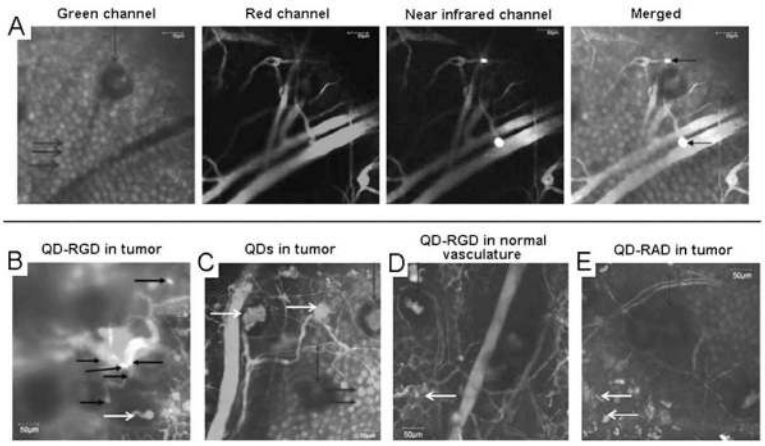

Fig. 8.

Direct visualization of binding of QD800-RGD to tumor vessel endothelium and controls. (**A**) Panel displays different output channels of the identical imaging plane along the row. In the green channel, individual EGFP-expressing cancer cells are visible, while the red channel outlines the tumor's vasculature *via* injection of Angiosense dye. The NIR channel shows intravascularly administered QDs which remain in the vessels. Binding events are visible by reference to bright white signal. These are demarcated by arrows in the rightmost merged image, in which all three channels have been overlaid. (**B**) Merged image in a different mouse using QD800-RGD. Individual cells are not generally visible. (**C–E**) Typical images of no binding in each control condition: (**C**) Tumor neovasculature containing unconjugated QDs, (**D**) normal vasculature containing QD800-RGD, and (**E**) tumor neovasculature containing QD800-RAD. Reprinted with permission from ref. [70]. Copyright 2008, American Chemical Society.

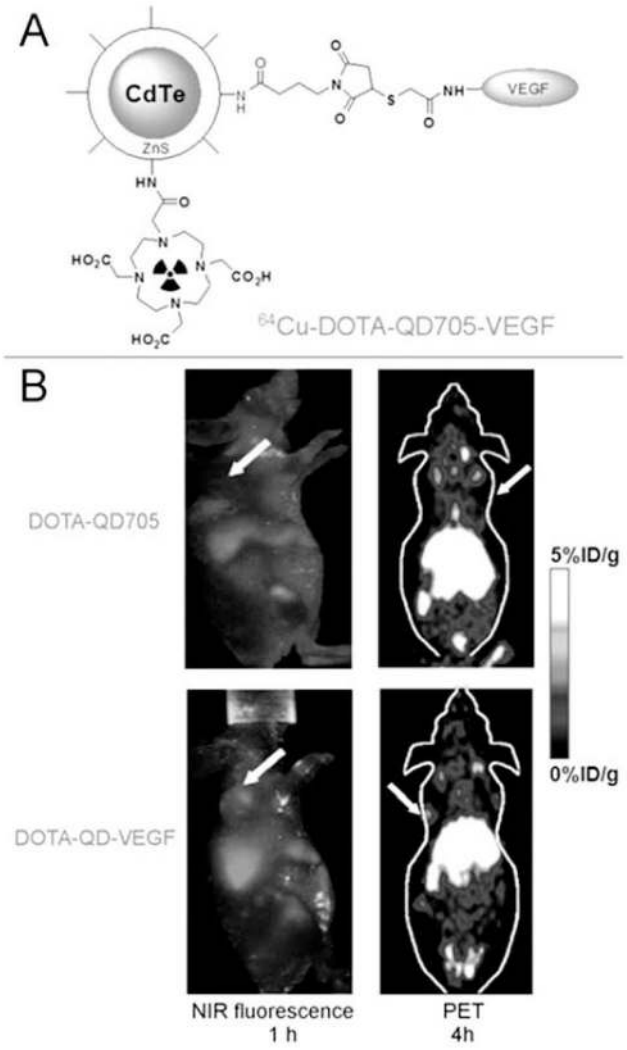

Fig. 9.

Dual-modality optical and PET imaging of tumor VEGFR expression using radiolabeled NIR QDs. (**A**) The structure of ^{64}Cu-DOTA-QD705-VEGF conjugate. (**B**) *In vivo* fluorescence imaging and coronal PET imaging of U87MG tumor-bearing mice (arrows) at 1 h and 4 h after the tail vein injection of DOTA–QD705 and DOTA-QD705–VEGF, respectively.

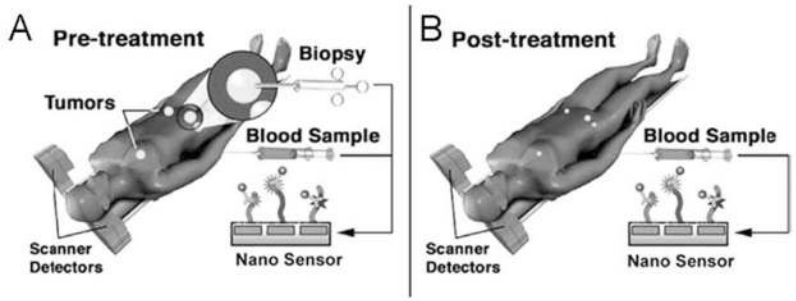

Fig. 10.

The *ex vivo* diagnostics of patients using NIR-emitting QDs as nanosensors. (**A**) Before treatment, patients can have their tumors biopsied and blood samples drawn for protein profiling by ex vivo nanosensors to predict their response to a given therapy. (**B**) During the treatment, patient response will be evaluated by blood analysis and molecular imaging to ensure the accurate differentiation of responders from non-responders.