POTENTIAL CLINICAL RELEVANCE

Nanomedicine: Nanotechnology, Biology, and Medicine
7 (2011) 385–402

Review Article

nanomedjournal.com

Quantum dots, lighting up the research and development of nanomedicine

Yunqing Wang, PhD, Lingxin Chen, PhD*

Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, China
Key Laboratory of Coastal Zone Environmental Processes, CAS, Yantai, China
Shandong Provincial Key Laboratory of Coastal Zone Environmental Processes, Yantai, China

Received 9 September 2010; accepted 17 December 2010

Abstract

Quantum dots (QDs) have proven themselves as powerful inorganic fluorescent probes, especially for long term, multiplexed imaging and detection. The newly developed QDs labeling techniques have facilitated the study of drug delivery on the level of living cells and small animals. Moreover, based on QDs and fluorescence imaging system, multifunctional nanocomplex integrated targeting, imaging and therapeutic functionalities have become effective materials for synchronous cancer diagnosis and treatment. In this review, we will summarize the recent advances of QDs in the research of drug delivery system from the following aspects: surface modification strategies of QDs for drug delivery, QDs as drug nanocarriers, QD-labeled drug nanocarriers, QD-based fluorescence resonance energy transfer (FRET) technique for drug release study as well as the development of multifunctional nanomedicines. Possible perspective in this field will also be discussed.

From the Clinical Editor: This review discusses the role and significance of quantum dots (QDs) from the following aspects: surface modification strategies of QDs for drug delivery, QDs as drug nanocarriers, QD-labeled drug nanocarriers, QD-based fluorescence resonance energy transfer (FRET) technique for drug release study as well as the development of multifunctional nanomedicines.
© 2011 Elsevier Inc. All rights reserved.

Key words: Quantum dots; Nanomedicine; Fluorescence imaging; Multifunctional drug delivery system

Nanomedicine is referred as the application of nanotechnology to disease treatment, diagnosis, monitoring, and to the control of biological systems at the level of single molecules or molecular assemblies.[1] The major goal in this area is to design rational delivery and targeting of pharmaceutical, therapeutic, and diagnostic agents. Compared with conventional drugs, nanomedicine usually exhibits different response to light, magnetic or electronic irritation, and its physicochemical properties such as pH, temperature sensitivity also change obviously. Therefore, nanomedicine shows numerous advantages in the biological characteristics for targeted drug delivery and therapeutics, which overcome the limitations of molecular imaging and gene/drug delivery commonly arose in recent years. For example, it will protect drugs against degradation and enhances drug stability, prolong the circulation and target searching time, reduce the side effect and improve the distribution and metabolic process in tissues.[2]

During the past decades, a series of inorganic nanomaterials with unique physiochemical properties have been emerged and greatly promoted the development of nanomedicine. The luminescent quantum dots (QDs) are the most attractive stars in this field. QDs are semiconductor nanoparticles (NPs) comprising elements from the periodic groups II-VI or III-V. The size of QDs is ranging between 2 and 10 nm in diameter, which is close to or smaller than the dimensions of the exciton Bohr radius. As a result, the mobility of charge carriers (electrons and holes) is restricted within the nanoscale dimensions, and this quantum confinement effect endows QDs with unique optical and electronic features.[3] Compared with normal organic dyes, QDs process several advantages in fluorescence properties for biological applications as following: 1) QDs have broad excitation spectra together with narrow and symmetric emission spectra. Under the same excitation light, different QDs can simultaneously emit different colors, so the multicolor QD probes can be used to image and track multiple molecular targets simultaneously; 2) QDs have very large molar extinction coefficients in the order of 0.5–5.0 × 10^6 $M^{-1}cm^{-1}$, which is nearly

This work was supported by the following: the National Natural Science Foundation of China (grant number 20975089), Innovation Projects of the Chinese Academy of Sciences (grant number KZCX2-EW-206), Department of Science and Technology of Shandong Province of China (grant number 2008GG20005005), One Hundred Person Project of the Chinese Academy of Sciences.

No conflict of interest was reported by the authors of this paper.

*Corresponding author: Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003 China.

E-mail address: lxchen@yic.ac.cn (L. Chen).

1549-9634/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.nano.2010.12.006

10–50 times larger than that of organic dyes.[4] Therefore, QDs are able to absorb 10-50 times more photons than organic dyes at the same excitation photon flux, leading to a significant improvement in the probe brightness; individual QDs have been found to be 10–20 times brighter than organic dyes[5]; 3) the maximum emission wavelength of QDs can be controlled in a relatively simple manner by variation of particle size and composition, or through variation of surface coatings. It can be tuned at a variety of precise wavelengths from ultraviolet (UV) to near infrared (NIR). The emission of several QDs like CdHgTe, CdTeSe/CdS can be engineered in the range of 700–900 nm, which is a "transparent window" of biological species that can effectively eliminate the light absorbance and background interference of tissues and body fluids. Thus NIR QDs have found broad applications in living animal fluorescence imaging. 4) The stability with regard to photobleaching is much more superior to conventional fluorophores, which enable QDs to be applied for highly sensitive detection and long observation times in fluorescence microscopy. Based on the unique optical properties, Bruchez[6] and Nie[7] firstly applied QDs in fluorescence labeling of biological species in 1998. Up to now, QD-probe has developed into a kind of powerful tool in life sciences, playing an important role in molecular detection, cell labeling as well as in vivo imaging investigations.

The prosperity that QDs achieved in biolabeling inspired pharmaceutical researchers to apply them to the research and development of novel nanomedicines. There are two main aspects for the application of QDs in this field. The first is to develop fluorescent diagnostic nanoprobes for cancer detection and therapy after modification of certain targeting molecules. A single QD is large enough for conjugation to multiple ligands, leading to enhanced binding affinity to the targets. Together with their brightness and anti-bleaching advantage, the functionalized QDs can be used to capture and quantify a panel of biomarkers on intact cancer cells and tissue specimens sensitively and specifically, allowing a correlation of traditional histopathology and molecular signatures for the same material. The second application of QDs in nanomedicine is to label drug molecules or nanocarriers. With the aid of sensitive and fast responsive fluorescent imaging system that has been developing into a promising platform for drug investigation and screening, we can expect to acquire real-time information on distribution, transportation, drug release and pharmacodynamic mechanism of nanomedicine in biospecimens intuitively.

In this review, we will summarize the recent application of QDs in the research of nanomedicine from the following aspects: surface modification strategies of QDs for drug delivery and tumor diagnosis, QDs as drug nanocarriers, labeling other drug nanocarriers for *in vitro* and *in vivo* monitoring and pharmacokinetics evaluation, QD-based fluorescence resonance energy transfer (FRET) technique for drug release study as well as the development of multifunctional nanomedicines.

Surface modification strategies of quantum dots

Surface stabilization chemistry

It is commonly regarded that bare QDs are impractical for biological applications for several reasons. Firstly, QDs are water-insoluble in most of the synthesis strategies. Therefore, to make use of their optical properties in biological system, the surface has to be modified by a hydrophilic coating. Secondly, because of the large surface area-volume ratio, the QDs cores are highly reactive and suffer from very strong unspecific interactions with macromolecules, leading to the particle aggregation and fluorescence variation. Thirdly, the surface modification procedure can greatly reduce the toxicity of QDs. Because of their heavy metal composition, small size and active surface, QDs also draw a lot of attention for the toxicity and biocompatibility during biomedical application. It has been reported that QDs could induce biological toxicity mainly in the following two ways.[8] One is that slow oxidation process occurs for bare QDs under exposure to UV light and free cadmium ions will release into the surroundings, which will caused toxicity for the labeled living subjects. The other involves the creation of reactive oxygen species (ROS) such as free radicals (hydroxyl radical: ·OH and superoxide: $\cdot O_2^-$) and singlet oxygen ($^1O_2$), which are known to cause irreversible damage to nucleic acids, enzymes, and cellular components such as mitochondria and both plasma and nuclear membranes. After surface modification, the impermeable surrounding coating shell will effectively prevent the release of heavy metal ions and detect interaction between generating ROS and functional molecules in biological system, therefore reducing the QDs toxicity. So far, various surface stabilizing and coating materials and protocols have been applied for obtaining monodispersed, bio-inert and highly stable fluorescent QDs.

Among different surface modification strategies, ligand exchange method has been studied extensively. One of the easiest ways is the attachment of thiolated poly(ethylene glycol) polymers, which renders the water solubility and reduced unspecific cellular uptake of QDs.[9-12] Other polymers with varying chain lengths and number of binding dentates, such as PEGylated dihydrolipoic acid,[13-15] dendrimers,[16,17] and multidentate phosphine polymers,[18] are also used to stabilize QDs in aqueous solution. An alternative protocol for a solubilization and stabilization is ligand capping by various amphiphilic polymers such as poly(maleic anhydride alt-1-tetradecene),[19] triblock copolymer,[5] and alkyl-modified poly (acrylic acid)[20-22] to form highly stable polymer linking or micelle-like structures.[23-25] Coating QDs with polymers may increase the overall size of the assemblies by as much as 5-10 nm depending on the coating. Nevertheless, the major advantage of the hydrophobic interaction method is that ligand exchange reactions are avoidable. It is worth noting that this approach has already been explored using small organic molecules instead of polymers. The QDs are in that case coated with amphiphilic phospholipids,[26] calixarenes[27,28] or cyclodextrins[29-31] to achieve the same goal. Besides, the silica-coating method[32-36] has also been used to tailor QDs for gaining solubilization and functionalization to conjugate with biofunctional molecules. By microemulsion or reverse microemusion strategies, the silica-coated QDs with uniform sizes have been synthesized. The core-shell NPs display good photostability and low cytotoxicity requisite for biological use.

Attachment of molecules for targeting

Water-soluble QDs have to be cross-linked to biomolecules such antibodies, aptamers, or small molecule ligands to render them specific to biological targets and therefore be used for diagnosis and targeting drug delivery. Functionalization of QDs can be achieved by thiol exchange with biomolecules containing a sulfhydryl group or proteins and peptides with cysteine residues.[37] After incubation, equilibrium of the thiols on the QDs surface is achieved resulting in partial substitution of the initial coating molecules by the biomolecules. Stable covalent bonds can be formed by a mercapto acid (e.g. mercaptoacetic acid) coating of the QDs. The mercapto groups can bind to the QDs surface, while the carboxylic acid groups can form stable amide bonds with amines of various biomolecules with the aid of coupling reagents, such as carbodiimide and N-hydroxysuccinimide (NHS).[38,39] Another preferred strategy is the use of streptavidin modified QDs because they can easily be linked to biotin-tagged biomolecules.[40-42] Besides, electrostatic interactions between the QDs surface and macromolecules like peptides or proteins can also provide a facial way for coating and modification.[43] For QDs encapsulated in silica shell, a variety of organic functionalities can be readily attached with using well developed silane chemistry.[44,45] Up to now, various affinity reagents for modifying QDs, for example peptides, aptamers and small molecules, which specifically recognize certain over-expressed biomarkers on cancer cells, have been reported to producing cancer diagnosis probes and target drug delivery vesicles. Relevant information was summarized in Table 1.

Applications

QDs as drug nanocarriers

With the progress of surface modification technique in the past decade, QDs with water soluble capping stabilizer such as mercaptoacetic acid, mercaptoethylamine and polyethylene glycol polymer are readily to conjugate with drug molecules via covalent bonds or electrostatic interaction, forming complex nanomedicine with QDs as drug carriers. By monitoring the fluorescence signal of QDs, understanding of basic properties such as specific targeting, delivery efficiency and release rate of drug molecules within living cells and animals can be realized, which will help us to assure the diagnostic recognition and understand the mechanistic pathways of drug delivery. Moreover, complex drug delivery systems that combine QDs and therapeutic modalities in a single construct may offer advantages for the improvement of therapeutic effect and reduction of side effect for pharmaceuticals. So far, a certain small molecules or macromolecules like peptides and DNAs with potential medical value have been successfully studied through QDs based fluorescent imaging way.

Drug molecule tracking

As satisfactory fluorescence probes, QDs play an important role for investigating specific targeting interaction and pharmacokinetics of bioactive molecules at cell and animal level, which were key factors for design of nanomedicine for diagnosis and treatment. Yamomoto et al[68] set the first example on studying in vivo behavior of small molecule drug-QD nanocompex. They conjugated captopril (cap), an antihypertensive drug, to the hydrophobic QDs surface via liagnd exchange protocol and studied its distribution behavior in stroke-prone spontaneously hypertensive rats. The results showed that the administered cap-QD conjugates were capable of decreasing rat blood pressure to the same extent as the cap alone in the first 30 min and in vivo fluorescence of the QDs revealed that the conjugates mainly accumulated in the liver, lungs, and spleen. Another example was reported by Byrne et al.[69] They conjugated nonsteroidal anti-inflammatory drug naproxen to aqueous CdTe QDs and investigate their photophysical properties and biological behavior. These nanocomposites demonstrated interesting photophysical properties, good stability in an aggressive enzymatic medium, and displayed localization to the outer membrane of macrophage THP-1 cells. Similarly, Choi et al[70] developed two different types of QDs, one targeting prostate-specific membrane antigen (PSMA)-positive prostate cancer cells via the small molecule ligand GPI and one targeting integrin $\alpha_v\beta_3$-positive melanoma cells via the small molecule cRGD. Both in vitro and in vivo tests proved the tumor targeting ability of QDs functionalized small-molecule ligands. More importantly, in vivo fluorescence images showed that if the hydrodynamic diameter of the complex was less than 5.5 nm, which set an upper limit of 5–10 ligands per QD, they could go through a renal clearance. This study suggested a set of design rules for the clinical translation of targeted NPs that could be eliminated through the kidneys. Recently Kikkeri et al[59] described the in vivo distribution behavior of two sugars via QDs labeling way. As shown in Figure 1, they developed a new type of QDs coated with two sugars of mannose and galactosamine that were attracted to mannose receptors and ASGP-R in specific tissues and organs. In a study with mice, the coated QDs with either sugar accumulated selectively in the liver, which showed three times more concentrated in the mice livers than the regular PEG2000-QDs, demonstrating their higher specificity.

Macromolecules such as proteins share similar size with QDs and possess numerous active binding groups, which facilitate the QDs labeling reaction and their fluorescence investigation. Diagaradjane et al[71] evaluated the tumor targeting property, pharmacokinetic and biodistribution of epidermal growth factor (EGF) with the aid of QDs. They coupled NIR QDs to EGF using thiol-maleimide conjugation to create EGF-QD nanoprobes. As shown in Figure 2, in vivo fluorescence imaging showed three distinct phases of tumor influx, clearance and accumulation of nanocomplex in a tumor bearing nude mouse. Similarly, Koshman et al[72] localized QD-conjugated cardiac troponin C to the myofibrils and a nuclear peptide to the nucleus in living cardiac myocytes, which opened the possibility for live tracking of exogenous proteins and study of protein dynamics. Pang's group[51] used CdSe/ZnS QDs with maximum emission wavelength of 590 nm (QD590) linked to alpha-fetoprotein (AFP) monoclonal antibody (Ab) to detect AFP in cytoplasm of human hepatocellular carcinoma (HCC) cell line HCCLM6. For the in vivo studies, QD-AFP-Ab probes for targeted imaging of human HCC xenograft growing in nude mice were injected

Table 1
Recent examples of targeting molecules used for QDs modification

Modified molecule	Type of QD (stabilizer)	Conjugating configuration	Cell type/Target	Ref
Peptide				
TAT*	CdSe/ZnS (streptavidin)	Biotin-streptavidin interaction	A549 (lipid-raft-mediated macropinocytosis)	46
TAT	CdSe/ZnS (TOPO†)	Ligand exchange of cysteine-TAT	HepG2 (perinuclear region/lysosome)	47
$(His)_8$-Trp-Leu-Ala-Aib-Ser-Gly-$(Arg)_8$-amide	CdSe/ZnS (-COOH)	Metal-affinity driven self-assembly	COS-1, HEK293/T17 (endolysosome)	42
Allatostatin (AST1, APSGAQRLYG FGL-NH_2)	QD605 (streptavidin)	Biotin QD streptavidin interaction	A431 (argalanin receptor mediated endocytosis)	48
Chlorotoxin (CTX)/ dendrotoxin-1 (DTX-1)	ITK QD 525/655 (NH_2-PEG, Invitrogen)	N-succinimidyl iodoacetate and 2-iminothiolane reaction	C6 glioma cells (potassium channel)	49
Protein				
Lectin	CdSe (-COOH)	NHS‡-EDC§ reaction	Leukemia cells (specific target oligosaccharides)	50
Alpha-fetoprotein antibody	CdSe/ZnS (thioglycolic acid)	NHS-EDC reaction	HCC cell line HCCLM6 (Alpha-fetoprotein)	51
Cholera toxin B	CdSe/ZnS,CdTe/CdSe/ZnS (-COOH, Invitrogen)	EDC reaction	NIH 3T3, hMSC, MDSC, M21, MH15 (gangliosides)	52
Aptamer				
AS1411, TTA1, MUC-1	QDs 605,655,705 (-COOH, Invitrogen)	EDC reaction	PC-3, HeLa, C6, NPA (intracellular)	53
GBI-10	CdSe (polyamidoamine dendrimer)	EDC reaction	U251 glioblastoma cells (membrane)	54
TLS9a	QDs (streptavidin)	Biotin-streptavidin interaction	Mouse liver hepatoma cell (membrane)	55
Anti-PSMA aptamers A9	CdTe (biotinylated PEG)	Avidin-biotin interation	LNCaP and PC3 cells (PSMA)	56
Carbohydrate				
N-(2-aminoethyl) gluconamide hydrochloride	CdTe/CdS (-COOH)	NHS-EDC reaction	HeLa (intracellular)	57
Hyaluronic acid	CdSe/CdS/ZnS (N-(2-aminoethyl)-6, 8-dimercaptooctanamide, amine-DHLA)	Electrostatic interaction	HeLa (HA receptor CD44)	58
D-galactose	CdSe/ZnS (-COOH)	NHS reaction	HepG2 (intracellular)	59
Lactose	CdSeS/ZnS (TOPO)	1-thiol-β-D-lactose ligand exchange	Leukocytes (β_2 integrin) (CD11b/CD18)	60
Other small molecule				
HaloTag protein ligand	QD655 (streptavidin, Invitrogen)	Biotin-streptavidin interaction	COS7 (HaloTag protein ligand mediated membrane labeling)	61
GPI/cRGD	CdSe/ZnCdS (cysteine)	NHS-EDC reaction	Prostate cancer cell (PSMA)/melanoma cell (integrin $\alpha_v\beta_3$)	62
Hoechst 33342	CdTe (N-acetylcysteine)	Electrostatic interaction		63
Polyarginine-pyrenebutyrate	QDs655 (Invitrogen)	Biotin-streptavidin linkage	BS-C-1 monkey kidney cells (pyrenebutyrate increased uptake in cytosol)	64
β-CD-L-Arg	CdSe/ZnSe (-COOH)	Electrostatic interaction	ECV-304 (cytoplasmic localization)	65
Folate	InP/ZnS (-COOH)	NHS-DCC¶ reaction	KB cells (folate receptor)	66
	QDs (NH_2-PEG)	NHS-DCC reaction	KB cells (folate receptor)	67

* human immunodeficiency virus-1 transactivator protein.
† trioctylphosphine oxide.
‡ N-hydroxysuccinimide.
§ N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide.
¶ N,N'-dicyclohexylcarbodiimide.

into the tail vein, which showed good sensitivity, biocompatibility and tumor specificity.

Hyaluronic acid (HA) is an endogenous anionic glycosaminoglycan that plays a structural role as part of the connective tissue matrix and participates in various cell-to-cell interactions. It is usually used as tissue healing drug and biomaterial scaffold in tissue engineering research. Besides, HA can also used for the prognosis of some malignant tumors.[73] Therefore, HA-QDs can be useful reporter probes that monitor tumor progressions, or screen anticancer drug efficacies. Kim et al investigated the in vivo distribution behavior by preparing HA-NIR QDs conjugates and subcutaneously injecting them to nude mice.[74] According to the real-time bioimaging, it was found that conjugates with 35 mol% HA content maintaining enough binding sites for HA receptors were mainly accumulated in the liver, while those with 68 mol% HA content losing much of HA characteristics

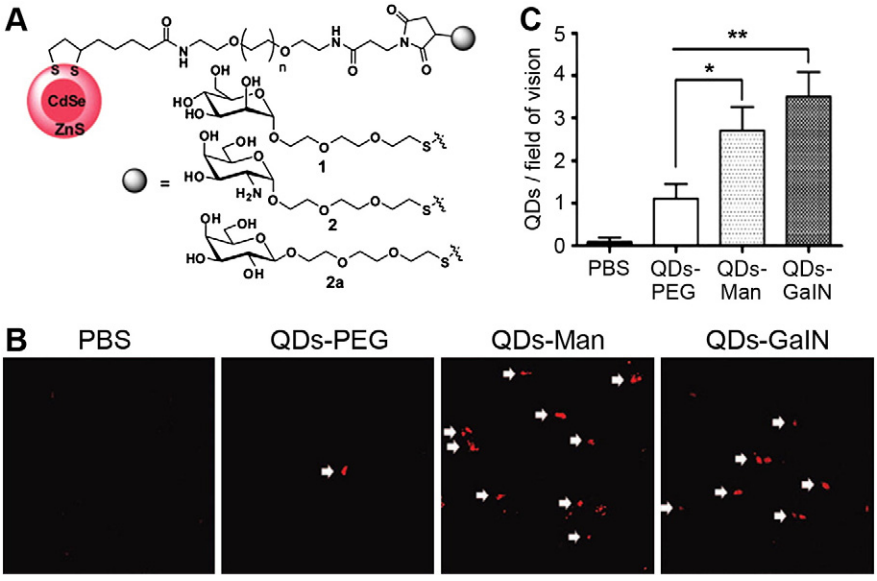

Figure 1. **(A)** Quantum dots and sugars used in the study. **(B)** Fluorescence images of paraffin sections of the livers obtained from mice injected with PBS or 2.5 nmol of either PEG2000 QDs or QDs capped with D-mannose or D-galactosamine. Arrows indicate QDs sequestered to liver tissue. **(C)** Statistical analysis of QD sequestration in the liver was performed by counting 10 microscopic fields of vision for each mouse. Reproduced from Kikkeri et al. [59] with permission from American Chemical Society.

were evenly distributed to the tissues in the body. Based on this result, they further compared the retention behavior of the conjugates in a normal and diseased liver.[75] Interestingly, it revealed that the clearance of conjugates was relatively slow in a cirrhotic liver. Furthermore, immunofluorescence and flow cytometric analyses of dissected liver tissues showed the target-specific delivery of HA derivatives to liver sinusoidal endothelial cells and hepatic stellate cells. The results were thought to reflect the feasibility of HA derivatives as novel drug delivery macromolecules for the treatment of hepatitis, liver cirrhosis and liver cancer.

The above works provided a new thought for the targeting behavior and in vivo investigation of drug molecules in an in vivo optical imaging way. However, the maintenance of biological activity of the linking molecules is an important issue that should be taken into consideration during the experiment designing. On one hand, the targeting moieties in the molecules must not be occupied in the coupling reaction with QDs. On the other hand a proper linking molecule served as spacer is essential to eliminate the steric hindrance of the much larger QDs in the targeting recognition process.

Promoting drug cellular internalization

Besides the application for molecule fluorescence localization, QDs also show great potential to promote drug delivery into cells and therefore increase the therapeutic effect. The most successful examples were reported on the labeling and delivery of small interfering RNAs (siRNAs). SiRNAs are small double stranded therapeutic RNA molecules containing a "sense" and an "antisense" strand that by a regulatory mechanism of RNA interference, which results in the degradation of a target mRNA and inhabitation of specific proteins synthesis. Derfus et al[76] applied a PEGlyated QD core as a carrier, and successively conjugated siRNA and tumor-homing peptides (F3) as functional groups on the QDs surface. Delivery of these F3/siRNA-QDs to HeLa cells and siRNA release from their endosomal entrapments were monitored by QDs fluorescence. Moreover, it was found that conjugation chemistry was a key factor for maintaining silencing efficiency. SiRNA attached to the particle by disulfide cross-linkers showing greater silencing efficiency than when attached by a nonreducible thioether linkage. Walther et al[77] developed fluorescent delivery system for oligonucleotide drug by incorporating branched hCT-derived carrier peptide hCT (18–32)-k7 on the surface of QDs, which could successful intracellular transport Cy3 labeled RNA and exhibit no effect on cell viability.

The QD-assisted gene delivery approaches mentioned above mainly focused on the enhancement of transfection efficiency, knockdown of non-oncogenes such as the gene coding for green fluorescent protein. Toward the goal to develop methods for monitoring the effects of siRNA-mediated target-gene silencing, Jung et al[78] described the synthesis and target-specific delivery of multifunctional siRNA-QD constructs for selectively inhibiting the expression of epidermal growth factor receptor variant III (EGFRvIII) in target human U87 glioblastoma cells, and subsequently monitoring the resulting down-regulated signaling pathway with high efficiency.

Besides the function of drug transporting and fluorescence imaging, Yezhelyev et al[79] firstly reported that QDs nanocarreirs could also improve the curative effect of siRNA. As shown in Figure 3, they modified tertiary amine polymer on the QDs surface, forming “proton-sponge” coatings, and then adsorbed siRNA via electrostatic force. The nanocomplexes not only allowed real-time tracking of siRNA delivery process such as cellular penetration, endosomal release, carrier

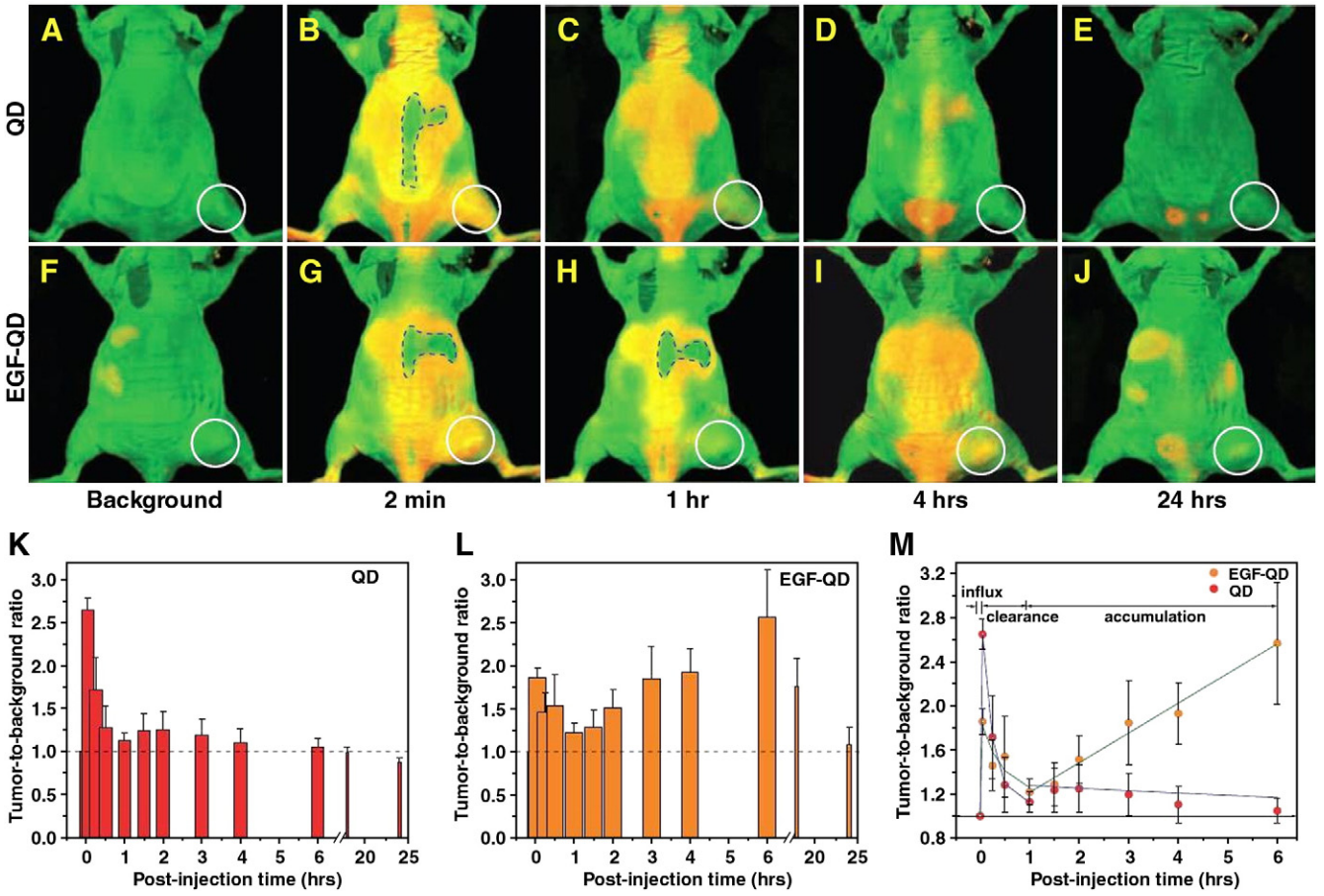

Figure 2. In vivo imaging of EGFR-expressing tumors using EGF-QD nanoprobes. **(A)** to **(E)**, representative NIR fluorescence images at 0 and 3 min, and 1, 4, and 24 h after i.v. injection of QD nanoparticles. **(F)** to **(J)**, corresponding images after i.v. injection of EGF-QD nanoprobes. **(K)** and **(L)**, tumor-to-background ratios from the mice injected with QD nanoparticles (n = 7) and EGF-QD nanoprobes (n = 8), respectively. **(M)**, the influx, clearance, and accumulation/equilibration phases of EGF-QD nanoprobes and QD nanoparticles kinetics within tumor are represented by best-fit lines (green and blue, respectively). Reproduced from Diagaradjane et al. [71] with permission from American Association for Cancer Research.

unpacking, and intracellular transport, but also demonstrated dramatic improvement in gene silencing efficiency by 10–20-fold and simultaneous reduction in cellular toxicity by 5–6-fold, when compared directly with existing transfection agents for MDA-MB-231 cells.

Drug release investigation

The study of drug release properties is a crucial aspect for the evaluation and screening of nanomedicine. In a traditional way, the researchers apply chromatographic techniques such as HPLC-MS to determine the overall content of drugs in cells or animals to investigate drug release behavior. However, this method usually suffers from the following problem in real applications. First, the test subject must be executed for complicated sample pretreatment; hence it is hard to acquire real time drug release information in living subjects. Second, chromatographic method cannot distinguish and separately detect the free (released) drug and nanocarrier encapsulated drug. Third, it is still difficult to quantitatively analyze trace amount of certain pharmaceuticals with weak detective signal in complicated biological background. Fortunately, the recently developed QD-based FRET strategy offered a novel method to resolve these problems.

When QDs donors and chromophore acceptors are in close proximity and their emission spectrum and absorption spectrum are fully overlapped, QDs in the electronic excited state may transfer energy to the acceptors, resulting in the fluorescence quenching of QDs and enhancement of the chromophores. The FRET efficiency is closely related with the spatial distance between QDs and acceptors, a closer distance will lead to a stronger FRET. Therefore, this distance regulating FRET technique allows the successful application of QDs in immunoanalysis and nanosensors fabrication,[80] and makes great progress on inter-cellular drug release study of nanomedicine in recent years.

In the research of polyplex-mediated gene delivery system, a critical barrier is the timely unpacking of polyplexes within the target cell to liberate DNA for efficient gene transfer. Up to now it is still a major challenge to gain a mechanistic understanding of the rate-limiting steps. Ho YP et al[81] made a valuable attempt to clarify this question relying on QD-FRET technique. As shown in Figure 4, the component plasmid DNA and polymeric gene carrier were individually labeled with QDs and Cy5 dyes,

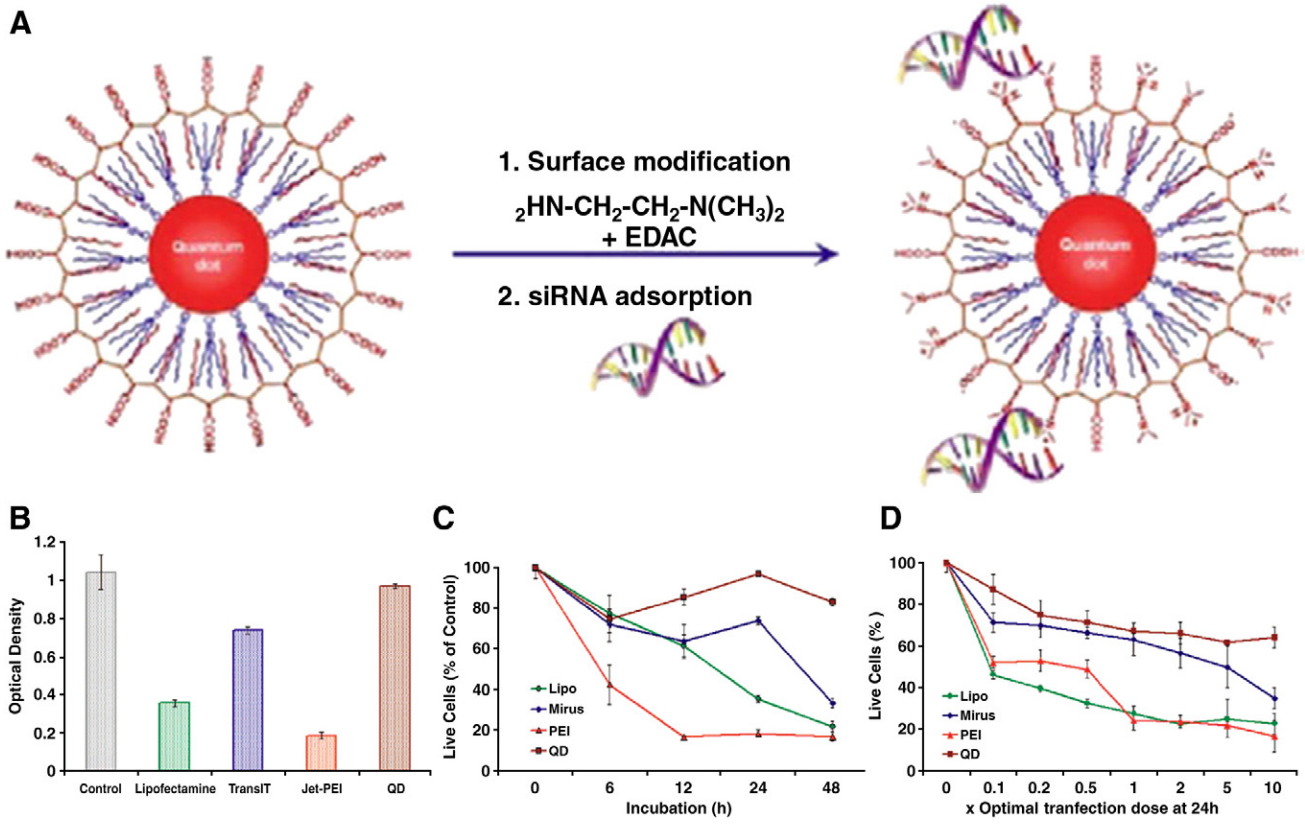

Figure 3. **(A)** Chemical modification of polymer-encapsulated QDs to introduce tertiary amine groups, and adsorption of siRNA on the particle surface by electrostatic interactions. **(B)** Cytotoxicity data obtained from QDs and three transfection reagents (Lipofectamine 2000, TransIT, and JetPEI) at their optimal transfection efficiencies (100 nM for QDs). Data points were obtained at 24 h, and the proton-sponge coated QDs were nearly nontoxic to MDA-MB-231 cells. **(C)** Cellular toxicity data as a function of transfection time obtained from QDs and conventional reagents at siRNA concentrations for optimal transfection efficiency. **(D)** Dose-dependent toxicity data for QDs and conventional agents. The x-axis indicates the fold of siRNA concentration relative to the optimal concentration for transfection. Reproduced from Yezhelyev et al.[79] with permission from American Chemical Society.

respectively, as a donor and acceptor pair for FRET. In a compact nanocomplex, the proximity of QDs and Cy5 caused high FRET efficiency and resultant increasing fluorescence of the Cy5 acceptor. The intracellular uptake and dissociation of polyplexes resulted in the decrease of Cy5 signal and increase of QDs signal, which was captured over time by confocal microscopy. From quantitative image-based analysis, distributions of released plasmid within the endo/-lysosomal, cytosolic, and nuclear compartments formed the basis for constructing a three-compartment first-order kinetics model. By using the same approach, this group further compared the polyplex unpacking kinetics for three different polymers of chitosan, PEI and polyphosphoramidate, and the result correlated well with transfection efficiencies.[82] In another work, Lee et al[83] reported polyelectrolyte complexes composed by QDs conjugated positively charged PEI and Cy5 labeled vascular endothelial growth factor siRNA (cy5-VEGF siRNA). In addition, PEI conjugated QDs were further modified with a protein transduction domain from human transcriptional factor, Hph-1. From confocal microscopic FRET analysis, it could be visualized that the two siRNA/QD-PEI complexes with and without Hph-1 showed markedly different intracellular uptake behaviors and unpacking kinetics of cy5-siRNA. As mentioned above, QD-FRET enabled detection of polyplex stability combined with image-based quantification is a valuable method for studying mechanisms involved in polyplex unpacking and trafficking within live cells.

Instead of chemically modified acceptor dyes on the macromolecules, Lim et al[84] investigated a novel FRET pair with QDs as donors and DNA labeling dyes of BOBO-3, which can intercalate into double-stranded nucleic acid chains, as acceptors by using DNA as a linker. Using this FRET pair, it was able to monitor the configuration changes and the fate of the DNA nanocomplexes during intracellular delivery, thereby providing an insight into the mechanistic study of gene delivery. In another example, Bagalkot et al reported bi-FRET system composed of a QD, an aptamer, and the small molecular anticancer drug doxorubicin (Dox) for in vitro targeted imaging, therapy and sensing of drug release.[85] As illustrated in Figure 5, aptamers were conjugated to QDs to serve as targeting units, and Dox was attached to the stem region of the aptamers, taking advantage of the nucleic acid binding ability of Dox. Two donor-quencher pairs of FRET occurred in this construct, as the QDs fluorescence was quenched by Dox, and Dox was quenched by the double-stranded RNA aptamers. As a result, after taking up by tumor cells, gradual release of Dox from the conjugate was

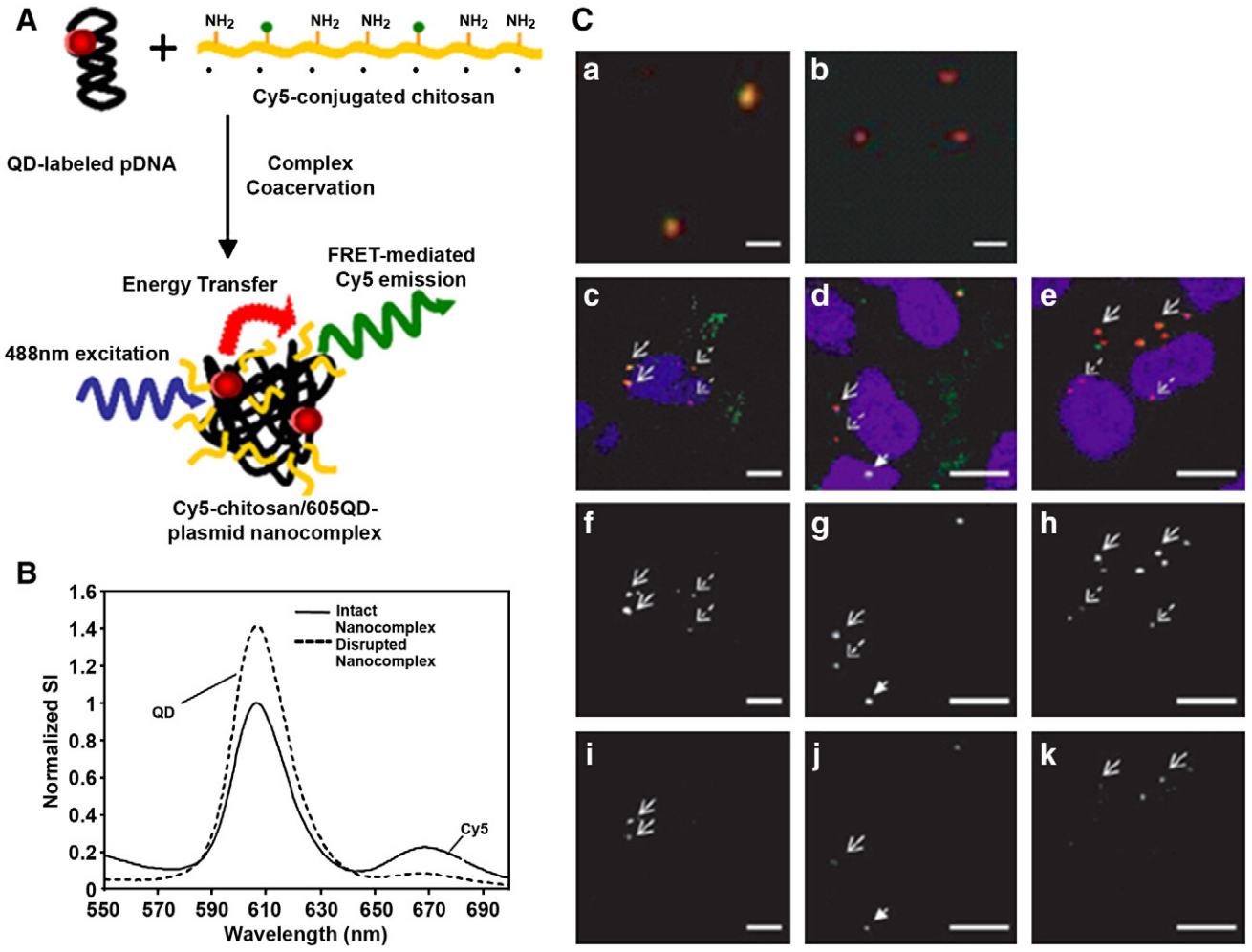

Figure 4. **(A)** Schematic of polyplex synthesis by condensing QD-labeled anionic pDNA with Cy5-conjugated chitosan gene carriers. **(B)** By spectrofluorometry, intact nanocomplexes exhibited FRET-mediated Cy5 emission centered at 670 nm, while disrupted nanocomplexes exhibited weak Cy5 emission and recovery of QD signal intensity. **(C)** Live-cell imaging of quantum dot-fluorescence resonance energy transfer (QD-FRET) polyplexes. **(a)** Fluorescent image of intact QD-FRET chitosan polyplexes mounted on a coverslip. Upon excitation of the QDs, individual polyplexes exhibited energy transfer as indicated by the colocalization (orange/yellow) of QD (red) and Cy5 (green) signals. **(b)** After complete disruption, energy transfer is abrogated and the QD signal is recovered. At 4 hours post-transfection with QD-FRET polyplexes, **(c-e)** composite images from confocal imaging of live HEK293 cells, and the corresponding gray scale images of the individual **(f-h)** QD and **(i-k)** Cy5 channels are shown separately. Reproduced from Ho et al. [81] with permission from Elsevier.

found to "turn on" the fluorescence of both QDs and Dox, providing a means to sense the release of the drug.

Most QDs-FRET system reported based on specific changes of the donor/acceptor distance. Recently, Fernández-Argüelles et al[86] designed novel FRET sensors that detect spectral changes of the acceptor (under the influence of analyte binding) at fixed CdSe/ZnS QDs donor/acceptor distance by the introduction of the organic dye acceptor into the polymer coating. This approach allows for short acceptor donor separation and thus for high-energy transfer efficiencies. Although the pharmaceutical application of this structure was not mentioned, it enlightened us that if the drug molecules which can dramatically affect the FRET efficiency of QDs and the immobilized dyes were loaded in the complex, the QD-dye pairs would give apparent fluorescence responsive with the drug release. This idea may be helpful for the dynamic monitoring framework dissolve and drug release process for QD-based polymeric NP formulations.

Quantum dots as tags for other drug nanocarriers

The research and development of various drug nanocarriers is an important part for the advance of nanomedicine. General drug molecules can be encapsulated in or attached to the surfaces of nanocarriers, which offer enormous advantages such as to reduce of dosage, minimize side effect, assure of the pharmaceutical potency and enhance drug stability. However, owing to the small size, various classes of composing materials and the complicated nanostructure of nanocarriers, the tracking techniques and the property evaluation methods of loaded nanocarriers in vitro and in vivo are different from those of conventional molecular drugs. The sensitive and fast responsive fluorescent imaging method using QDs as probes can dynamically monitor the behavior of labeled nanocarriers at both cell and living animal level, and is developing into a promising platform for their property investigation and screening. Comparing with various

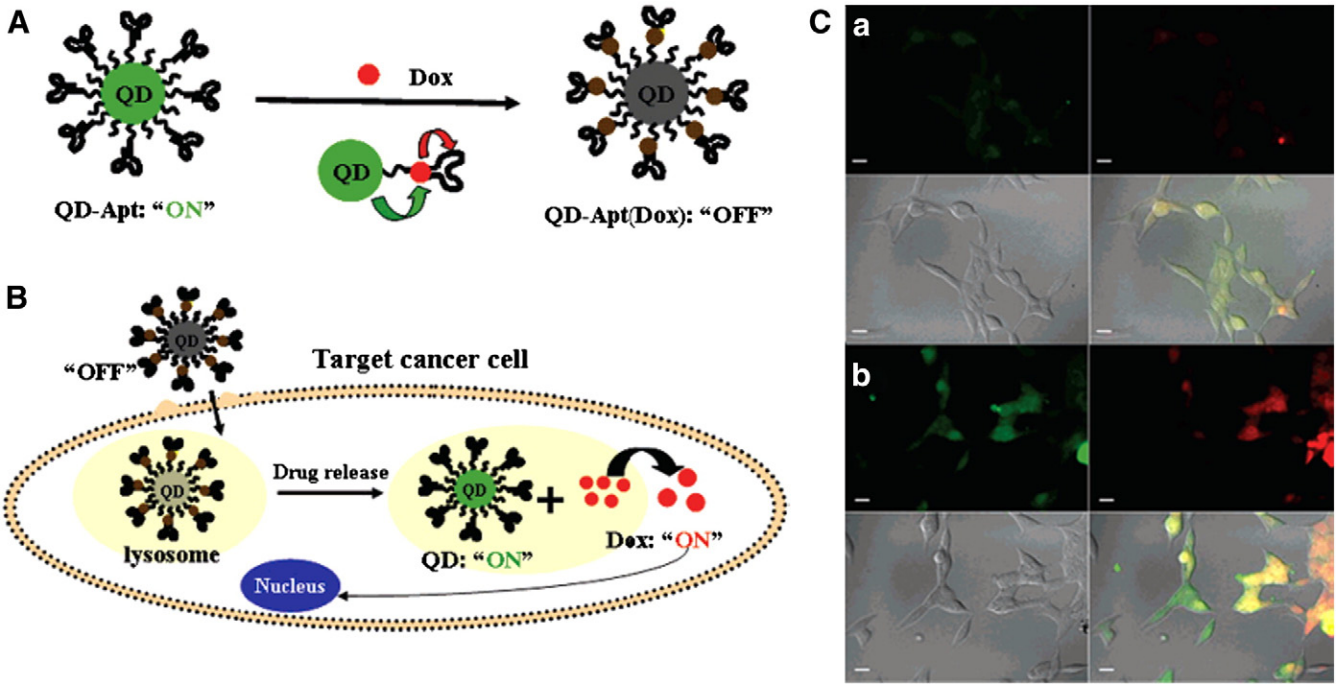

Figure 5. **(A)** Schematic illustration of QD-Apt(Dox) bi-FRET system. **(B)** Schematic illustration of specific uptake of QD-Apt(Dox) conjugates into target cancer cell through PSMA mediate endocytosis. The release of Dox from the QD-Apt(Dox) conjugates induces the recovery of fluorescence from both QD and Dox ("ON" state), thereby sensing the intracellular delivery of Dox and enabling the synchronous fluorescent localization and killing of cancer cells. **(C)** Confocal laser scanning microscopy images of PSMA-expressing LNCaP cells after incubation with 100 nM QD-Apt(Dox) conjugates for 0.5 h at 37°C, washing two times with PBS buffer, and further incubation at 37°C for **(a)** 0 h and **(b)** 1.5 h. Dox and QD are shown in red and green, respectively, and the lower right images of each panel represent the overlay of Dox and QD fluorescence. Reproduced from Bagalkot et al. [85] with permission from American Chemical Society.

kinds of small molecule fluorescence reagents, QDs exhibit much superior optical properties such as the size dependent multi-color for multiplex labeling, the resistance to be bleached for long-term tracking as well as tunable emission wavelength to NIR region for in vivo imaging. To date, many types of drug delivery nanosystems have been labeled with QDs via numerous synthesis methods and studied by using fluorescent imaging method (Table 2).

Liposomes

liposomes are tiny drug vesicles usually composed by phospholipid bilayer. The depth of the bilayer is in the range of 3-4 nm that near the diameter of small QDs, which offers the possibility for their entrapment. Therefore the liposome can be fluorescently labeled in such a simple way. For instance, Al-Jamal et al[87] reported the labeling of zwitterionic dioleoylphosphatidylcholine (DOPC) and cationic 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) unilamellar liposomes with TOPO capped CdSe/ZnS QDs. Cryo-TEM proved that 2 nm QDs in core size were self-assembled into the bilayers of vesicles via hydrophobic interaction (Figure 6, *A*). Confocal laser scanning microscopy (CLSM) images indicated that the QD-loaded vesicles were able to intracellularly transport inside the human epithelial lung cells (A549). Moreover, after injection in vivo intratumorally, the fluorescence of human cervical carcinoma (C33a) xenografts showed that cationic DOTAP liposomes led to enhanced retention compared with DOPC liposomes. In another work, similar QDs labeling method was applied to investigate the cell targeting distinctions of liposomes with difference lipid composition.[88] The fluorescence imaging results revealed that liposome of DOTAP:1,2-Dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC) (25:75) entered into cells within seconds after mixing while the counterparts of DOTAP: 1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine-N-[methoxy(polyethylene glycol 2000)] (DPPE-PEG2000):DMPC (25:0.5:74.5) remained on the cell membrane after 1 h incubation.

Besides encapsulation of hydrophobic QDs in the bilayer of liposomes mentioned above, covalent linking method was also applied in Weng' group for preparation of QD-conjugated immunoliposomes (QD-ILs).[91] They first synthesized doxorubicin loaded liposomes containing amine-functionalized *N*-(polyethylene glycol)-1,2-distearoyl-*sn*-glycero-3-phosphoethanola-mine (PEG-DSPE), and further conjugated carboxyl terminated CdSe/ZnS QD as fluorescent probe and anti-HER2 scFv protein as targeting moiety (Figure 6, *B*). Both flow cytometry and CLSM revealed efficient receptor-mediated endocytosis in target cells. After injection in MCF-7/HER2 xenograft mouse models, localization of QD-ILs at tumor sites was confirmed by in vivo fluorescence imaging.

Polymeric nanoparticles

Biodegradable polymeric NPs have attracted considerable attention as potential drug delivery systems in view of their

Table 2
Type and feature of QD-labeled nanocarriers

Nanocarrier	QDs (surface ligand)	QDs location (labeling method)	Advantage/application	Ref
Liposome	CdSe/ZnS (TOPO*)	Liposome bilayer (solvent emulsification-evaporation-trasonication)	Enhanced optical stability and internalized in A549 cells and in vivo tumor labeling	87
	CdSe (TOPO)	Liposome bilayer (solvent emulsification-evaporation-trasonication)	Monitoring the encapsulation behavior of liposomes with different surface charge	88
	Evident Tech QDs (PEG-COOH)	Hydrophilic cavity (solvent emulsification-evaporation-trasonication)	Uptaking by living cells enhanced penetration and retention into the tumor interstitium	89
	Invitrogen QDs 655 (streptavidin)	Hydrophilic cavity (solvent emulsification-evaporation-trasonication)	Directly delivered QDs to cell cytosol	90
	CdSe/ZnS (Mercaptoacetic acid)	Attaching on the outside surface (EDC reaction)	Doxorubicin-loaded, tumor cell-selective internalization and tumor in vivo imaging	91
	Evident Tech QDs (PEG-COOH)	Hydrophilic cavity (solvent emulsification-evaporation-trasonication)	Delivery to solid tumor in vivo	92
	CdTe (Mercaptoacetic acid)	Hydrophilic cavity (solvent emulsification-evaporation-trasonication)	Sentinel lymph node mapping in vivo	93
PLGA NP†	Qdot 655ITK	The interior of the polymeric matrix (solvent exaction-evaporation technique)	Improving cell imaging quality and extending the half-life of the QDs	94
	Qdot 525, 655ITK (PEG-NH_2)	Attaching on the surface (EDC reaction)	Monitoring nuclear translocation of NPs	95
	Invitrogen QDs 655 (PEG-NH_2)	Attaching on the surface (EDC reaction)	Without changing the proliferation and differentiation capability of labeled hMSCs	96
	Evident Tech CdSe/ZnS (TOPO)	The interior of the polymeric matrix (emulsion-diffusion-evaporation method)	DNA loading and ultrasensitive detection of Langerhans cell migration	97
PLA NP	CdSe (TOPO)	The interior of the polymeric matrix (emulsion-diffusion-evaporation method)		98
Nanogel	CdTe (Mercaptoacetic acid)	The interior of the gel matrix (hydrogen bond interaction)	Monitoring the nanogel swelling and deswelling by temperature-responsive fluorescence	99
	Qdot655 (Protein A, Goat anti-Fluorescein, streptavidin)	The interior of the gel matrix (electrostatic force)	Labeling cells with much higher cellular uptake efficiency than that of cationic liposomes	100
	CdSe/CdS (Thiol-modified poly(ethylene oxide)s	Attaching on the surface on gels (incubation reactions)	Non-specific binding to fibroblasts	101
	CdS ((NIPAM-AAm-PBA)	In situ synthesis in NIPAM-AAm-PBA‡ nanogel	Reversible fluorescence quenching for the optical detection of glucose	102
Chitosan NP	CdSe/ZnS (Mercaptoacetic acid)	The interior of the chitosan matrix (electrostatic force)	Producing bi-functional nanobeads with fluorescent and paramagnetic properties	103
	CdSe/ZnS (Mercaptoacetic acid)	The interior of the chitosan matrix (EDC reaction)	-	104
	CdSe/ZnS (Mercaptoacetic acid)	The interior of the chitosan matrix (electrostatic force)	Multicolor nanobeads for cell labeling	105
	CdSe/ZnS (Mercaptoacetic acid)	The interior of the chitosan matrix (electrostatic force)	siRNA delivery and monitoring transfection efficiency	106
	ZnO (oleic acid)	The interior of the chitosan matrix (electrostatic force)	Enhanced optical stability and loaded with anti-cancer drugs	107
Gelatin NP	CdTe (Mercaptoacetic acid)	The interior of the gelatin matrix (two-step desolvation method)	-	108
	CdHgTe (Mercaptoacetic acid)	The interior of the gelatin matrix (in situ QDs synthesis)	Monitoring in vivo distribution of gelatin NPs	109
Carbon nanotube	CdTe (Mercaptoacetic acid)	Attaching on the surface (electrostatic force)	Monitoring CNTs deliver oligodeoxynucleotides into cells	110
	EviTags 600 CdSe/ZnS (-NH_2)	Covalent linked on the surface (NHS-EDC reaction)	MWCNTs-QDs exhibited strong luminescent emissions *in vivo*	111
	Invitrogen QDs 800 (PEG-NH_2)	Covalent linked on the surface (NHS-EDC reaction)	Anti-cancer drug delivery and monitoring the in vivo distribution	112
	Invitrogen QDs 800 CdSeTe/ZnS (PEG-NH_2)	Covalent linked on the surface (NHS-EDC reaction)	-	113
Solid lipid NP	CdSe/ZnS (TOPO)	The interior of lipid (solvent emulsification-evaporation-trasonication)	-	114
	CdSe (TOPO)	The interior of lipid (solvent emulsification-evaporation-trasonication)	Monitoring target delivery to folate receptor expressing cells	115
Dendrimer	CdSe/ZnS (TOPO)	The interior (solvent emulsification-evaporation)	HeLa cell imaging	17

* Trioctylphosphine oxide.
† Nanoparticle.
‡ N-isopropylacrylamide-acrylamidephenylboronic acid.

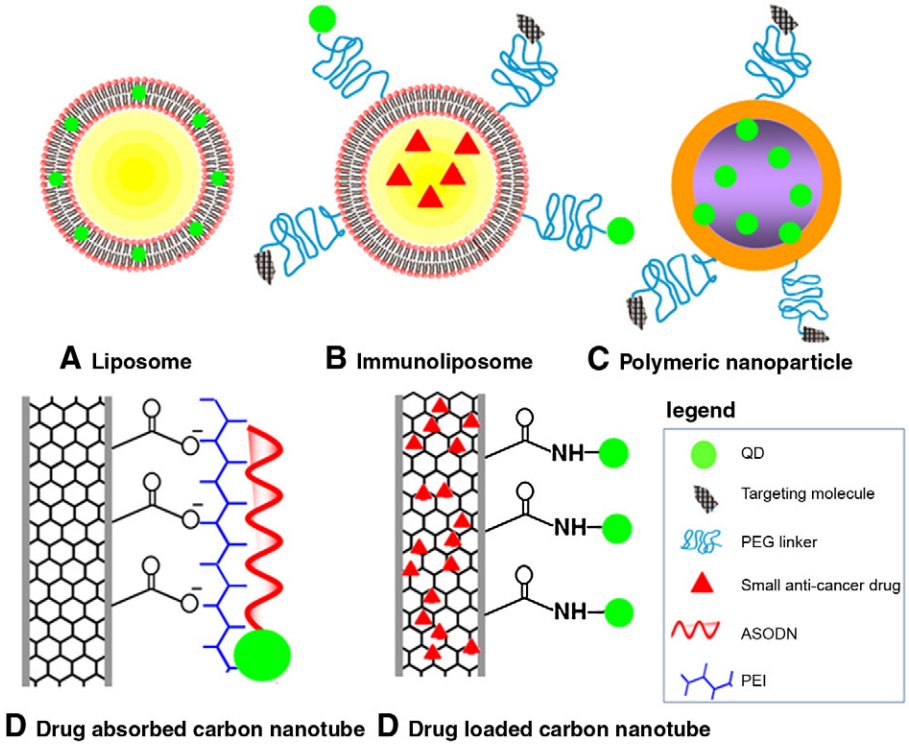

Figure 6. Schematic illustration of QD-labeled drug nanocarriers.

applications in the controlled released of drugs, their ability to target particular organs/tissues and deliver drugs through a peroral route of administration.[116] Active research is now focused on the preparation of fluorescent QD-labeled NPs using hydrophilic polymers like chitosan, gelatin for drug delivery investigation (Figure 6, *C*).

Zhang Yong' group firstly presented a novel and easy way to encapsulate QDs into chitosan NP drug carriers. As a macromolecule with protonated amino groups in the repeating hexosaminide residue, chitosan stays positively charged under weakly acidic conditions, forming a long and intertwined chain of positive charges along its back-bone onto which the negatively-charged QDs are electrostatically attracted to. Relying on this feature, they successfully prepared multicolored QD-labeled[105] and QD-Gd-DTPA-embedded fluorescent-magnetic dual functional chitosan NPs.[103] Furthermore, they used labeled chitosan NPs as the carrier of HER2/neu siRNA. The target delivery and transfection of the siRNA in HER2-overexpressing SKBR3 breast cancer cells could be monitored by the presence of fluorescent QDs.

Other than the commonly used natural hydrophilic polymers, a number of synthetic biodegradable polymers were also applied to prepare QD-tagged drug delivery NPs through emulsion-evaporation method. For example, Nehilla et al[117] synthesized surfactant-free copolymer poly(lactide-co-glycolide) (PLGA) NPs co-loaded with QDs and hydrophobic drug coenzyme Q10 molecules. Confocal imaging studies showed that the NPs were taken up by PC12 cells after one day in vitro. Gao et al[118] developed a QD-based imaging platform for brain imaging by incorporating QDs into the core of poly(ethyleneglycol)-poly (lactic acid) NPs, which was then functionalized with wheat germ agglutinin and delivered into the brain via nasal application, holding considerable potential for the treatment of various central nervous system diseases. Folate-decorated, QD-embedded NPs using locally synthesized biodegradable poly (lactide)-vitamin E TPGS (PLA-TPGS) and vitamin E TPGS-carboxyl (TPGS-COOH) copolymers were also reported by Pan et al.[119] This formulation was successfully applied for targeted and sustained imaging for cancer diagnosis, and showed lower in vitro cytotoxicity compared with the free QDs.

Carbon nanotubes

Within the family of nanomaterials, carbon nanotubes (CNTs) have emerged as a new alternative and efficient tool for transporting and translocating therapeutic molecules. CNTs have a high purity, large surface area, and can load bioactive peptides, proteins, nucleic acids and drugs, and deliver their cargos to cells and organs. Moreover, functionalized CNTs display low toxicity and are not immunogenic, such systems hold great potential in the field of nanobiotechnology and nanomedicine. Recently, researchers integrated two nanomaterials of CNTs and QDs for the design of novel fluorescent nanomedicines and applied them to the in vivo investigation.

Jia's group explored a novel double functionalized multi-walled carbon nanotube (MWCNT) drug delivery system.[110] CdTe QDs as fluorescent probes were first covalently linked to antisense oligodeoxynucleotides (ASODNs) as a therapeutic gene, forming QD-ASODNs nanocomplex. Then carboxylized MWCNTs were modified with polyethylenimine (PEI), followed

by the layer-by-layer assembling of QD-ASODNs via electrostatic force (Figure 6, *D*). With the aid of CLSM, they found that PEI coating of CNTs greatly affected the therapeutic properties of system, which led to decreasing of the cellular toxicity and increasing delivery efficiency of ASONNs.

Different from the labeling through electrostatic interaction, Shi et al[111] directly conjugated amino group functionalized CdSe/ZnS QDs with emission wavelength of 600 nm on the surface of MWCNTs through amide linkages and studied in vivo distribution behavior of the complex. Bright fluorescent signal could be observed through an in vivo imaging system after the complex was injected into the back and abdomen of a nude mouse. This group further selected NIR CdSeTe/ZnS QDs with longer emission wavelength of 752 nm and labeled MWCNT that loaded by antitumor drug of paclitaxel[112] (Figure 6, *E*). In vivo imaging of live mouse was achieved by intravenously injecting QD-conjugated MWCNT for the first time. After six days' circulation, it was found that liver, kidney, stomach and intestine exhibited bright fluorescence, indicating MWCNT nanocarriers mainly accumulated in these organs.

Polymeric micelles

Polymeric micelles are made up of polymer chains and are usually spontaneously formed by self-assembly in a liquid, which typically have a core-shell structure. The core of the micelles, which is either the hydrophobic part or the ionic part of the NPs, can hold nanocrystals or therapeutic drug molecules, while the shell prevents interactions with the solvent and make the loaded micelles thereby stable in aqueous solution.

In 2002, individual QD was firstly encapsulated in phospholipid block-copolymer micelle and demonstrated both in vitro and in vivo imaging.[23] From then on QD-labeled micelle nanomedicine was inspired and studied deeply. For instance, Fu et al[120] developed a novel ZnS QDs/poly(N-isopropylacrylamide) (PNIPAM) hybrid micelles obtained by localizing free radical polymerization of NIPAM and crosslinker N,N'-methylenebis(acrylamide) at the peripheral of poly(ε-caprolactone) (PCL) NPs, followed by biodegradation of PCL with an enzyme of the Lipase PS. The QDs not only rendered micelle structure the fluorescence signal but also revealed thermosensitive reversible properties (swelling and deswelling change at about 32°C) by the slight red shift in photoluminescence spectra. Papagiannaros et al[121] introduced a new nanosized imaging agent for effective visualization of tumors based on poly (ethylene glycol)-phospholipid micelle encapsulated QDs (QD-Ms). NIR in vivo images showed that QD-Ms had a higher fluorescent signal and better tendency to accumulate in the tumor area compared with the commercially available PEGylated QDs. In another work, NIR QD-loaded micelles were applied for targeted cancer imaging and therapy.[122] QDs were modified by 10,12-pentacosadiynoic acid (PCDA)-PEG and PCDA-herceptin conjugates to demonstrate water-solubility and target-specific properties. After injected intravenously into a tumor-bearing nude mouse, it was observed that the micelles distributed rapidly throughout the animal body including the tumor in real time, exhibiting ability for both active and passive targeting, imaging and treatment of cancers in the early stage.

Nanogels

Nanogels have also attracted considerable attention as multifunctional polymer-based drug delivery systems. One advantage of nanogel is that with optimization of their molecular composition, size and morphology, they can be tailor-made to sense and respond to environmental changes in order to ensure spatial and stimuli-controlled drug release in vivo.[123] Nanogels can be designed to facilitate the encapsulation of QDs with two methods. One is to mix the as-prepared nanogels and QDs to load them making the use of concentration gradient diffusion; the other is to add QDs to the raw reaction materials before crosslinking and they are entrapped in nanogels during their synthesis process.

Gong et al[99] first reported the QD-PNIPAM nanogel complex prepared through the former method with the aid of hydrogen bonding between the ligands capped on CdTe QDs and the PNIPAM chains. Interestingly, although QDs in the nanogel increased the cross-linking degree of the PNIPAM network, the volume of the resultant composite spheres remained tunable against temperature. Therefore, if QDs with different size were loaded, Förster energy transfer between QDs could be initiated by increasing the environmental temperature, creating a temperature-responsive emission. Hasegawa's group[100,124] proposed monodispersed hybrid NPs prepared by simple mixing QDs with nanogels of cholesterol-bearing pullulan (CHP) modified with amino groups. The $CHPNH_2$-QD NPs were examined effectively internalized into the various human cells, and the efficiency of cellular uptake was much higher than that of a conventional carrier of cationic liposome. These results indicated that $CHPNH_2$ nanogels had a potential as a research tool in the studies of intracellular delivery system. Wu et al[125] reported on in-situ immobilization of CdSe QDs and anticancer drug temozolomide in the interior of the pH and temperature dual responsive hydroxypropylcellulose-poly(acrylic acid) (HPC-PAA) nanogels. The hybrid nanogels integrated the functional building blocks for optical pH-sensing, cancer cell imaging and controlled drug release into a single NP system, which can offer broad opportunities for combined diagnosis and pH-triggered sustained-release of the drug molecules.

Other drug nanocarriers

With the goal of identifying an improved delivery scheme for intracellular and in vivo tracking and anticancer therapy, several other delivery systems containing drugs and QDs probes have also been prepared. For example, lipid NPs containing QDs entrapped in a lipid shell, and post-loaded with a folate-lipid conjugate for tumor targeting was also reported.[115] Selective binding and uptake of lipodots by J6456-FR cells was observed in vivo after intra-peritoneal injection in mice bearing ascitic J6456-FR tumors. Low cytotoxic ZnO QD-based nonviral vectors with the dual functions of delivering plasmid DNA and labeling cells were fabricated by capping the surface of ZnO QDs with poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA).[126] The polycation-modified ZnO QDs were capable of condensing plasmid DNA into nanocomplexes and mediating an efficient transfer of plasmid DNA into COS-7 cells

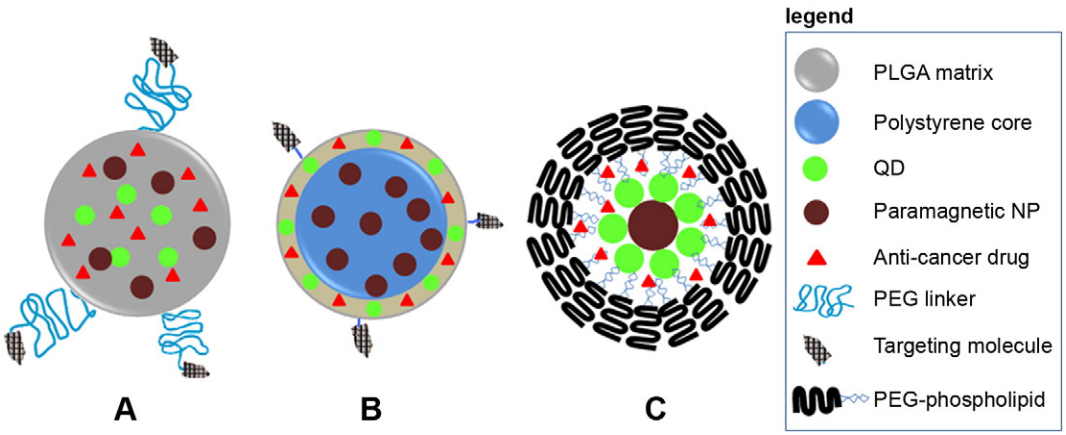

Figure 7. Schematic illustration of QD-based multifunctional nanomedicines.

with much lower cytotoxicity, meanwhile allowing real-time imaging of gene transfection.

Development of multifunctional nanomedicine

Real time, noninvasive diagnosis and therapy of disease, especially for tumors, is a significant scientific question that is closely related to the improvement of life quality and health of human beings. Multifunctional nanomedicine integrates target labeling, drug delivery and result reporting abilities, allowing diseases to be monitored and treated simultaneously. It holds considerable promise as the next generation of medicine, and has been attracted much attention of researchers in recent years. Certain inorganic NPs with unique optical properties such as colloidal gold, iron oxide nanocrystals, especially QDs, can greatly improve the ability to detect diseases at much earlier stages, and offers a new opportunity for development of novel multifunctional drug formulations in the future.

Up to now, a lot of researchers have developed florescent nanoprobes by modifying various tumor targeting agents on QDs surface, realizing tumor diagnosis at cell and living animal level.[127] However, the applications of QDs for in vivo imaging are limited by tissue penetration depth, quantification problems, and a lack of anatomic resolution and spatial information. To address these problems, several research groups have led efforts to couple QD-based fluorescent imaging with other imaging modalities that are not limited by penetration depth, such as magnetic resonance imaging (MRI)[128-133] and positron emission tomography (PET).[134-136] For example, Mulder et al[130] developed a fluorescent-MRI dual modality tumor imaging probe by chemically incorporating paramagnetic gadolinium complexes in the RGD peptide conjugated lipid coating layer of QDs. Cai et al[134] modified QDs with an amine functionalized surface with RGD peptides and radioactive ^{64}Cu chelators for integrin $\alpha_v\beta_3$-targeted PET/NIR fluorescence imaging. The dual-mode imaging, tissue homogenate fluorescence measurement, and immunofluorescence staining were all performed with U87MG tumor-bearing mice to quantify the probe uptake in the tumor and major organs.

Based on these works, Kim et al[137] designed a multifunctional polymer nanomedicine platform for simultaneous cancer-targeted MRI or fluorescent imaging and magnetically-guided drug delivery. As shown in Figure 7, *A*, this platform was composed of four components. 1) biodegradable PLGA NPs matrix for loading and subsequent controlled release of hydrophobic therapeutic agents into cells; 2) incorporated Fe_3O_4 superparamagnetic magnetite nanocrystals for magnetically guided delivery and T_2 MRI contrast agents and CdSe/ZnS QDs for optical imaging; 3) Dox was used as a therapeutic agent for cancers; 4) cancer-targeting folate conjugated onto the PLGA NPs to target KB cancer cells. The cancer cells targeted with the multifunctional polymer nanomedicine were detectable through MRI or confocal microscopy.

Cho et al[138] recently reported another QDs based multifunctional nanomedicine with novel architecture (Figure 7, *B*). They conjugated NIR QDs onto the surface of a nanocomposite consisting of a spherical polystyrene matrix (about 150 nm) and the internally embedded, high fraction of 10 nm superparamagnetic Fe_3O_4 NPs. For drug storage, the chemotherapeutic agent paclitaxel was loaded onto the surfaces of multifunctional nanocarriers by using a layer of PLGA. Anti-prostate specific membrane antigen (anti-PSMA) was further conjugated for targeting. Specific detection studies of anti-PSMA-conjugated nanocarrier binding activity in LNCaP prostate cancer cells were carried out and considerable targeting effects were observed.

Up to now, most multifunctional nanocomposites have been used for in vitro cell targeting. In vivo studies, in particular for cancer imaging and therapy, have been limited owing to the poor stability or short systemic circulation times in living animals. Aiming to this problem, Park et al[139] described tumor targeting, long-circulating, micellar hybrid NPs (MHNs) that contain MNs, QDs, and the anticancer drug Dox within a single poly(ethylene glycol)-phospholipid micelle modified with F3 peptide (Figure 7, *C*), and provide the first example of simultaneous targeted drug delivery and dual-mode NIR fluorescence imaging and MRI of diseased tissue in vitro and in vivo (Figure 8). The PEG coating of micelles prevented them from recognition and endocytosis by reticuloendothelial system, and prolonged the

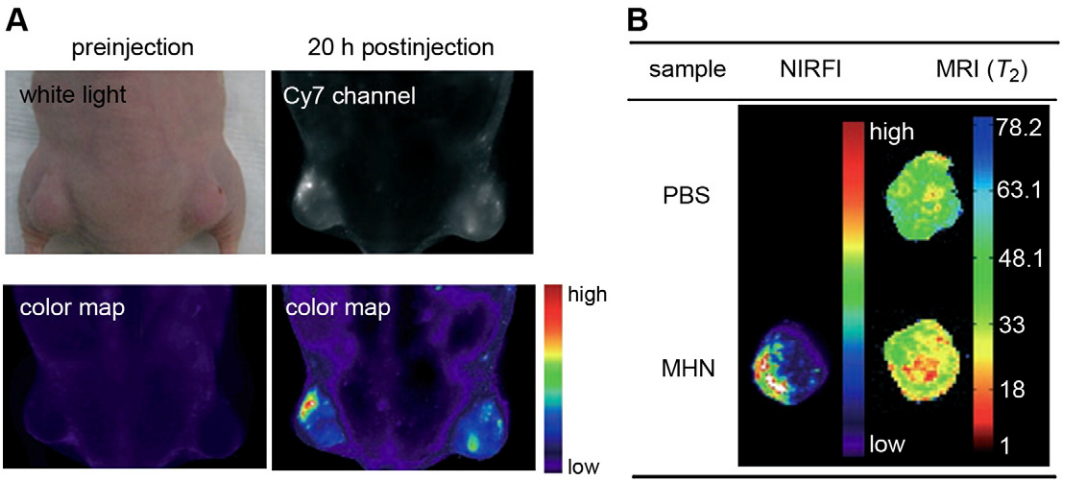

Figure 8. **(A)** NIR fluorescence images showing the passive accumulation of MHNs containing QDs (emission at 800 nm, MHN(800)) in a mouse with MDA-MB-435 tumors. The mouse was imaged preinjection and 20 h postinjection. **(B)** Image table describing the results of multimodal imaging (by MRI and NIR fluorescence) of the tumor harvested from the mouse in **(A)**. Reproduced from Park et al. [139] with permission from Wiley-VCH Verlag.

circulation and targeting time, which was a key factor for the successful application in vivo.

Future perspectives

With the joint effort of scientists in the field of chemistry, biology, medical engineering and pharmaceutical sciences, QDs have been received as technological improvements with characteristics that could greatly improve biological imaging and gained prominent achievement in the research of nanomedicine. Nevertheless, a number of new questions have also been raised. In the near future, there are several areas of research that are particularly promising and should be paid enough attention to:

(1) Development of novel type of QDs and surface coating method with high biosafety. It should be realized that the application of QDs in nanomedicine was only limited in the level of cells and experimental animals. Although most works reported that QDs did not cause apparent influence on physiological status of living subjects, the compatibility and long-term toxicity of heavy metals composed QDs were still major concerns, and hence they were prohibitive to any patient studies. If alternative QDs can be prepared from relatively non-toxic materials, or the toxic components can be inertly protected from exposure and subsequently cleared from the body, it will accelerate the pace for their usage in drug screening and the further clinical relevance of QDs could also be foreseeable. The recently emerging carbon dots,[140,141] carbogenic QDs,[142,143] silica QDs,[144,145] ZnO QDs[126,146] as new types of safe and cheap luminescent QDs labels, have an inspiring prospect in the clinical applications.

(2) The influence of labeling QDs on the inherent property of nanomedicines. It is still on the early stage for the investigation of nanomedicines via QDs labeling. Most works mainly focused on the labeling method and the in vitro or in vivo behavior of fluorescent nanocomplex obtained from optical imaging system, however, the influence of labeling QDs on the inherent property of nanomedicines, was rarely reported and need to be deeply explored. For example, it is important to make clear the pharmacokinetics and pharmacodynamics differences between QDs labeled and original drugs; the stability and drug loading capacity variations of drug nanocarriers after QDs labeling.

(3) Quantitative analysis for QDs fluorescence imaging. The commonly used graphical analysis function set in fluorescence imaging system, especially the in vivo imaging system, can only offer semiquantitative results, which cannot fulfill the quantitative requirement of biopharmaceutical analysis in living animals. Moreover, the acquisition of accurate multi-channel image signal still has technical problem at whole animal level, hindering the FRET analysis. Therefore, QDs-FRET based drug release study can only be carried out in the cells, similar works performed in living animals were not reported until now. These problems need to be solved with the multidisciplinary collaboration of pharmaceutical analysis, computer image processing and chemometrics.

References

1. Webster TJ. Nanomedicine-what's in a definition. Int J Nanomedicine 2006;1:115-6.
2. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer. Nat Nanotechnol 2007;2:751-60.
3. Gao X, Yang L, Petros JA, Marshall FF, Simons JW, Nie S. In vivo molecular and cellular imaging with quantum dots. Curr Opin Biotechnol 2005;16:63-72.
4. Leatherdale CA, Woo WK, Mikulec FV, Bawendi MG. On the absorption cross section of CdSe nanocrystal quantum dots. J Phys Chem B 2002;106:7619-22.
5. Gao X, Cui Y, Levenson RM, Chung LW, Nie S. In vivo cancer targeting and imaging with semiconductor quantum dots. Nat Biotechnol 2004;22:969-76.

6. Bruchez Jr M, Moronne M, Gin P, Alivisatos AP. Semiconductor nanocrystals as fluorescent biological labels. Science 1998;281:2013-6.
7. Chan WC, Nie S. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science 1998;281:2016-8.
8. Tsay JM, Michalet X. New light on quantum dot cytotoxicity. Chem Biol 2005;12:1159-61.
9. Uyeda HT, Mednitz IL, Jaiswal JK, Simon SM, Mattoussi H. Synthesis of compact multidentate ligands to prepare stable hydrophilic quantum dot fluorophores. J Am Chem Soc 2005;127:3870-8.
10. Mei BC, Susumu K, Medintz IL, Mattoussi H. Polyethylene glycol-based bidentate ligands to enhance quantum dot and gold nanoparticle stability in biological media. Nat Protoc 2009;4:412-23.
11. Stewart MH, Susumu K, Mei BC, Medintz IL, Delehanty JB, Blanco-Canosa JB, et al. Multidentate poly(ethylene glycol)ligands provide colloidal stability to semiconductor and metallic nanocrystals in extreme conditions. J Am Chem Soc 2010;132:9804-13.
12. Schipper ML, Iyer G, Koh AL, Cheng Z, Ebenstein Y, Aharoni A, et al. Particle size, surface coating, and PEGylation influence the biodistribution of quantum dots in living mice. Small 2009;5:126-34.
13. Smith AM, Nie SM. Minimizing the hydrodynamic size of quantum dots with multifunctional multidentate polymer ligands. J Am Chem Soc 2008;130:11278-9.
14. Susumu K, Mei BC, Mattoussi H. Multifunctional ligands based on dihydrolipoic acid and polyethylene glycol to promote biocompatibility of quantum dots. Nat Protoc 2009;4:424-36.
15. Tan SJ, Jana NR, Gao SJ, Patra PK, Ying JY. Surface-ligand-dependent cellular interaction, subcellular localization, and cytotoxicity of polymer-coated quantum dots. Chem Mater 2010;22:2239-47.
16. Lemon BI, Crooks RM. Preparation and characterization of dendrimer-encapsulated CdS semiconductor quantum dots. J Am Chem Soc 2000; 122:12886-7.
17. Liu JA, Li HB, Wang W, Xu HB, Yang XL, Liang JG, et al. Use of ester-terminated polyamidoamine dendrimers for stabilizing quantum dots in aqueous solutions. Small 2006;2:999-1002.
18. Kim SW, Kim S, Tracy JB, Jasanoff A, Bawendi MG. Phosphine oxide polymer for water-soluble nanoparticles. J Am Chem Soc 2005; 127:4556-7.
19. Pellegrino T, Manna L, Kudera S, Liedl T, Koktysh D, Rogach AL, et al. Hydrophobic nanocrystals coated with an amphiphilic polymer shell: a general route to water soluble nanocrystals. Nano Lett 2004;4: 703-7.
20. Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, et al. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat Biotechnol 2003;21: 41-6.
21. Luccardini C, Tribet C, Vial F, Marchi-Artzner V, Dahan M. Size, charge, and interactions with giant lipid vesicles of quantum dots coated with an amphiphilic macromolecule. Langmuir 2006;22:2304-10.
22. Yu WW, Chang E, Falkner JC, Zhang JY, Al-Somali AM, Sayes CM, et al. Forming biocompatible and nonaggregated nanocrystals in water using amphiphilic polymers. J Am Chem Soc 2007;129:2871-9.
23. Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A. In vivo imaging of quantum dots encapsulated in phospholipid micelles. Science 2002;298:1759-62.
24. Tomczak N, Jańczewski D, Han MY, Vancso GJ. Designer polymer-quantum dot architectures. Progress in Polymer Science 2009;34: 393-430.
25. Wu YZ, Chakrabortty S, Gropeanu RA, Wilhelmi J, Xu Y, Er KS, et al. pH-Responsive quantum dots via an albumin polymer surface coating. J Am Chem Soc 2010;132:5012-4.
26. Geissbuehler I, Hovius R, Martinez KL, Adrian M, Thampi KR, Vogel H. Lipid-coated nanocrystals as multifunctionalized luminescent scaffolds for supramolecular biological assemblies. Angew Chem Int Ed 2005;44:1388-92.
27. Jin T, Fujii F, Sakata H, Tamura M, Kinjo M. Amphiphilic psulfonatocalix[4]arene-coated CdSe/ZnS quantum dots for the optical detection of the neurotransmitter acetylcholine. Chem Commun 2005:4300-2.
28. Jin T, Fujii F, Yamada E, Nodasaka Y, Kinjo M. Control of the optical properties of quantum dots by surface coating with calix[n]arene carboxylic acids. J Am Chem Soc 2006;128:9288-9.
29. Feng J, Ding SY, Tucker MP, Himmel ME, Kim YH, Zhang SB, et al. Cyclodextrin driven hydrophobic/hydrophilic transformation of semiconductor nanoparticles. Appl Phys Lett 2005;033108:86.
30. Rakshit S, Vasudevan S. Resonance energy transfer from β-Cyclodextrin-capped ZnO:MgO nanocrystals to included Nile red guest molecules in aqueous media. ACS Nano 2008;2:1473-9.
31. Freeman R, Finder T, Bahshi L, Willner I. β-Cyclodextrin-Modified CdSe/ZnS Quantum Dots for Sensing and Chiroselective Analysis. Nano Lett 2009;9:2073-6.
32. Yi DK, Selvan ST, Lee SS, Papaefthymiou GC, Kundaliya D, Ying JY. Silica-coated nanocomposites of magnetic nanoparticles and quantum dots. J Am Chem Soc 2005;127:4990-1.
33. Kim J, Lee JE, Lee J, Yu JH, Kim BC, An K, et al. Magnetic fluorescent delivery vehicle using uniform mesoporous silica spheres embedded with monodisperse magnetic and semiconductor nanocrystals. J Am Chem Soc 2006;128:688-9.
34. Selvan ST, Patra PK, Ang CY, Ying JY. Synthesis of silica-coated semiconductor and magnetic quantum dots and their use in the imaging of live cells. Angew Chem Int Ed 2007;46:2448-52.
35. Koole R, van Schooneveld MM, Hilhorst J, de Mello Donegá C, 't Hart DC, van Blaaderen A, et al. On the incorporation mechanism of hydrophobic quantum dots in silica spheres by a reverse microemulsion method. Chem Mater 2008;20:2503-12.
36. Han RC, Yu M, Zheng Q, Wang LJ, Hong YK, Sha YL. A facile synthesis of small-sized, highly photoluminescent, and monodisperse CdSeS QD/SiO_2 for live cell imaging. Langmuir 2009;25: 12250-5.
37. Shi LF, Rosenzweig N, Rosenzweig Z. Luminescent quantum dots fluorescence resonance energy transfer-based probes for enzymatic activity and enzyme inhibitors. Anal Chem 2007;79:208-14.
38. Srinivasan C, Lee J, Papadimitrakopoulos F, Silbart LK, Zhao M, Burgess DJ. Labeling and intracellular tracking of functionally active plasmid DNA with semiconductor quantum dots. Mol Ther 2006;14: 192-201.
39. Choi Y, Kim HP, Hong SM, Ryu JY, Han SJ, Song R. In situ visualization of gene expression using polymer-coated quantum-dot-DNA conjugates. Small 2009;5:2085-91.
40. Lidke DS, Nagy P, Heintzmann R, Arndt-Jovin DJ, Post JN, Grecco HE, et al. Quantum dot ligands provide new insights into erbB/HER receptor-mediated signal transduction. Nat Biotechnol 2004;22: 198-203.
41. Dahan M, Levi S, Luccardini C, Rostaing P, Riveau B, Triller A. Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking. Science 2003;302:442-5.
42. Medintz IL, Pons T, Delehanty JB, Susumu K, Brunel FM, Dawson PE, et al. Intracellular delivery of quantum dot-protein cargos mediated by cell penetrating peptides. Bioconjugate Chem 2008;19:1785-95.
43. Hanaki KI, Momo A, Oku T, Komoto A, Maenosono S, Yamaguchi Y, et al. Semiconductor quantum dot/albumin complex is a long-life and highly photostable endosome marker. Biochem Biophys Res Commun 2003;302:496-501.
44. Bottini M, D'Annibale F, Magrini A, Cerignoli F, Arimura Y, Dawson MI, et al. Quantum dot-doped silica nanoparticles as probes for targeting of T-lymphocytes. Int J Nanomedicine 2007;2:227-33.
45. Zhang TT, Stilwell JL, Gerion D, Ding LH, Elboudwarej O, Cooke PA, et al. Cellular effect of high doses of silica-coated quantum dot profiled with high throughput gene expression analysis and high content cellomics ceasurements. Nano Lett 2006;6:800-8.
46. Chen B, Liu QL, Zhang YL, Xu L, Fang XH. Transmembrane delivery of the cell-penetrating peptide conjugated semiconductor quantum dots. Langmuir 2008;24:11866-71.

47. Wei YF, Jana NR, Tan SJ, Ying JY. Surface coating directed cellular delivery of TAT-functionalized quantum dots. Bioconjugate Chem 2009;20:1752-8.
48. Anas A, Okuda T, Kawashima N, Nakayama K, Itoh T, Ishikawa M, et al. Clathrin-mediated endocytosis of quantum dot-peptide conjugates in living cells. ACS Nano 2009;3:2419-29.
49. So MK, Yao HQ, Rao JH, Orndorff RL, Rosenthal SJ. Neurotoxin quantum dot conjugates detect endogenous targets expressed in live cancer cells. Nano Lett 2009;9:2589-99.
50. Zhelev Z, Ohba H, Bakalova R, Jose R, Fukuoka S, Nagase T, et al. Fabrication of quantum dot-lectin conjugates as novel fluorescent probes for microscopic and flow cytometric identification of leukemia cells from normal lymphocytes. Chem Commun 2005: 1980-2.
51. Chen LD, Liu J, Yu XF, He M, Pei XF, Tang ZY. The biocompatibility of quantum dot probes used for the targeted imaging of hepatocellular carcinoma metastasis. Biomaterials 2008;29:4170-6.
52. Chakraborty SK, Fitzpatrick JAJ, Phillippi JA, Andreko S, Waggoner AS, Bruchez MP, et al. Cholera toxin B conjugated quantum dots for live cell labeling. Nano Lett 2007;7:2618-26.
53. Kang WJ, Chae JR, Cho YL, Lee JD, Kim S. Multiplex imaging of single tumor cells using quantum-dot-conjugated aptamers. Small 2009;22:2519-22.
54. Li ZM, Huang P, He R, Lin J, Yang S, Zhang XJ, et al. Aptamer-conjugated dendrimer-modified quantum dots for cancer cell targeting and imaging. Mater Lett 2010;64:375-8.
55. Zhang J, Jia X, Lv XJ, Deng YL, Xie HY. Fluorescent quantum dot-labeled aptamer bioprobes specifically targeting mouse liver cancer cells. Talanta 2010;81:505-9.
56. Chu TC, Shieh F, Lavery LA, Levy M, Richards-Kortum R, Korgel BA, et al. Labeling tumor cells with fluorescent nanocrystal-aptamer bioconjugates. Biosen Bioelectron 2006;21:1859-66.
57. Jiang XZ, Ahmed M, Deng ZC, Narain R. Biotinylated glyco-functionalized quantum dots: synthesis, characterization, and cytotoxicity studies. Bioconjugate Chem 2009;20:994-1001.
58. Bhang SH, Won N, Lee TJ, Jin H, Nam J, Park J, et al. Hyaluronic acid-quantum dot conjugates for in vivo lymphatic vessel imaging. ACS Nano 2009;3:1389-98.
59. Kikkeri R, Lepenies B, Adibekian A, Laurino P, Seeberger PH. In vitro imaging and in vivo liver targeting with carbohydrate capped quantum dots. J Am Chem Soc 2009;131:2110-2.
60. Yu M, Yang Y, Han RC, Zheng Q, Wang LJ, Hong YK, et al. Polyvalent lactose-quantum dot conjugate for fluorescent labeling of live leukocytes. Langmuir 2010;26:8534-9.
61. So M, Yao HQ, Rao JH. HaloTag protein-mediated specific labeling of living cells with quantum dots. Biochem Biophys Res Commun 2008;374:419-23.
62. Choi HS, Liu WH, Liu FB, Nasr K, Misra P, Bawendi MG, et al. Design considerations for tumour-targeted nanoparticles. Nat Nanotechnol 2010;5:42-7.
63. Zhao D, He ZK, Chan PS, Wong RNS, Mak NK, Lee AWM, et al. NAC-capped quantum dot as nuclear staining agent for living cells via an in vivo steering strategy. J Phys Chem C 2010;114:6216-21.
64. Jablonski AE, Humphries WH, Payne CK. Pyrenebutyrate-mediated delivery of quantum dots across the plasma membrane of living cells. J Phys Chem B 2009;113:405-8.
65. Zhao MX, Xia Q, Feng XD, Zhu XH, Mao ZW, Ji LN, et al. Synthesis, biocompatibility and cell labeling of L-arginine-functional β-cyclodextrin-modified quantum dot probes. Biomaterials 2010;31:4401-8.
66. Bharali DJ, Lucey DW, Jayakumar H, Pudavar HE, Prasad PN. Folate-receptor-mediated delivery of InP quantum dots for bioimaging using confocal and two-photon microscopy. J Am Chem Soc 2005;27: 11364-71.
67. Song EQ, Zhang ZL, Luo QY, Lu W, Shi YB, Pang DW. Tumor cell targeting using folate-conjugated fluorescent quantum dots and receptor-mediated endocytosis. Clin Chem 2009;55:955-63.
68. Manabe N, Hoshino A, Liang YQ, Goto T, Kato N, Yamamoto K. Quantum dot as a drug tracer in vivo. IEEE Trans Nanobiosci 2006;5: 263-7.
69. Byrne SJ, le Bon B, Corr SA, Stefanko M, Connor CO, Gun'ko YK, et al. Synthesis, characterisation, and biological studies of CdTe quantum dot-naproxen conjugates. ChemMedChem 2007;2: 183-6.
70. Choi HS, Liu WH, Liu FB, Nasr K, Misra P, Bawendi MG, et al. Design considerations for tumor-targeted nanoparticles. Nat Nanotechnol 2010;5:42-7.
71. Diagaradjane P, Cardona JMO, Colón-Casasnovas NE, Deorukhkar A, Shentu S, Kuno N, et al. Imaging epidermal growth factor receptor expression in vivo-pharmacokinetic and biodistribution characterization of a bioconjugated quantum dot nanoprobe. Clin Cancer Res 2008; 14:731-41.
72. Koshman YE, Waters SB, Walker LA, Los T, de Tombe P, Goldspink PH, et al. Delivery and visualization of proteins conjugated to quantum dots in cardiac myocytes. J Mol Cell Cardiol 2008;45:853-6.
73. Gunthert U, Hofmann M, Rudy W, Reber S, Zoller M, Haubmann I, et al. A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. Cell 1991;65:13-24.
74. Kim J, Kim KS, Jiang G, Kang H, Kim S, Kim BS, et al. In vivo real-time bioimaging of hyaluronic acid derivatives using quantum dots. Biopolymers 2008;89:1144-53.
75. Kim KS, Hur W, Park SJ, Hong SW, Choi JE, Goh EJ, et al. Bioimaging for targeted delivery of hyaluronic acid derivatives to the livers in cirrhotic mice using quantum dots. ACS Nano 2010;4: 3005-14.
76. Derfus AM, Chen AA, Min DH, Ruoslahti E, Bhatia SN. Targeted quantum dot conjugates for siRNA delivery. Bioconjug Chem 2007;18: 1391-6.
77. Walther C, Meyer K, Rennert R, Neundorf I. Quantum dot-carrier peptide conjugates suitable for imaging and delivery applications. Bioconjugate Chem 2008;19:2346-56.
78. Jung J, Solanki A, Memoli KA, Kamei K, Kim H, Drahl MA, et al. Selective inhibition of human brain tumor cells through multifunctional quantum-dot-based siRNA delivery. Angew Chem Int Ed 2010;49: 103-7.
79. Yezhelyev MV, Qi L, O'Regan RM, Nie S, Gao X. Proton-sponge coated quantum dots for siRNA delivery and intracellular imaging. J Am Chem Soc 2008;130:9006-12.
80. Gill R, Zayats M, Willner I. Semiconductor quantum dots for bioanalysis. Angew Chem Int Ed Engl 2008;47:7602-25.
81. Ho Y, Chen H, Leong K, Wang T. Evaluating the intracellular stability and unpacking of DNA nanocomplexes by quantum dots-FRET. J Control Release 2006;116:83-9.
82. Chen H, Ho Y, Jiang X, Mao H, Wang T, Leong KW. Quantitative comparison of intracellular unpacking kinetics of polyplexes by a model constructed from quantum dot-FRET. Mol Ther 2008;16: 324-32.
83. Lee H, Kim IK, Park TG. Intracellular trafficking and unpacking of siRNA/Quantum Dot-PEI complexes modified with and without cell penetrating peptide: confocal and flow cytometric FRET Analysis. Bioconjugate Chem 2010;21:289-95.
84. Lim TC, Bailey VJ, Ho YP, Wang TH. Intercalating dye as an acceptor in quantum-dot-mediated FRET. Nanotechnology 2008; 075701:19.
85. Bagalkot V, Zhang L, Levy-Nissenbaum E, Jon S, Kantoff PW, Langer R, et al. Quantum dot-aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer. Nano Lett 2007;7: 3065-70.
86. Fernandez-Argüelles MT, Yakovlev A, Sperling RA, Luccardini C, Gaillard S, Medel AS, et al. Synthesis and characterization of polymer-coated quantum dots with integrated acceptor dyes as FRET-based nanoprobe. Nano Lett 2007;7:2613-7.

87. Al-Jamal WT, Al-Jamal KT, Tian B, Lacerda L, Bomans PH, Frederik PM, et al. Lipid-quantum dot bilayer vesicles enhance tumor cell uptake and retention in vitro and in vivo. ACS Nano 2008;2:408-18.
88. Gopalakrishnan G, Danelon C, Izewska P, Prummer M, Bolinger PY, Geissbühler I, et al. Multifunctional lipid/quantum dot hybrid nanocontainers for controlled targeting of live cells. Angew Chem Int Ed Engl 2006;45:5478-83.
89. Al-Jamal WT, Al-Jamal KT, Bomans PH, Frederik PM, Kostarelos K. Functionalized-quantum-dot-liposome hybrids as multimodal nanoparticles for cancer. Small 2008;4:1406-15.
90. Dudu V, Ramcharan M, Gilchrist ML, Holland EC, Vazquez M. Liposome delivery of quantum dots to the cytosol of live cells. J Nanosci Nanotechnol 2008;8:2293-300.
91. Weng KC, Noble CO, Papahadjopoulos-Sternberg B, Chen FF, Drummond DC, Kirpotin DB, et al. Targeted tumor cell internalization and imaging of multifunctional quantum dot-conjugated immunoliposomes in vitro and in vivo. Nano Lett 2008;8:2851-7.
92. Al-Jamal WT, Al-Jamal KT, Tian B, Cakebread A, Halket JM, Kostarelos K. Tumor targeting of functionalized quantum dot-liposome hybrids by intravenous administration. Mol Pharmaceutics 2009;6:520-30.
93. Chu MQ, Zhuo S, Xu J, Sheng QN, Hou SK, Wang RF. Liposome-coated quantum dots targeting the sentinel lymph node. J Nanopart Res 2010;12:187-97.
94. Pan J, Wang Y, Feng SS. Formulation, characterization, and in vitro evaluation of quantum dots loaded in poly (lactide)-vitamin E TPGS nanoparticles for cellular and molecular imaging. Biotechnol Bioeng 2008;101:622-33.
95. Cheng FY, Wang SP, Su CH, Tsai TL, Wu PC, Shieh DB, et al. Stabilizer-free poly(lactide-co-glycolide) nanoparticles for multimodal biomedical probes. Biomaterials 2008;29:2104-12.
96. Kuo WS, Hwang SM, Sei HT, Ku YC, Hsu LF, Cheng FY, et al. Stabilizer-free poly(lactide-co-glycolide) nanoparticles conjugated with quantum dots as a potential carrier applied in human mesenchymal stem cells. J Chin Chem Soc 2009;56:940-8.
97. Lee PW, Hsu SH, Tsai JS, Chen FR, Huang PJ, Ke CJ, et al. Multifunctional core-shell polymeric nanoparticles for transdermal DNA delivery and epidermal Langerhans cells tracking. Biomaterials 2010;31:2425-34.
98. Guo G, Liu W, Liang J, Xu H, He Z, Yang X. Preparation and characterization of novel CdSe quantum dots modified with poly (D,L-lactide) nanoparticles. Mater Lett 2006;60:2565-8.
99. Gong YJ, Gao MY, Wang DY, Möhwald H. Incorporating fluorescent CdTe nanocrystals into a hydrogel via hydrogen bonding: toward fluorescent microspheres with temperature-responsive properties. Chem Mater 2005;17:2648-53.
100. Hasegawa U, Nomura SM, Kaul SC, Hirano T, Akiyoshi K. Nanogel-quantum dot hybrid nanoparticles for live cell imaging. Biochem Biophys Res Commun 2005;331:917-21.
101. Salcher A, Nikolic MS, Casado S, Vélez M, Weller H, Juàrez BH. CdSe/CdS nanoparticles immobilized on pNIPAm-based microspheres. J Mater Chem 2010;20:1367-74.
102. Wu WT, Zhou T, Shen J, Zhou SQ. Optical detection of glucose by CdS quantum dots immobilized in smart microgels. Chem Commun 2009:4390-2.
103. Tan W, Zhang Y. Multifunctional quantum-dot-based magnetic chitosan nanobeads. Adv Mater 2005;17:2375-80.
104. Nie Q, Tan W, Zhang Y. Synthesis and characterization of monodisperse chitosan nanoparticles with embedded quantum dot. Nanotechnology 2006;17:140-4.
105. Tan W, Huang N, Zhang Y. Ultrafine biocompatible chitosan nanoparticles encapsulating multi-coloured quantum dots for bioapplications. J Colloid Interface Sci 2007;310:464-70.
106. Tan W, Jiang S, Zhang Y. Quantum-dot based nanoparticles for targeted silencing of HER2/neu gene via RNA interference. Biomaterials 2007;28:1565-71.
107. Yuan Q, Hein S, Misra RDK. New generation of chitosan-encapsulated ZnO quantum dots loaded with drug:synthesis, characterization and in vitro drug delivery response. Acta Biomaterialia 2010;6:2732-9.
108. Wang YQ, Chen HY, Ye C, Hu YZ. Synthesis and characterization of CdTe quantum dots embedded gelatin nanoparticles via a two-step desolvation method. Mater Lett 2008;62:3382-4.
109. Wang YQ, Ye C, Wu LH, Hu YZ. Synthesis and characterization of self-assembled CdHgTe/gelatin nanospheres as stable near infrared fluorescent probes in vivo. J Pharm Biomed Anal 2010;53:235-42.
110. Jia N, Lian Q, Shen H, Wang C, Li X, Yang Z. Intracellular delivery of quantum dots tagged antisense oligodeoxynucleotides by functionalized multiwalled carbon nanotubes. Nano Lett 2007;7:2976-80.
111. Shi D, Guo Y, Dong Z, Lian J, Wang W, Liu G, et al. Quantum-dot-activated luminescent carbon nanotubes via a nano scale surface functionalization for in vivo imaging. Adv Mater 2007;19:4033-7.
112. Guo Y, Shi D, Cho HS, Dong Z, Kulkarni A, Pauletti GM, et al. In vivo imaging and drug storage by quantum-dot-conjugated carbon nanotubes. Adv Funct Mater 2008;18:2489-97.
113. Shi D, Cho HS, Huth C, Wang F, Dong ZY, Pauletti GM, et al. Conjugation of quantum dots and Fe_3O_4 on carbon nanotubes for medical diagnosis and treatment. Appl Phys Lett 2009;223702:95.
114. Liu W, He Z, Liang J, Zhu Y, Xu H, Yang X. Preparation and characterization of novel fluorescent nanocomposite particles:CdSe/ZnS core-shell quantum dots loaded solid lipid nanoparticles. J Biomed Mater Res A 2008;84:1018-25.
115. Schroeder JE, Shweky I, Shmeeda H, Banin U, Gabizon A. Folate-mediated tumor cell uptake of quantum dots entrapped in lipid nanoparticles. J Control Release 2007;124:28-34.
116. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release 2001;70:1-20.
117. Nehilla BJ, Allen PG, Desai TA. Surfactant-free, drug-quantum-dot coloaded poly(lactide-co-glycolide) nanoparticles: towards multifunctional nanoparticles. ACS Nano 2008;2:538-44.
118. Gao XL, Chen J, Chen JY, Wu BX, Chen HZ, Jiang XG. Quantum dots bearing lectin-functionalized nanoparticles as a platform for in vivo brain imaging. Bioconjugate Chem 2008;19:2189-95.
119. Pan J, Feng SS. Targeting and imaging cancer cells by Folate-decorated, quantum dots (QDs)-loaded nanoparticles of biodegradable polymers. Biomaterials 2009;30:1176-83.
120. Fu HK, Kuo SW, Huang CF, Chang FC, Lin HC. Preparation of the stimuli-responsive ZnS/PNIPAM hollow spheres. Polymer 2009;50:1246-50.
121. Papagiannaros A, Levchenko T, Hartner W, Mongayt D, Torchilin V. Quantum dots encapsulated in phospholipid micelles for imaging and quantification of tumors in the near-infrared region. Nanomed Nanotechnol Biol Med 2009;5:216-24.
122. Nurunnabi M, Cho KJ, Choi JS, Huh KM, Lee YK. Targeted near-IR QDs-loaded micelles for cancer therapy and imaging. Biomaterials 2010;31:5436-44.
123. Raemdonck K, Demeester J, Smedt SD. Advanced nanogel engineering for drug delivery. Soft Matter 2009;5:707-15.
124. Fukui T, Kobayashi H, Hasegawa U, Nagasawa T, Akiyoshi K, Ishikawa I. Intracellular delivery of nanogel-quantum dot hybrid nanoparticles into human periodontal ligament cells. Drug Metab Lett 2007;1:131-5.
125. Wu WT, Aiello M, Zhou T, Berliner A, Banerjee P, Zhou SQ. In-situ immobilization of quantum dots in polysaccharide-based nanogels for integration of optical pH-sensing, tumor cell imaging, and drug delivery. Biomaterials 2010;31:3023-31.
126. Zhang P, Liu G. ZnO QD@PMAA-co-PDMAEMA nonviral vector for plasmid DNA delivery and bioimaging. Biomaterials 2010;31:3087-94.
127. Zhang H, Yee D, Wang C. Quantum dots for cancer diagnosis and therapy: biological and clinical perspectives. Nanomedicine 2008;3:83-91.

128. van Tilborg GAF, Mulder WJM, Chin PTK, Storm G, Reutelingsperger CP, Nicolay K, et al. Annexin A5-conjugated quantum dots with a paramagnetic lipidic coating for the multimodal detection of apoptotic cells. Bioconjug Chem 2006;17:865-8.
129. Santra S, Yang HS, Holloway PH, Stanley JT, Mericle RA. Synthesis of water dispersible fluorescent, radio-opaque, and paramagnetic CdS : Mn/ZnS quantum dots: a multifunctional probe for bioimaging. J Am Chem Soc 2005;127:1656-7.
130. Mulder WJM, Koole R, Brandwijk RJ, Storm G, Chin PTK, Strijkers GJ, et al. Quantum dots with a paramagnetic coating as a bimodal molecular imaging probe. Nano Lett 2006;6:1-6.
131. Jin T, Yoshioka Y, Fujii F, Komai Y, Seki J, Seiyama A. Gd^{3+}-functionalized near-infrared quantum dots for in vivo dual modal (fluorescence/magnetic resonance) imaging. Chem Commun 2008: 5764-6.
132. Cormode DP, Skajaa T, van Schooneveld MM, Koole R, Jarzyna P, Lobatto ME, et al. Nanocrystal core high-density lipoproteins: a multimodality contrast agent platform. Nano Lett 2008;8:3715-23.
133. Lim YT, Cho MY, Kang JH, Noh YW, Cho JH, Hong KS, et al. Perfluorodecalin/[InGaP/ZnS quantum dots] nanoemulsions as ^{19}F MR/optical imaging nanoprobes for the labeling of phagocytic and nonphagocytic immune cells. Biomaterials 2010;31:4964-71.
134. Cai WB, Chen K, Li Z, Gambhir SS, Chen X. Dual-functional probe for PET and near-infrared fluorescence imaging of tumor vasculature. J Nucl Med 2007;48:1862-70.
135. Ducongé F, Pons T, Pestourie C, Hérin L, Thézé B, Gombert K, et al. Fluorine-18-labeled phospholipid quantum dot micelles for in vivo, multimodal imaging from whole body to cellular scales. Bioconjugate Chem 2008;19:1921-6.
136. Chen K, Li ZB, Wang H, Cai WB, Chen XY. Dual-modality optical and positron emission tomographyimaging of vascular endothelial growth factor receptor on tumor vasculature using quantum dots. Eur J Nucl Med Mol Imaging 2008;35:2235-44.
137. Kim J, Lee JE, Lee SH, Yu J, Lee JH, Park TG, et al. Designed fabrication of a multifunctional polymer nanomedical platform for simultaneous cancer-targeted imaging and magnetically guided drug delivery. Adv Mater 2008;20:478-83.
138. Cho HS, Dong ZY, Pauletti GM, Zhang JM, Xu H, Gu HC, et al. Fluorescent, superparamagnetic nanospheres for drug storage, targeting, and imaging: a multifunctional nanocarrier system for cancer diagnosis and treatment. ACS Nano 2010;4:5398-404.
139. Park JH, von Maltzahn G, Ruoslahti E, Bhatia SN, Sailor MJ. Micellar hybrid nanoparticles for simultaneous magnetofluorescent imaging and drug delivery. Angew Chem Int Ed Engl 2008;47: 7284-8.
140. Yang ST, Cao L, Luo PG, Lu FS, Wang X, Wang HF, et al. Carbon dots for optical imaging in vivo. J Am Chem Soc 2009;131: 11308-9.
141. Sun YP, Zhou B, Lin Y, Wang W, Fernando KAS, Pathak P, et al. Quantum-sized carbon dots for bright and colorful photoluminescence. J Am Chem Soc 2006;128:7756-7.
142. Bourlinos AB, Stassinopoulos A, Anglos D, Zboril R, Karakassides M, Giannelis EP. Surface functionalized carbogenic quantum dots. Small 2008;4:455-8.
143. Zhao QL, Zhang ZL, Huang BH, Peng J, Zhang M, Pang DW. Facile preparation of low cytotoxicity fluorescent carbon nanocrystals by electrooxidation of graphite. Chem Commun 2008:5116-8.
144. Sokolov I, Naik S. Novel fluorescent silica nanoparticles:towards ultrabright silica nanoparticles. Small 2008;4:934-9.
145. Shiohara A, Hanada S, Prabakar S, Fujioka K, Lim TH, Yamamoto K, et al. Chemical reactions on surface molecules attached to silicon quantum dots. J Am Chem Soc 2010;132:248-53.
146. Xiong HM, Xu Y, Ren QG, Xia YY. Stable aqueous ZnO@polymer core-shell nanoparticles with tunable photoluminescence and their application in cell imaging. J Am Chem Soc 2008;130: 7522-3.