

Functional Quantum Dots for Promising Cancer Diagnosis and Therapy

Dongxiu He^{1,2#}, Danxia Wang^{2#}, Wenjie Quan² and Cui-yun Yu^{1,2,*}

¹Hunan Province Cooperative Innovation Center for Molecular Target New Drug Study, Hengyang, 421001, China

²Institute of Pharmacy & Pharmacology, Department of Pharmacy, University of South China, Hengyang, 421001, China

[#]Contributed equally.

Corresponding author: CY Yu, Hunan Province Cooperative Innovation Center for Molecular Target New Drug Study, Hengyang, 421001, China, Tel: +86 734 8282614; fax: +86 734 8282914; **E-mail:** yucuiyunusc@aliyun.com; yucuiyunusc@hotmail.com

Received date: 7 July 2015; **Accepted date:** 29 July 2015; **Published date:** 5 August 2015.

Citation: He D, Wang D, Quan W, Yu C (2015) Functional Quantum Dots for Promising Cancer Diagnosis and Therapy. *J Nanomed Nanosurg* 1(1): doi <http://dx.doi.org/10.16966/2470-3206.103>

Copyright: © 2015 He D, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract:

Functional nanoparticles are considered to have the potential as novel molecular probes for both carcinoma imaging and targeting delivery, which plays a critical role in cancer diagnosis and therapy. Functional quantum dots (QDs), conjugated with types of targeting ligands or anticancer drug/gene to simultaneously cancerous image, treat cancer through selective binding to the receptors over-expressed on cancerous cells and tissue surface, were introduced. A series of significant progress in the fields of the fabrication of functional QDs and their applications in cancer diagnosis and therapy were summarized in this review.

Keywords: Functional quantum dots; Fabrication; Applications; Cancer diagnosis; Cancer therapy

Introduction

The diagnosis and treatment of cancer remains a key challenge for biomedical technology. The diagnosis and therapy of cancer at the cellular level will be greatly improved with the development of techniques that enable the delivery of molecular probes and therapeutic agents into cells and cellular compartments. Functional nanoparticles, one of the cutting-edge materials of the twenty-first century, are considered to have the potential as novel molecular probes for both imaging and targeting delivery, which plays a critical role in biomedicine. One major merit of using functional nanoparticles is that one can control and tailor properties in a very predictable manner to meet the needs of specific application. Recently, functional nanoparticles have received great interest in biomedical applications [1], especially their future application in the field of carcinoma diagnosis and therapy [2-5].

QDs are semiconductor inorganic nonmaterials ranging from 1-10 nm. QDs, containing elements found in groups II-IV (eg, CdSe, CdTe, CdS, and ZnSe) or III-V (eg, InP and InAs) of the periodic table, have shown great potential interest to medical scientists because of their unique advantages over traditional fluorescent dyes, such as broad excitation spectra, narrow and symmetric photoluminescence bands, large two-photon absorption cross-section, size-tunable absorption and photoluminescence spectra, exceptional photo stability, high quantum yield, and versatility in surface modification [3]. QDs have initially been used for carcinoma cell imaging. Additionally, QDs provide a versatile nanoscale scaffold for designing multifunctional nanoparticles with both imaging and drug delivery vehicles [4]. Herein, QDs, conjugated with types of targeting ligands or anticancer drug/gene to simultaneously cancerous image, cancer treatment through selective binding to the receptors over-expressed on cancerous cells and tissue surface, have the potential to considerably improve the efficiency of fluorescence imaging and target delivery [6-7]. This article provides a brief review on the recent developments of the fabrication of functional QDs and promising applications in cancerous image and cancer treatment.

Fabrication of functional QDs

Although functional QDs as contrast agent or drug delivery vehicle

brings about many benefits to us, its fabrication is still a big challenge, because an ideal contrast agent or drug delivery vehicle must meet the need of increasing imaging or curative effect and simultaneously reducing the unintended severely side effects. In addition, to gather more information during specific cancer imaging or target delivery, QDs must be coupled with molecules which have the capabilities of recognizing the target. Surface chemistry modifications on the QDs are necessary for biomedical application. These surface modifications can also reduce the nonspecific binding, help prevent aggregation, and be critical to achieving specific target imaging and delivery. However, they often reduce the fluorescence quantum yield of the QDs.

Several recent studies have already discussed the synthesis and the biomedical imaging and targeting delivery applications of functional QDs [8-10]. There are many parameters that have to be taken into consideration and several fabrication methods that can be chosen in the design of the ideal functional QDs for cancer imaging (diagnosis) and targeting delivery (cancer therapy) (Figure 1). The main parameters are as following: i) the toxicity; ii) the fluorescence properties, including its quantum yield, its absorption cross-section, its lifetime, its resistance; iii) the ability to modify its surface chemistry without losing the QDs fluorescence; and iv) the specific target.

The toxicity of the functional QDs strongly depends on especially QDs' composition and surface chemistry [11]. The fluorescence properties of the functional QDs rely on the surface chemical modifications and properties of the QDs. The design to obtain low toxicity, ideal fluorescence, slow clearance and targeting as well as the strategies currently available to fabricate them will be firstly introduced.

Toxicity and fluorescence of the functional QDs

Both low toxicity and ideal fluorescence are the primary factors which should be taken into account. Many efficient attempts were made to reduce the toxicity and preserve a good fluorescence of the QDs.

One attempt to reduce the toxicity was to choose non-toxic and biodegradable nano-materials as drug/gene carriers and to find the substitutions for heavy metal-based QDs. Up to now, non-toxic elements

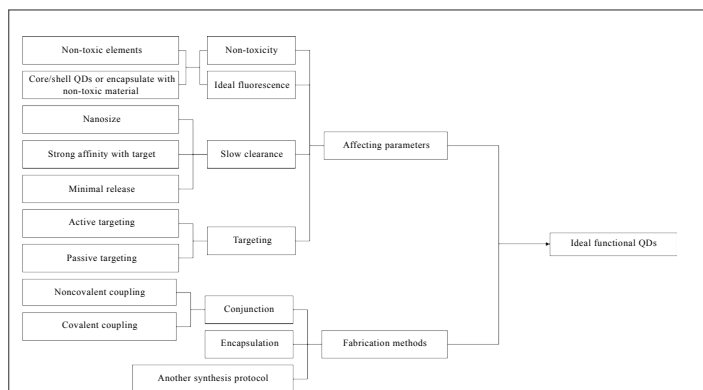


Figure 1: Schematic diagram of an ideal functional QDs used for diagnosis and therapy.

such as silica, zinc, sulfur and copper were often used in the synthesis of the QDs [5,12-14]. Due to the low toxicity, graphene quantum dots were also pursued as possible alternatives in the field of cancer imaging [15,16]. Nevertheless, low photoluminescence quantum yield was the main concern.

Another attempt to reduce the toxicity and preserve a good fluorescence of the functional QDs was developed to synthesize core/shell QDs or encapsulate QDs by non-toxic materials [11,16-24]. QDs encapsulated in non-toxic materials including cross linked dendrimers [11], silica [11,16-19], nanogel [20,21], nucleotide [22], and copolymer [23,24] were verified to remarkably reduce the cytotoxicity of QDs. Moreover, shells or non-toxic materials offer platform for conjugating molecule for cell and tissue targeting. QDs became stable once silica shell formed on their surface and excess surfactants were removed [11,25]. PEGylated phospholipids anchoring onto carbon nanotube surface have shown better targeting efficacy, better entrapment in spleen and liver without any degradation and toxicity [26].

Slow clearance of the functional QDs

In general, emission in the near infrared (NIR) range (700-1000 nm) is characterized by far more penetration power than emission in the visible spectrum, thus making it possible to visualize the structure of living tissues *in vivo* [12-14,27]. Once a large particle enters the vasculature, it is likely to be removed through the reticuloendothelial system, however, if the size of the particles is small enough i.e., in nanosize (<100 nm) they would bypass this process. Additionally, there are possible interactions of functional QDs with blood components and stimulation of immune response. Herein, the functional QDs applied for deep tissues infrared imaging or targeting delivery must meet more criteria: near infrared emitting, nanosize, and strong affinity for their target and minimal release back into the blood or lymph circulation. Most of these functional QDs are coated with long chain PEG for minimization of non-specific adsorption and prolonged circulation [11].

Passive and active targeting of the functional QDs

The entry of functional QDs into the cell requires transit across the lipid bilayer. This may occur through endocytosis or receptor-mediated uptake, which are also known as passive or active transport. Passive targeting is much slower and less efficient than active targeting. When passive targeting is used, nanocomposites can be easily accumulated at tumor site with high concentration due to the pathophysiological differences between normal tissues and tumor tissues known as enhanced permeability and retention (EPR) effect [28]. While the functional QDs have been successfully active targeted to different tissues, such as blood or lymphatic vessel [12,29], or liver [30], it involves adding molecules

such as antibodies, peptides or other ligands to the QDs surface. Because tumor cells often express unique proteins on their membranes that can be identified by the specific ligand, antibody or targeting-peptide binding [31-33].

Recently, QDs conjugated with targeting moieties that include aptamer [6], lectin [23], hyaluronic acid [29], folic acid [34], transferrin [35], antibodies against tumor-specific antigens [36,37], were fabricated. Fabrication methods of the functional QDs for cancerous imaging and targeting delivery can be classified into several categories listed below.

Synthesis protocols

Conjunction: Conjunction, which can be grouped into two general strategies: noncovalent coupling [38] and covalent coupling [39,40], is a common fabrication method. Some functional QDs have been fabricated by noncovalent coupling such as electrostatic interactions [20] or π - π stacking interactions [31]. While more functional QDs have been prepared by noncovalent coupling [6,8,22,34,39,40]. For example, CdSe/ZnS-QDs coated with a polymer shell and covalently functionalized with streptavidin were attached onto pristine side-walls of CNTs by the surface amino groups of the streptavidin. Based on the strong affinity between biotin and streptavidin, these hydrophilic functional QDs was used as a multivalent intracellular fluorescent imaging in Jurkat T leukemia cells to which biotinylated mouse anti-human CD3 antibody bonded to surface CD3 receptors [41].

To fabricate the functional QDs, other intermediate molecules including 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and/or N-hydroxysuccinimide (NHS) have been involved [6, 11, 38]. QDs were first covalently coupled with aptamer specific for mutated MUC1 mucin overexpressed in ovarian carcinoma cells, followed by doxorubicin covalently coupled with QDs -MUC1 aptamer conjugates by EDC and NHS [6].

Encapsulation:

Encapsulation is a physical loading technique. The functional QDs for carcinoma imaging and targeting delivery were encapsulated in various materials such as silica [16-19,42,43], glycopolymer [23], micelle [12, 44], and liposome [45]. Among of them, silica has been applied most to encapsulate both hydrophobic and hydrophilic QDs because silica encapsulation has the following advantages: dramatically reducing non-specific adsorption on the QDs, reducing the release of harmful ions, and decreasing environmental interferences on the brightness of QDs [19,43,46,47]. Silica encapsulation method studies recently focused on sol-gel process (so-called Stöber method) [46,47] and reverse microemulsion method [43].

Another synthesis protocol

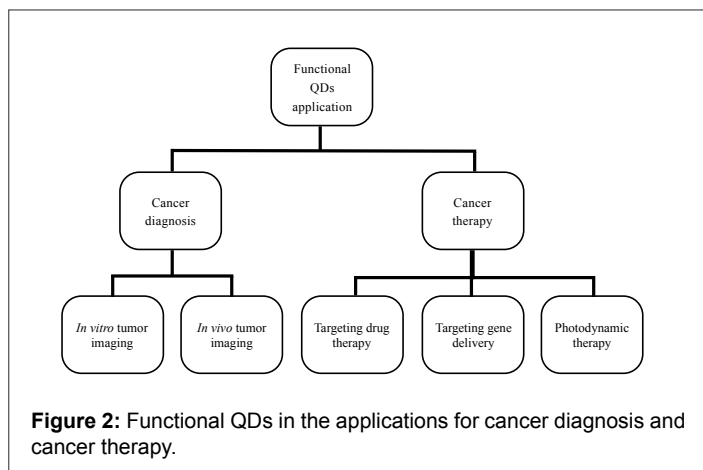
Wu's group reported that the hybrid nanogels were prepared by in-situ immobilization of CdSe QDs in the interior of the pH and temperature dual responsive hydroxypropylcellulose-poly (acrylic acid) (HPC-PAA) semi-interpenetrating polymer networks [21].

Applications of functional QDs

Over the past decade, functional QDs have been used in many different aspects of biomedical field. First used for bioimaging [6,22], they later became useful tools for bioanalysis [1], then biolabeling and medical therapy [2-5]. Here, we will focus on the two main applications of functional QDs: cancer diagnosis and cancer therapy (Figure 2).

Cancer diagnosis applications

Early diagnosis plays an important role in cancerous diagnosis. In most cases, detection of stage I cancers is associated with a higher than



90% 5-year survival rate [48] due to availability of curative treatment. Fluorescence labeling/imaging techniques are especially valuable tools in the arsenal of clinical diagnosis. Functional QDs have been wide used for improving fluorescence imaging [6,22,37,38] due to the photochemical stability and high fluorescence intensity of QDs.

In vitro tumor imaging: One of the most advancing applications of functional QDs is *in vitro* imaging of cancer cells. Many research groups applied functional QDs for *in vitro* fluorescence imaging of human cancer cells derived from ovarian carcinoma [6], melanoma [22], breast cancer [36], pancreatic cancer [37], glioblastoma [38], ovarian epidermoid carcinoma [49], lung adenocarcinoma [50], and hepatocellular carcinoma [24,51].

Zhang and his colleagues [36] found that QDs conjugated with anti-type 1 insulin-like growth factor receptor (IGF1R) is a promising candidate for targeting and imaging in breast cancer cells. The key in this targeting was the detection of up-regulated IGF1R in MCF-7 breast cancer cells by QD-anti-IGF1R conjugate.

Epidermal growth factor receptor (EGFR or HER1) or human epidermal growth factor 2 (HER2) is transmembrane glycoprotein that constitutes one of four members of the erbB family of tyrosine kinase receptors, is over-expressed in many cancers and can bind to epidermal growth factor (EGF) [52]. Based on that, the QD-EGF conjugate can be utilized for various cancer cells fluorescence imaging because EGFR is over-expressed in many cancers. Nakane and coworkers [53] reported most KPL-4 cells (breast cancer cells) were stained by the anti-HER2 antibody conjugated PbS QDs, whereas only very weak fluorescence signals were observed when stained with bovine serum albumin (BSA) conjugated PbS QDs after the incubation of anti-HER2 antibody conjugated PbS QDs and BSA-conjugated PbS QDs. This result clearly shows the specific targeting imaging ability of PbS QDs to HER2 surface receptors in KPL-4 cells.

Kawashima et al. [49] also explored intermolecular interactions involved in the lateral propagation of cell-signaling by EGFR single-molecules in human ovarian epidermoid carcinoma cells (A431) using nanocomposites loaded CdSe/ZnS QDs. Kawashima found that CHO and A431 cells were efficiently labeled by QD-EGF conjugates due to the specific binding of EGF to EGFR.

In research by Yong [37], they selectively detected human pancreatic cancer cells using QDs conjugated with anti-Claudin-4 antibody and anti-prostate stem cell antigen (anti-PSCA). These conjugates were recognized by the membrane proteins Claudin-4 and PSCA which are over-expressed in both primary and metastatic pancreatic cancer cells. This result clearly shows fluorescence images of human pancreatic cancer cells (MiaPaCa) treated with various nanocomposites loaded QDs. From these results, it

clearly showed that the nanocomposites loaded InP/ZnS QDs can serve as a potential biocompatible targeted nanoprobe to specifically diagnose human pancreatic cancer cells.

In vivo tumor imaging: The basic principles underlying *in vitro* targeting of cancer cells can be applied *in vivo*. However, *in vivo* applications of functional NIR QDs are more complicated and challenging. One main challenge for *in vivo* targeting and imaging using the functional QDs is their biodistribution and pharmacokinetics. Additionally, the type and structure of organic/bioorganic shells of QDs determine their biocompatibility and are crucial for their application in imaging *in vivo*, due to the effects of the shell on the following properties: colloidal stability, solubility in physiological fluids, influence of the basic physiological parameters, and cytotoxicity [11]. Chen *et al.* have monitored the dynamic distribution of CdHgTe/SiO₂ nanocomposites *in vivo* by near infrared fluorescence imaging system. They also show that CdHgTe/SiO₂ nanocomposites acted as a novel fluorescence probe have a maximum fluorescence emission of 785 nm and high photo-stability and the hydrodynamic diameter of CdHgTe/SiO₂ nanocomposites could be adjusted to 122.3 nm [54]. Another main challenge for *in vivo* targeting and imaging is the fluorescence emitting property of the functional QDs. Visible emitting QDs provide poor signal-to background ratio in deep tissue and when imaging targets in small animals [23]. While NIR QDs offer several advantages for the non-invasive visualization of living tissues because of its deeper photon penetration, low absorption and scattering. So, functional NIR QDs are considered to have the potential as novel probe for tumor diagnostics, for example, carcinoma cell labeling or tracking [55-57], tumor markers targeted imaging [58,59], tumor vessel imaging [60,61].

Carcinoma cell labeling or tracking in living organisms was monitored efficiently and sensitively, which may provide tools to locate tumors and metastases or map tumor margins during surgery. This could be achieved by functional QDs, then administrating conjugated QDs for *in vivo* targeted imaging of various human cancer cells such as oral squamous carcinoma cell [52], brain cancer [55], hepatocarcinoma cell [56], and colon carcinoma cell [57]. Fang *et al.* reported that *in vivo* fluorescence imaging of a breast tumor in a mouse were taken through anti-HER2 antibody conjugated PbS QDs injected into the tail vein. Immediately after the injection of QDs, 2nd-NIR fluorescence signals for the QDs were observed over the whole body with time: after about 1 h QDs were detected in the liver and after 48 h the breast tumor was clearly detected [51]. Yu *et al.* subcutaneously implanted HCCLM6 cancer cells in mice, and intravenously injected the QDs conjugated with an antibody to alpha-fetoprotein (anti-AFP). The QD-anti-AFP conjugate was effectively accumulated in human hepatocarcinoma cells, which have demonstrated that the QD-anti-AFP conjugate was an ideal candidate for *in vivo* targeted imaging of HCCLM6 human hepatocarcinoma cells [56].

Tumor markers play a critical role in the detection and diagnosis of cancer [58]. Biomarker assays may be useful for screening and diagnosis of cancer if a set of tumor markers can be quantified and statistically differentiated between cancerous cells and normal cells [58].

Angiogenesis, the formation of new blood vessels from preexisting vasculature, is essential for tumor growth and progression. Integrin $\alpha\beta_3$, which binds to RGD-containing components of the interstitial matrix, plays a key role in tumor angiogenesis and metastasis [60]. Functional QDs represent excellent tool for tumor vessel imaging [61] or multimodal molecular imaging of angiogenesis [62].

Cancer therapy applications

Targeting drug therapy applications: Recently, targeted therapy has been widely explored. Drug/gene targeting by nanoparticles or nanocapsules offers the following enormous advantages, as examples:

reduces dosage, ensures the pharmaceutical effects, and minimizes side-effects, and enhances drug stability. QDs are newer luminescent nanoparticles with rich surface chemistry and unique optical properties that make them useful as visualization probes or carriers for traceable targeted delivery and therapy applications [63]. Thus, it has been developed functional QDs that can perform imaging and drug delivery tasks without the need for external dyes [64]. We will briefly describe developments of functional QDs as probes and carriers in targeting drug delivery applications.

By directly noncovalent coupling or covalent coupling drug molecules to the QD surface, drug-conjugated QDs can be delivered to specific sites and subsequently release drug molecules from the QD surface in response to local biological conditions such as pH or the presence of enzymes. Several research groups have demonstrated the integration of drug molecules with conjugated QDs and targeting moieties for targeting drug delivery *in vitro* and *in vivo*. Chakravarthy *et al.* [65] found that doxorubicin (Dox) can be effectively released from the functional CdSe/CdS/ZnS QDs conjugated with Dox and accumulated in the cell nucleus. They also demonstrated that the functional QDs can provide targeted macrophage-selective therapy for the treatment of pulmonary disease. And, several researches recently suggested that QDs conjugated therapeutic antibody [66] or drug [67] can be used as a suitable nanoparticles for breast cancer treatment. Xu *et al.* [66] demonstrated for the first time that the functional QDs conjugated with anti-GRP78 scFv could be efficiently internalized by cancer cells, thus up-regulate phosphatase-AKT-ser473 and possess biological anti-tumor activity by inhibition of breast cancer growth in a xenograft model. Fibrinogen (fib) coated CdTe/ZnTe and paclitaxel (PTX) [PTX-fib-QDs] can target MCF-7 cells and effectively deliver PTX towards breast cancer cells via the $\alpha 5\beta 1$ -integrins [67]. Furthermore, Jeyadevia's study revealed that using TGA-CdTe QDs as nanocarrier of quercetin could enhance the anti-arthritis effect of quercetin even at a lower concentration of the drug in rheumatic complications [68].

All these studies have laid an important foundation in the functional QDs as probes and carriers for target drug delivery research and applications.

Targeting gene delivery applications: Gene therapy has emerged as a powerful strategy for cancer treatment over the past several decades because of the genetic link associated with tumor development and progression. The delivery of nucleic acid therapeutics to down-regulate or replace mutated genes, and to silence unexpected gene expression, is becoming an attractive approach to suppressing tumor cell growth and invasion. There have been intensive efforts to develop safe and efficient gene delivery carriers to provide high transfection efficiency at the desired target.

Besides the delivery of small molecule drugs, QDs have also been proved to have prospect for delivery of more intricate genes, such as small interfering RNA (siRNA). The short and double-stranded therapeutic siRNA works by silencing the expression of unwanted, disease-causing genes. Nevertheless, free forms of them owe high negative charge and are easy to degrade in body environment. Thus, in order to achieve optimal function in physiological conditions, they must be delivered via combination with cationic nanocarriers. Because of appropriately surface functionalized with cationic moieties, nanocomposites including QDs are good choices of siRNA carriers as they not only render these genetic drugs with physiological stability and target specificity, but also the entire nanocomposites can be optically traced. Therefore, nanocomposites loaded with QDs were specifically designed to overcome barriers in siRNA delivery such as siRNA protection, cellular penetration, endosomal release, carrier unpacking, intracellular transport and gene silencing. Li *et al.* confirmed that functional QDs could efficiently deliver siRNA into HeLa cells and

silence a target gene, and the nanocomposites loaded with QDs could also be used as fluorescence probes, allowing real-time tracking and localization of QDs during delivery and transfection *in vivo* [69]. More importantly, multifunctional QDs have been demonstrated to deliver an active siRNA to knockdown EGFRvIII receptors in human glioma cells, and subsequently monitor the resulting down-regulated signaling pathway with high efficiency [70], suggesting that functional QDs could be designed to deliver gene to a specified target cell type.

Photodynamic therapy of cancer: Photodynamic therapy (PDT) is still considered to be a new and promising invasive antitumor strategy that can exert a selective cytotoxic activity toward malignant cells. The antitumor strategy involves administration of a photosensitizing (PS) agent followed by irradiation at a wavelength corresponding to an absorbance band of the photosensitizing agent. Clinical studies revealed that PDT can be curative, particularly in early stage tumors because sensitizer may lead to direct tumor cell death, damage to the microvasculature, and induction of a local inflammatory reaction [71]. QDs can be designed to have fluorescence peaks at different wavelengths by adjusting their size. The unique property of the QDs allows QDs to be fabricated for specific functions such as Fluorescence Resonance Energy Transfer (FRET), in which a specific emission wavelength can transfer energy to drug molecules or photosensitizers. Consequently, FRET phenomenon can be potentially used for the development of novel therapeutic approaches for cancer treatment. Additionally, photo stability of QDs is the most promising property for PDT. Recent reports showed that QDs can be used in photodynamic and radiation therapy of cancer [72,73].

Although targeted delivery of QDs in cancer cells and tumor milieu by using QDs conjugated with anticancer antibodies or other bio molecules have become possible recently, compared with conventional PS drugs such as porphyrins and phthalocyanines, the efficiency of QDs to produce ROI under direct photoactivation is low. Herein, preparation of conjugates between QDs and conventional PS drugs, investigation of energy transfer efficiencies from QDs to PS drugs and ROI production by the conjugates are being widely investigated.

Conclusions

Real-time imaging and targeting delivery have been hot topics in cancerous diagnosis and therapy fields. Current investigation of the functional QDs *in vitro* and *in vivo* has offered less invasive imaging and targeting drug delivery. However, up to date the clinical applications of the functional QDs have been limited due to side-effects. Various novel nanoparticles such as CNTs have been explored with recent developments in the preparation and characterization techniques of nanoparticles. The emergence of functional QDs, targeting moieties, and other nanoparticles enabled to improve imaging and targeting delivery applications because of their better biocompatibility, lower toxicity and longer circulation time *in vivo*, which were better applied for high-resolution noninvasive cancer diagnosis and therapy.

Acknowledgements

The authors gratefully acknowledged financial supports from National Natural Science Foundation of China (81471777, 81102409) and financial supports from Natural Science Foundation of Hunan province (13JJ6096).

References

1. Irina YG, Elena SS, Valentina VG, Tang DP, Sarah DS (2015) Synthesis and bioanalytical applications of nanostructures multiloading with quantum dots. *Trends in Anal Chem* 66: 53-62.
2. Barbara B, Frank CJMVV, Boguslaw T (2013) Applications of Nanoparticles for MRI Cancer Diagnosis and Therapy. *J Nanomater* 2013: 12 pages.

3. Valizadeh A, Mikaeili H, Samiei M, Farkhani SM, Zarghami N, et al. (2012) Quantum dots: synthesis, bioapplications and toxicity. *Nanoscale Res Lett* 7: 480-494.
4. Liu Y, Miyoshi H, Nakamura M (2007) Nanomedicine for drug delivery and imaging: a promising avenue for cancer therapy and diagnosis using targeted functional nanoparticles. *Int J Cancer* 120: 2527-2537.
5. Yuan L, Tang Q, Yang D, Zhang J, Zhang F, et al. (2011) Preparation of pH-responsive mesoporous silica nanoparticles and their application in controlled drug delivery. *J Phys Chem C* 115: 9926-9932.
6. Ronak S, Oleh T, Olga G, Tamara M (2011) Tumor targeted quantum dot-mucin 1 aptamer-doxorubicin conjugate for imaging and treatment of cancer. *J Control Release* 153: 16-22.
7. Ye F, Asa B, Heba A, Manuchehr AV, Ibrahim ES, et al. (2014) Biodegradable polymeric vesicles containing magnetic nanoparticles, quantum dots and anticancer drugs for drug delivery and imaging. *Biomaterials* 35: 3885-3894.
8. Madani SY, Shabani F, Dwek MV, Seifalian AM (2013) Conjugation of quantum dots on carbon nanotubes for medical diagnosis and treatment. *Int J Nanomed* 8: 941-950.
9. Jin S, Hu Y, Gu Z, Liu L, Wu H (2011) Application of Quantum Dots in Biological Imaging. *J Nanomater* 2011: 13 pages.
10. Aswathy RG, Yoshida Y, Maekawa T, Kumar DS (2010) Near-infrared quantum dots for deep tissue imaging. *Anal Bioanal Chem* 397: 1417-1435.
11. Gao JH, Chen K, Xie R, Xie J, Lee S, et al. (2010) Ultrasmall near-infrared non-cadmium quantum dots for in vivo tumor imaging. *Small* 6: 256-261.
12. Erogbogbo F, Yong KT, Roy I, Hu R, Law WC, et al. (2010) In vivo targeted cancer imaging, sentinel lymph node mapping and multi-channel imaging with biocompatible silicon nanocrystals. *ACS Nano* 5: 413-423.
13. Karolian K, Przybyl M, Ziolczyk P, Sowinski P, Miller E (2014) Fluorescence properties of 3-amino phenylboronic acid and its interaction with glucose and ZnS: Cu quantum dots. *Spectrochim Acta A Mol Biomol Spectrosc* 129: 320-325.
14. Tan L, Huang C, Peng R, Tang Y, Li W (2014) Development of hybridorganic-inorganic surface imprinted Mn-doped ZnS QDs and their application as a sensing material for target proteins. *Biosens Bioelectron* 61: 506-511.
15. Chong Y, Ma Y, Shen H, Tu XL, Zhou X, et al. (2014) The in vitro and in vivo toxicity of graphene quantum dots. *Biomaterials* 35: 5041-5048.
16. Wen T, Yang B, Guo Y, Sun J, Zhao C, et al. (2014) Organosilane-functionalized graphene quantum dots and their encapsulation into bi-layer hollow silica spheres for bioimaging applications. *Phys Chem Chem Phys* 16: 23188-23195.
17. Zhu Y, Li Z, Chen M, Cooper HM, Xu ZP (2013) Tuning core-shell SiO₂@CdTe@SiO₂ fluorescent nanoparticles for cell labeling. *J Mater Chem B* 1: 2315-2323.
18. Huang Y, Hu L, Zhang T, Zhong H, Zhou J, et al. (2013) Mn₃[Co(CN)₆]₂@SiO₂ Core shell Nanocubes: Novel bimodal contrast agents for MRI and optical imaging. *Sci Rep-UK* 3: 2647-2654.
19. Vibin M, Vinayakan R, John A, Raji V, Rejiya CS, et al. (2010) Cytotoxicity and fluorescence studies of silica-coated CdSe quantum dots for bioimaging applications. *J Nanopart Res* 13: 2587-2596.
20. Li Z, Xu W, Wang Y, Shah BR, Zhang C, et al. (2015) Quantum dots loaded nanogels for low cytotoxicity, pH-sensitive fluorescence, cell imaging and drug delivery. *Carbohydr Polym* 121: 477-485.
21. Wu W, Aiello M, Zhou T, Berliner A, Banerjee P, et al. (2010) In-situ immobilization of quantum dots in polysaccharide-based nanogels for integration of optical pH-sensing, tumor cell imaging, and drug delivery. *Biomaterials* 31: 3023-3031.
22. Xiao Q, Qiu T, Huang S, Liu Y, He Z (2012) Preparation and biological effect of nucleotide-capped CdSe/ZnS quantum dots on *Tetrahymena thermophila*. *Biol Trace Elem Res* 147: 346-353.
23. Pei D, Li Y, Huang Q, Ren Q, Li F, et al. (2015) Quantum dots encapsulated glycopolymer vesicles: Synthesis, lectin recognition and photoluminescent properties. *Colloid Surface B* 127: 130-136.
24. Camblin M, Detampel P, Kettiger H, Wu D, Balasubramanian V, et al. (2014) Polymersomes containing quantum dots for cellular imaging. *Int J Nanomed* 9: 2287-2298.
25. Hu X, Zrazhevskiy P, Gao X (2009) Encapsulation of single quantum dots with mesoporous silica. *Ann Biomed Eng* 37: 1960-1966.
26. Schipper ML, Nakayama-Ratchford N, Davis CR, Kam NWS, Chu P, et al. (2008) A pilot toxicology study of single-walled carbon nanotubes in a small sample of mice. *Nat Nanotechnol* 3: 216-221.
27. Cassette E, Helle M, Bezdetnaya L, Marchal F, Dubertret B, et al. (2013) Design of new quantum dot materials for deep tissue infrared imaging. *Adv Drug Deliver Rev* 65: 719-731.
28. Jee JP, Na JH, Lee S, Kim SH, Choi K, et al. (2012) Cancer targeting strategies in nanomedicine: Design and application of chitosan nanoparticles. *Curr Opin Solid St M* 16: 333-342.
29. Bhang SH, Won N, Lee T, Jin H, Nam J, et al. (2009) Hyaluronic acid-quantum dot conjugates for in vivo lymphatic vessel imaging. *ACS Nano* 3: 1389-1398.
30. Kikkeri R, Lepenies B, Adibekian A, Laurino P, Seeberger PH (2009) In vitro imaging and in vivo liver targeting with carbohydrate capped quantum dots. *J Am Chem Soc* 131: 2110-2112.
31. Lammers T, Hennink WE, Storml G (2008) Tumour-targeted nanomedicines: principles and practice. *Brit J Cancer* 99: 392-397.
32. Yao NH, Xiao WW, Wang XB, Marik J, Park SH, et al. (2009) Discovery of targeting ligands for breast cancer cells using the one-bead one-compound combinatorial method. *J Med Chem* 52: 126-133.
33. Byrne JD, Betancourt T, Brannon-Peppas L (2008) Active targeting schemes for nanoparticle systems in cancer therapeutics. *Adv Drug Deliver Rev* 60: 1615-1626.
34. Zhao Y, Liu S, Li Y, Jiang W, Chang Y, et al. (2010) Synthesis and grafting of folate-PEG-PAMAM conjugates onto quantum dots for selective targeting of folate-receptor-positive tumor cells. *J Colloid Interf Sci* 350: 44-50.
35. Wang Y, Chen J, Yan X (2013) Fabrication of Transferrin Functionalized Gold Nanoclusters/Graphene Oxide Nanocomposite for Turn-On Near-Infrared Fluorescent Bioimaging of Cancer Cells and Small Animals. *Anal Chem* 85: 2529-2535.
36. Zhang H, Sachdev D, Wang C, Hubel A, Gaillard-Kelly M, et al. (2009) Detection and downregulation of type I IGF receptor expression by antibody-conjugated quantum dots in breast cancer cells. *Breast Cancer Res Tr* 114: 277-285.
37. Yong KT, Ding H, Roy I, Law WC, Bergey EJ, et al. (2009) Imaging pancreatic cancer using bioconjugated InP quantum dots. *ACS Nano* 3: 502-510.
38. Das A, Hall E, Wai CM (2014) Noncovalent Attachment of PbS Quantum Dots to Single- and Multiwalled Carbon Nanotubes. *J Nanotechnol* 2014: 1-7.
39. Li ZM, Huang P, He R, Lin J, Yang S, et al. (2010) Aptamer-conjugated dendrimer-modified quantum dots for cancer cell targeting and imaging. *Mater Lett* 64: 375-378.
40. Zhang YY, Qin W, Tang H, Yan F, Tan L, et al. (2011) Efficient assembly of multi-walled carbon nanotube-CdSe/ZnS quantum dot hybrids with high biocompatibility and fluorescence property. *Colloids Surf B Biointerfaces* 87: 346-352.
41. Bottini M, Cerignoli F, Dawson MI, Magrini A, Rosato N, et al. (2006) Full-length single-walled carbon nanotubes decorated with streptavidin-

- conjugated quantum dots as multivalent intracellular fluorescent nanoprobe. *Biomacromolecules* 7: 2259-2263.
42. Zhu Y, Li Z, Chen M, Cooper HM, Lu QGM (2012) Synthesis of robust sandwich-like SiO₂@CdTe@SiO₂ fluorescent nanoparticles for cellular imaging. *Chem Mater* 24: 421-423.
 43. Wang S, Li C, Yang P, Ando M, Murase N (2012) Silica encapsulation of highly luminescent hydrophobic quantum dots by two-step microemulsion method. *Colloids Surf A Physicochem Eng Asp* 395: 24-31.
 44. Papagiannaros A, Levchenko T, Hartner W, Mongayt D, Torchilin V (2009) Quantum dots encapsulated in phospholipid micelles for imaging and quantification of tumors in the near-infrared region. *Nanomed Nanotechnol* 5: 216-224.
 45. Chen CS, Yao J, Durst RA (2006) Liposome encapsulation of fluorescent nanoparticles: Quantum dots and silica nanoparticles. *J Nanopart Res* 8: 1033-1038.
 46. Yang P, Zhang L, Li X, Zhang Y, Liu N, et al. (2012) Surface modification of SiO₂ beads with multiple hydrophobic quantum dots for bioapplications. *J Non-Cryst Solids* 358: 3069-3073.
 47. Yang P, Murase N, Yu J (2011) SiO₂ beads with quantum dots: Preparation and stability investigation for bioapplications. *Colloids Surf A Physicochem Eng Asp* 385: 159-165.
 48. Weissleder R (2006) Molecular imaging in cancer. *Science* 312: 1168-1171.
 49. Kawashima N, Nakayama K, Itoh K, Itoh T, Ishikawa M, et al. (2010) Reversible dimerization of EGFR revealed by single-molecule fluorescence imaging using quantum dots. *Chemistry* 16: 1186-1192.
 50. Zhang C, Ji X, Zhang Y, Zhou G, Ke X, et al. (2013) One-Pot Synthesized Aptamer-Functionalized CdTe:Zn²⁺ Quantum Dots for Tumor-Targeted Fluorescence Imaging in Vitro and in Vivo. *Anal Chem* 85: 5843-5849.
 51. Fang M, Peng CW, Yuan JP, Zhang ZL, Pang DW et al. (2013) Coevolution of the tumor microenvironment revealed by quantum dot-based multiplexed imaging of hepatocellular carcinoma. *Future Oncol* 9: 1029-1037.
 52. Yang K, Zhang FJ, Tang H, Zhao C, Cao YA, et al. (2011) In-vivo imaging of oral squamous cell carcinoma by EGFR monoclonal antibody conjugated near-infrared quantum dots in mice. *Int J Nanomedicine* 6: 1739-1745.
 53. Nakane Y, Tsukasaki Y, Sakata T, Yasuda H, Jin T (2013) Aqueous synthesis of glutathione-coated PbS quantum dots with tunable emission for non-invasive fluorescence imaging in the second near-infrared biological window (1000-1400 nm). *Chem Commun* 49:7584-7586.
 54. Chen HY, Cui SS, Tu ZZ, Gu YQ, Chi XM (2012) In vivo Monitoring of Organ-Selective Distribution of CdHgTe/SiO₂Nanoparticles in Mouse Model. *J Fluoresc* 22: 699-706.
 55. Fatehi D, Baral TN, Abulrob A (2014) In Vivo Imaging of Brain Cancer Using Epidermal Growth Factor Single Domain Antibody Bioconjugated to Near-Infrared Quantum Dots. *J Nanosci Nanotechnol* 14: 5355-5362.
 56. Yu X, Chen L, Li K, Xiao S, Luo X, et al. (2007) Immunofluorescence detection with quantum dot bioconjugates for hepatoma in vivo. *J Biomed Opt* 12:014008.
 57. Diagaradjane P, Orenstein-Cardonna JM, Colon-Casanovas NE, Eeorukhkar A, Shentu S, et al. (2008) Imaging epidermal growth factor receptor expression in vivo: Pharmacokinetic and biodistribution characterization of a bioconjugated quantum dot nanoprobe. *Clin Cancer Res* 140: 731-741.
 58. Peng CW, Tian Q, Yang GF, Fang M, Zhang ZL, et al. (2012) Quantum-dots based simultaneous detection of multiple biomarkers of tumor stromal features to predict clinical outcomes in gastric cancer. *Biomaterials* 33: 5742-5752.
 59. Zhang L, Xia J, Zhao Q, Liu L, Zhang Z (2010) Functional graphene oxide as a nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs. *Small* 6: 537-544.
 60. Smith BR, Cheng Z, De A, Koh AL, Sinclair R, et al (2008) Real-time intravital imaging of RGD-quantum dot binding to luminal endothelium in mouse tumor neovasculature. *Nano Lett* 8: 2599-2606.
 61. Hu R, Yong KT, Roy I, Ding H, Law WC, et al. (2010) Functionalized near-infrared quantum dots for in vivo tumor vasculature imaging. *Nanotechnology* 21: 145105-145114.
 62. Mulder WJ, Strijkers GJ, Nicolay K, Griffioen AW (2010) Quantum dots for multimodal molecular imaging of angiogenesis. *Angiogenesis* 13: 131-134.
 63. Harush-Frenkel O, Altschuler Y, Benita S (2008) Nanoparticle-cell interactions: drug delivery implications. *Crit Rev Ther Drug Carrier Syst* 25: 485-544.
 64. Wen CJ, Sung CT, Aljuffali IA, Huang IA, Fang JY (2013) Nanocomposite liposomes containing quantum dots and anticancer drugs for bioimaging and therapeutic delivery: a comparison of cationic, PEGylated and deformable liposomes. *Nanotechnology* 24: 325101.
 65. Chakravarthy KV, Davidson BA, Helinski JD, Ding H, Law WC, et al. (2011) Doxorubicin-conjugated quantum dots to target alveolar macrophages and inflammation. *Nanomedicine* 7: 88-96.
 66. Xu W, Liu L, Brown NJ, Christian S, Hodrnby D (2012) Quantum Dot-Conjugated Anti-GRP78 scFv Inhibits Cancer Growth in Mice. *Molecules* 17: 796-808.
 67. Rejinold NS, Baby T, Nair SV, Jayakumar R (2013) Paclitaxel Loaded Fibrinogen Coated CdTe/ZnTe Core Shell Nanoparticles for Targeted Imaging and Drug Delivery to Breast Cancer Cells. *J Biomed Nanotechnol* 9: 1657-1671.
 68. Jeyadevi R, Sivasudha T, Rameshkumar A, Ananth DA, Aseeravatham GS, et al. (2013) Enhancement of anti arthritic effect of quercetin using thioglycolic acid-capped cadmium telluride quantum dots as nanocarrier in adjuvant induced arthritic Wistar rats. *Colloids Surf B: Biointerfaces* 112: 255-263.
 69. Li JM, Zhao MX, Su H, Wang YY, Tan CP, et al. (2011) Multifunctional quantum-dot-based siRNA delivery for HPV18 E6 gene silence and intracellular imaging. *Biomaterials* 32: 7978-7987.
 70. Jung JJ, Solanki A, Memoli KA, Dr KK, Kim H, et al. (2010) Selective inhibition of human brain tumor cell proliferation via multifunctional quantum dot-based siRNA delivery. *Angew Chem Int Edit Engl* 49: 103-107.
 71. Agostinis P, Berg K, Cengel MD K, Foster TH, Girotti AW, et al. (2011) Photodynamic therapy of cancer: An update. *CA-Cancer J Clin* 61: 250-281.
 72. Juzenas P, Chen W, Sun YP, Coelho MA, Generalov R, et al. (2008) Quantum dots and nanoparticles for photodynamic and radiation therapies of cancer. *Adv Drug Deliv Rev* 60: 1600-1614.
 73. Markovic ZM, Ristic BZ, Arsikin KM, Klisic DG, Harhaji-Trajkovic LM, et al. (2012) Graphene quantum dots as autophagy-inducing photodynamic agents. *Biomaterials*. 33: 7084-7092.