Using of Quantum Dots in Biology and Medicine

19

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Abstract

Quantum dots are nanoparticles, which due to their unique physical and chemical (first of all optical) properties, are promising in biology and medicine. There are many ways for quantum dots synthesis, both in the form of nanoislands self-forming on the surfaces, which can be used as single-photon emitters in electronics for storing information, and in the form of colloidal quantum dots for diagnostic and therapeutic purposes in living systems. The paper describes the main methods of quantum dots synthesis and summarizes medical and biological ways of their use. The main emphasis is laid on the ways of quantum dots surface modification. Influence of the size and form of nanoparticles, charge on the surfaces of quantum dots, and cover type on the efficiency of internalization by cells and cell compartments is shown. The main mechanisms of penetration are considered.

Keywords

Quantum dots · Nanoparticles · Synthesis · Core · Shell

19.1 Introduction

One of the most intensively developing directions of nanotechnology is synthesis and practical use of the quantum dots (QDs). QDs are the fluorescent semiconductor nanocrystals consisting of atoms elements II–IV or III–V groups of Mendeleev periodic table and having the size less than the radius of Bohr's exciton for this material [1, 2]. They have the properties of controllable photoluminescence due to the effect of dimensional quantization [3]. Particular interest in QDs is explained by considerable difference of their exciton discrete spectrum from a bulk crystal spectrum of the same chemical. This difference results in the change of QDs optical properties in comparison with the bulk material. In particular, depending on chemical composition and QDs size the emission band may be on any site of a

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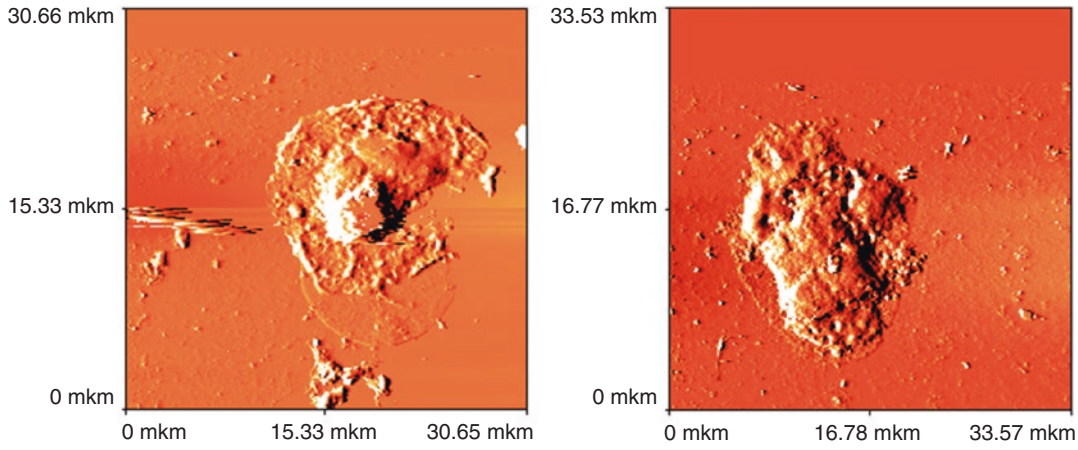

Fig. 19.1 The necrotic death of neutrophil granulocytes after incubation of cells with quantum dots CdSe/ZnS-MPA 620 (cells incubated with QDs in concentration 0.1 mg/ml for 30 min, after that fixed with glutaraldehyde 2.5%). Cells were scanned by atomic-force microscopy (NT-MDT)

spectrum from ultra-violet to infrared. This feature allows receiving marks of various colors for optical coding, to use QDs in optoelectronics, and apply them to study the structure of biological cells, by marking the different structure [4].

The progress in science and technology of QDs was observed after 1984 when Luís Brus received dependence between the size and width of the energy gap band for semiconductor nanoparticles [5]. Nearly a decade was necessary for further advance in the study of QDs. The results of these studies was the synthesis of colloidal QDs of CdX (X = S, Se, Te) with the reconstructed absorption band and emission. Up to now CdX are the most studied QDs having excellent optical and electrochemical properties. However, their use in biology and medicine was impossible because of toxicity of cadmium ion as a part of QDs core (Fig. 19.1).

To improve biocompatibility, and quantum yield of fluorescence as well as stability of these nanocrystals, they were encapsulated with the formation of nanocrystals of core-shell type. Efficiency of luminescence was considerably improved due to passivation on QDs surface of the semiconductor with a large of an energy gap band due to which leaching of metal ions from a core was blocked by this structure [5]. At first QDs of CdSe/ZnS and CdSe/CdS were most intensively studied. Later, many other QDs of "core-shell" type, such as CdSe/ZnSe, CdTe/CdS, CdTe/ZnS, and even CdTe/CdS/ZnS "core/shell/shell" were developed. In traditional QDs, cadmium is the main element of the composition. Nevertheless, it is well known that leakage of cadmium ions is the main cause of QDs cytotoxicity on the basis of cadmium which complicates their further use in vivo or in vitro. With increase in demand for more biocompatible QDs, there was a shift of priority towards synthesis of cadmium-free QDs, allowing their use in biology. QDs containing silicon (Si-QDs), containing carbon (C-QDs), graphene quantum dots (GQDs), Ag_2Se, Ag_2S, InP, $CuInS_2/ZnS$ allowing their use as luminescent probes for biosensors and bioimaging were developed.

19.2 Synthesis of QDs

QDs can be formed on a surface by a method of molecular-beam epitaxy growing with the subsequent etching. Such structures are used in semiconductor electronics as optical converters in light-emitted diode sources and photovoltaic cells [6]. However, obviously only colloidal QDs are used for biomedical studies and practical used. In solutions QDs are stabilized due to the ligands covering them, and depending on ligand structure they form either organic or

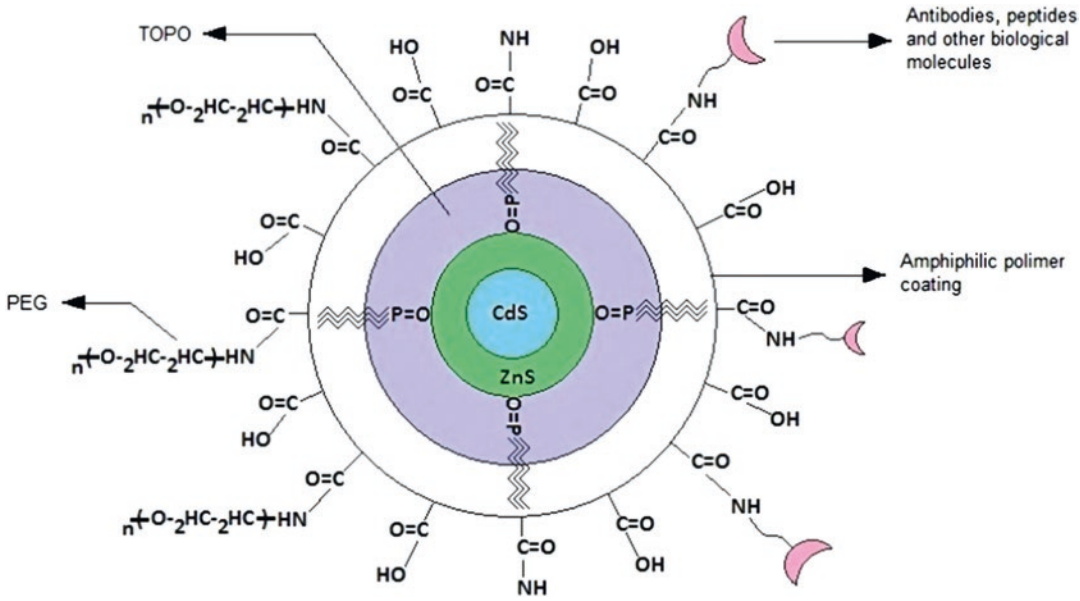

Fig. 19.2 Schematic representation of a quantum dot: a core in the center (blue), over it a shell (green), above a quantum dot is functionalized by various molecules

aqueous colloidal solutions [7]. In live systems QDs aqueous colloidal solutions are required, since, first, all reactions (including the marked QDs) proceed in the hydrophilic environment, secondly, it is necessary to get rid of toxic organic chemicals. The best of QDs proved to be QDs consisting of base material (core), usually either cadmium telluride (CdTe) or cadmium selenide (CdSe), covered with shell, for example, zinc sulfide (ZnS) or cadmium sulfide (CdS), as well as QDs of a complex composition a core-shell-shell, for example, CdTe/CdS/ZnS which have high fluorescence yield (Fig. 19.2).

Increase in quantum yield at synthesis of QDs "core-shell" type is due to:

(i) Passivation of uncompensated chemical bonds on a nanocrystal surface (trapping states in the energy gap band).
(ii) Protection of a QD's core from oxidation in external environment by covering.
(iii) Blocking of nonradiative recombination process (due to the development by the cover of a potential barrier for an exciton in QDs core) [6].

Trioctylphosphine oxide (TOPO) is usually used in QDs synthesis, but it giving them hydrophobic character. For transition to aqueous colloidal solution by means of various exchange reactions QDs surface is covered with a hydrophilic covering, for example mercaptoacids, polyethyleneglycol (PEG), bovine serum albumin (BSA) and others [8]. Ligand replacement is performed covering QDs with amphiphilic polymers, or making micellar encapsulation [3]. Conversion in an aqueous phase is often results in a considerable decrease in luminescence brightness [9]. Increase in QDs hydrodynamic diameter is another consequence of initial ligand replacement for a polymer coating [3]. QD direct synthesis in an aqueous phase is also possible, though in this case, quantum yield and stability are lower than in the QDs originally obtained in an organic phase. Besides, it is difficult to obtain QDs of different diameter in aqueous media, while it is possible to do it in organic media [7]. Anyhow, the used precursors, solvents, reaction temperature, injection parameters (when using injection methods of QDs colloidal synthesis) directly influence morphology, chemical and optical properties of QDs, their average size, its

standard deviation, place and spectral bandwidth, photoluminescence and its quantum yield [6]. Nucleic acids, DNA and RNA for example, can serve a matrix for semiconductor nanocrystals synthesis. The DNA specific groups can control synthesis and influence the emission properties of the received product. Thus, it was shown that in case of QDs PbS and CdS synthesis only GTF use as a mononucleotide ligands leads to formation of fluorescent nanocrystals [3].

Due to the active target teranostics development, functionalized QDs, i.e. QDs conjugated with biomolecules (Fig. 19.2) are widely used now. Most often: nucleic acids, peptides, proteins act as biomolecules [3]. Conjugation can be carried out due to hydrophobic or electrostatic interaction, however the most stable effect can be achieved only due to covalent binding, that is why it is most often used in the synthesis of functionalized QDs [7]. Both functionalized, and not functionalized QDs have should be stable and not subject to aggregation, their hydrodynamic diameter has to be less than 5.5 nm to provide a normal excretion by kidneys [3]. For stabilization of QDs they are quite often covered by PEG that has as positive effect (QDs are stable, do not aggregate, are not uptake by reticuloendothelial system), and negative (hydrodynamic diameter increases, an exit in a tumor is at a loss). Besides, it is necessary to remember that in a blood stream the serum proteins, such as albumine, immunoglobulins, proteins of complement system, fibrinogen, apolipoprotein, transferrin, hemoglobin envelop nanoparticles, causing their aggregation and forming a so-called "protein crown" [10]. It not only significantly increases the hydrodynamic diameter of QDs, but also carries out a peculiar role of "Trojan Horse", causing capture of nanoparticles by the mechanism of classical endocytosis [11]. It occurs first of all because "the protein crown" includes such classical opsonin as immunoglobulins and proteins of complement system in the structure. All features of QD interaction with living systems should be considered at synthesis of nanoparticles for medical and biological use.

19.3 The Prospects of QDs Modification for Biomedical Use

To maintain the smallest possible QD hydrodynamic diameter and at the same time not to lose the possibility of samples multiplex marking (now the wavelength of QD emission is regulated generally by the QD size (Fig. 19.3), it is sug-

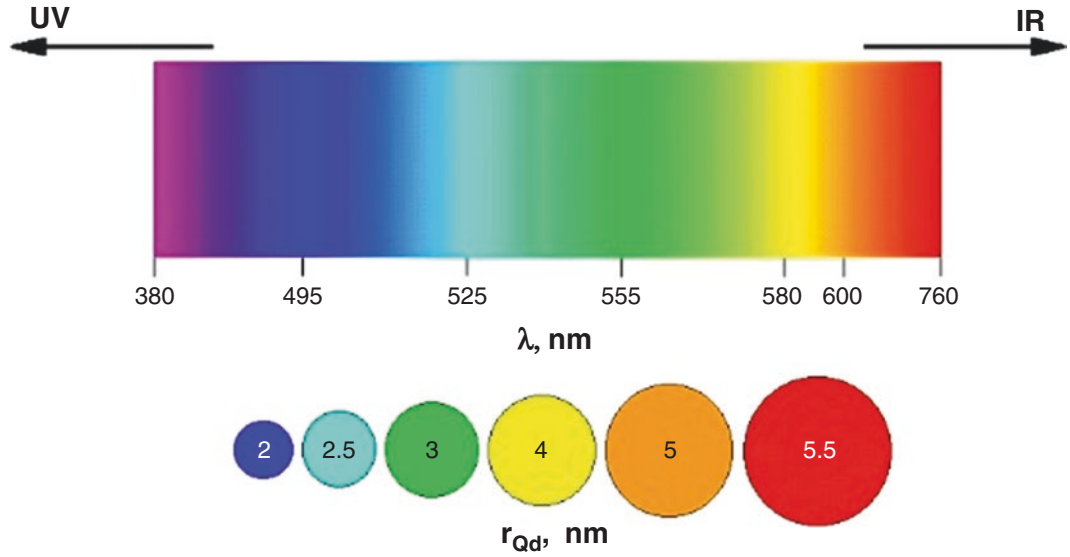

Fig. 19.3 Dependence of emission (in the visible light) from the size of quantum dots

gested to use $CdSe_xS_{1-x}$, $CdTe_xSe_{1-x}$, $Hg_xCd_{1-x}Te$ and $Hg_xCd_{1-x}Se$ cores instead of a classical CdSe core. It will allow to maintain constant and small diameter of QDs core, and to carry out fluorescence control by means of variations of core chemical composition.

Traditional cores are used as germinal structures for cultivation of QDs analogs of various form: rods, hexagonal, tetrapodal and star-like structures.

Use of polydentate ligands, which unlike classical ones (PEG and others) do not move from QDs surface outside but "envelop", promote QDs small size maintenance. Polydentate ligands use has the following advantages:

(i) The hydrophobic barrier layer is excluded;
(ii) QDs small hydrodynamic diameter remains;
(iii) High colloidal stability, resistance to photobleaching is provided;
(iv) High quantum yield is maintained;
(v) Due to QDs "enveloping" its nonspecific binding with organic molecules is excluded;
(vi) Preservation of small hydrodynamic diameter provides rapid renal clearance.

One of the main directions is modification of QDs chemistry for the decrease toxic effects, while preserving QDs unique optical properties [12].

19.4 Uses of QDs in Biology and Medicine

The point of the greatest interest for researchers is the possibility to study various biochemical, physical, kinetic processes in cells or the whole body by means of fluorescent marks. The existing organic fluorophors are of limited use owing to such restrictions as fast photobleaching, wide range of emission and necessity for constant selection of a suitable source of excitement. QDs do not have these disadvantages. The main advantages and disadvantages of QDs use in biology and medicine are summarized in Table 19.1.

QDs are used in classical biology for studying transport mechanisms in a cell [13], including endocytosis [14], functional heterogeneity of cells [15], diffusion movements of membrane transport proteins [16], intracellular organelle marking [17]. In medicine QDs are used for contrasting of blood and lymph vessels (including microvessels) [18], but first of all for multiplex molecular diagnostics and visualization *in vivo* [19–23].

One of earliest studies devoted to a possibility of QDs use for molecular diagnostics showed that conjugation of peptides with QDs leads to their selective accumulation in vessels of tumors and other tissues [23]. Later the studies showing a possibility of "QDs-peptide" conjugates use for specific visualization of tissues *in vivo* were performed. Thus, in the work by Cai et al. [24] QDs conjugated with tripeptide *arg-gly-asp* were used [24]. The last is an antagonist of the integrin $a_v\beta_3$ which, in turn, is selectively expressed on the surface of tumor cells and vessels. As a result of intravenous administration of conjugates of QDs with the specified tripeptide it was possible to get an ideal fluorescent picture of hypodermic glioblastoma in mice *in vivo*. QDs were also conjugated with monoclonal antibodies to membrane prostate-specific antigen for detection of a prostate cancer in mice *in vivo* [25]. In other study, QDs conjugates with antibodies against an α-fetoprotein were used to diagnose hepatoma *in vivo* [26]. The conjugates have to specifically interact with the target and do so in a stable manner, whilst possessing a low level of nonspecific binding [27]. Tada et al. [28], using a method of high-speed confocal microscopy of a skin fold, studied the movement of a single QD conjugated with an antibody to HER-2 in mice with breast cancer: QD circulation in blood vessel lumen, QD extravazation, its binding with a membrane antigen and the movement from tumor cell membrane to perinuclear zone were observed [28]. QDs enable not only to localize a tumor in the organism, but also to estimate the level of expression of various proteins, as well as the activity of individual cells and the processes that have an impact on tumor behavior and its response to the action of therapeutic agents [27]. The receptor part of signal proteins that are overexpressed on tumor cell membranes is used most often as a specific target. The level of expression of these cellular molecular oncomarkers, determined

Table 19.1 Advantages and shortcomings of quantum dots of biomedical practice

Advantages of quantum dots	Disadvantages of quantum dots
High photostability, resistance to photobleaching is 100–1000 times higher, than at organic fluorophores	Multiexponential decline of fluorescence and blinking of separate QDs
Narrow and symmetric peak of emission, Stokes' shift more than 200 nm (ease to detection)	High background level of deduction and accumulation of QDs in reticuloendothelial system
High quantum yield, long lifetime	Instability and increase hydrodynamic diameter after interaction with the serum proteins
Possibility of emission control by changing QDs size and structure (Fig. 19.3)	High toxicity of QDs when they using in *in vivo* systems
Excitation and emission in the visible light range (ease to detection), a possibility of emission in the field of "an optical window"	Incomplete elimination of QDs after injection into an organism
The wide absorption spectrum (operability) can be pumped up QDs of the different size and structure, and in a biological sample to investigate structures, marked by QDs of different color	Possibility of nonspecific binding with any organic molecules
Resistance to chemical and biological degradation	Instability of a colloid system of QDs in the wide range pH and ionic surrounding of the solutions that usually the main characteristic of biological systems
An opportunity to functionalized of QDs by biomolecules for creating of target-delivery system	"Binding" of optical and biomedical properties to hydrodynamic diameter

directly in the tumor tissue, characterizes the molecular profile of each individual tumor and is used to determine the immune status of the tumor and the individualization of therapeutic treatment [29]. But QDs can be using not only for specific diagnostic in target system, but also for nonspecific estimation of cancer cells motility and migration which are associated with metastases and the formation of secondary tumors. The cells nonspecifically incorporate QDs as they crawl over them, leaving behind QDs free zones representing the pattern of phagokinetic uptake of QDs [30]. Cancer cells uptake QDs more actively than normal cells. In particular, MDA-MB-231 tumor cells uptake more QDs than nontumorigenic MCF 10A cells [31].

Due to high quantum yield, QDs can be used not only for diagnostics, but also for photodynamic therapy of malignancies. Thus, QDs are bifunctional agents (therapeutic + diagnostic = theranostic). As antibodies, which carry out target delivery of QDs to tumor specific antigens (and sometimes in are therapeutic agents themselves) are not always type-specific; they are, as a rule, highly immunogenic. Therefore, it is well to use low-immunogenic specific molecules for target delivery. They can be covalent-bound with QDs, or connected via adapters. In case of large molecules (immunoglobulins), the probability of conformational changes of a molecule due to covalent binding with QDs is unlikely, while in case of small molecules use of adapter is essential for preservation of specificity and affinity of interaction. Biotin-streptavidin or barnase-barstar can be used as adapters ("molecular zipping") [27]. Now modular systems of delivery are being developed. For example, in work Wang et al. [32] reported on the synthesis of the multifunctional module uniting QDs (detection), magnetic nanoparticles (targeting in magnetic field) and paxitacel (the therapeutic agent) [32]. Biotin, which specifically binds with biotin receptor hyperexpressing on the surface of a malignantly transformed cell, may be used as a target molecule in such module. Bagalkot et al. [33] presented a novel and simple proof of concept QD-aptamer conjugate that can image and deliver anticancer drugs to prostate cancer cells and sense the delivery of drugs to the targeted tumor cells based on the mechanism of fluorescence resonance energy transfer (FRET) [33]. Their conjugate is comprised of three components:

(i) QDs, which function as fluorescent imaging vehicles;
(ii) RNA aptamers covalently attached to the surface of QD, which serve a dual function as targeting molecules and as drug carrying vehicles;
(iii) Doxorubicin (Dox), which is a widely used anthracycline drug with known fluorescent properties that intercalates within the double-stranded CG sequences of RNA and DNA as a therapeutic agent.

The assembly of this system results in the formation of a Bi-FRET complex: a donor-acceptor model FRET between QD and Dox, where the fluorescence of QD is quenched as a result of Dox absorbance, and a donor-quencher model FRET between Dox and aptamer, where Dox is quenched by double-stranded RNA aptamer. Therefore, both QD and Dox of the conjugate are in the fluorescence "OFF" state when the QD-Apt is loaded with Dox [QD-Apt(Dox)]. After the particle is taken up by targeted cancer cells, Dox is gradually released from the conjugate, which induces the activation of QD and Dox fluorescence to the "ON" state [33]. QDs can be used to sensitize either a photodynamic therapy agent via a FRET mechanism or molecular oxygen through a triplet energy transfer mechanism. Photodynamic therapy is a cancer treatment that takes advantage of the interaction between light and a photosensitizing agent to initiate apoptosis of cancer cells. The photosensitizing agent becomes activated by light but does not react directly with cells and tissues. Instead, it transfers its triplet state energy to nearby oxygen molecules to form reactive singlet oxygen ($^1O_2$) species, which cause cytotoxic reactions in the cells [34, 35]. Also QDs may increase therapeutic efficiency of radiotherapy by selectively scattering and/or absorbing X-rays and gamma rays causing localized damage to DNA and other targeted organelles of cancer cells and thus decreasing total radiation dose to minimize side effects of ionizing radiation on cancer patients [36]. Toxically potential of QDs also can be used for cancer treatment, because this kind of nanoparticles causes epigenetic changes. Choi et al. [37] showed that exposure of MCF-7 cells to hardly detectable intracellular QDs can cause epigenetic changes and trigger p53 posttranslational modifications and its translocation to mitochondria [37]. The activation of p53 results in upregulation of several p53-regulated proapoptotic genes: Puma, Noxa, and Bax. Thus, there are many different approaches for using QDs in the diagnosis and therapy of cancer. Depending on the delivering system, QDs can penetrate into the cell or localize only on their membrane. Total use of QDs for the diagnosis and treatment of cancer in dependence of the localization of the nanoparticles is shown in Fig. 19.4.

Unfortunately, tissue vital fluorescent diagnostics by means of QDs, which has been successfully demonstrated in several works for small laboratory animals, cannot be directly extrapolated to clinical practice because of the limited depth of optical signal penetration. In medicine, this technique can be used for identification of superficial tissue formations (skin and subcutaneous tumors), intraoperative diagnostics and visualization of the zones available at endoscopy [38]. QDs blinking caused by sporadic changes of radiating and nonradiating QDs state is another its drawback [39]. Blinking results in a rupture of QDs fluorescence which is especially important in the field of detection of one molecule, one QD. In some cases, for example, in immunocytologic studies, rupture in fluorescence helps to distinguish the signal coming from QDs from the signal received as an artifact. Hohng and Ha [40] assumed that QDs blinking can be suppressed as a result of passivation of QDs surface by thiol groups [40].

The interesting way of QDs use in ophthalmology is described in works of Chashchin et al. [41, 42]. QDs is suggested to be used for visualization of a vitreous body and epiretinal membranes at vitreoretinal interventions, for delivery medicines to eye tissues, including to the retina and cornea. The authors synthesized QDs on the basis of indium phosphide and intend to use them for retina photoreceptors stimulation in treatment of such pathologies as a pigmented retinitis, retina detachment, diabetic retinopathy and a macular degeneration.

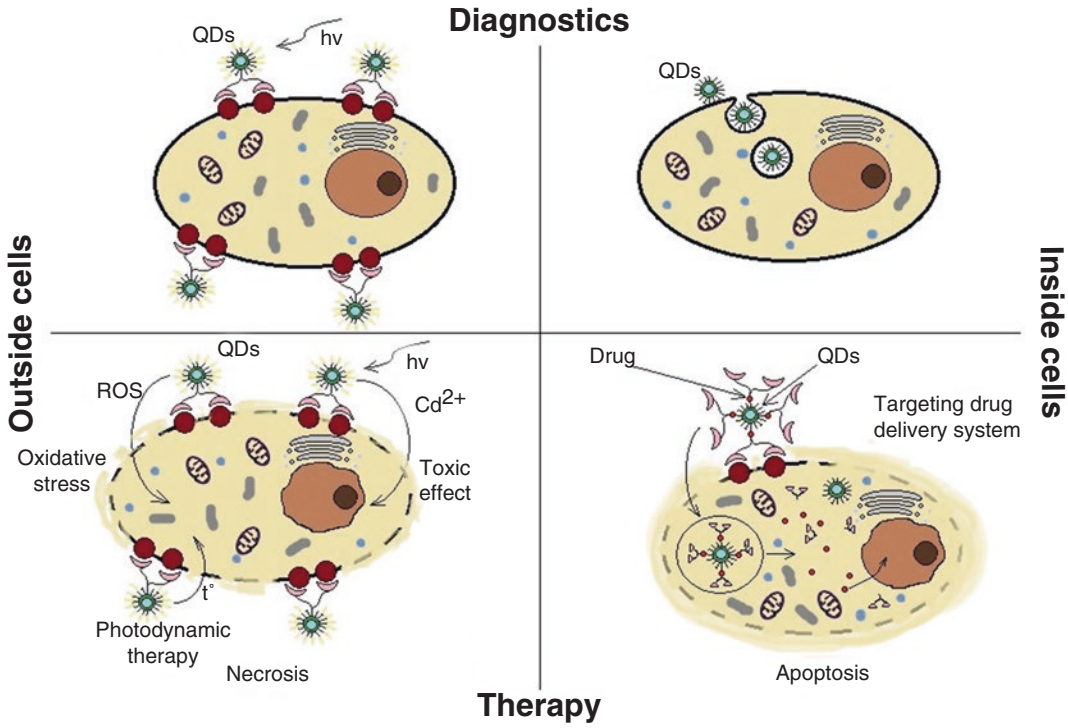

Fig. 19.4 Using of QDs for diagnostics (upper line) and therapy (lower line) of cancer: the upper left square-QDs due to specific delivery system bind to oncomarkers on the membranes of tumor cells, and after excitation, narrow-band diagnostic emission is observed, which allows localizing the tumor. This system is also used to assess the expression density of tumor markers; the upper right square–QDs can penetrate non specifically, directly into the malignant cell, depending on the degree of aggregation of nanoparticles, it can be raft-dependent, caveolae- dependent, clathrin-dependent, or independent penetration; the lower left square – the total possible therapeutic effect of QDs specifically interaction through antibodies with malignant cell oncomarkers (toxic effects, hyperthermic effects, sensitizing effects for photodynamic and radiotherapy); the lower right square – realization FRET and drug-delivery strategy into malignant cell by QDs

One of the main QDs disadvantages is their high toxicity described in many works [43, 44]. It is the main limiting factor for QDs use in medicine, however it is possible to use all QDs advantages in developing diagnostic systems *in vitro*. In particular, QD are used for the development of diagnostic test systems, and with their help, the following substances have already been detected: chloramphenicol in milk and IgE in blood serum [45], sulfamethazine in chicken meat [46], chlorpyriphos in drinking water [47], *Listeria monocytogenes* surface antigens [48], clenbuterol in pig urine [49], progesterone in cow milk [50]. However, despite great advantage of QDs conjugate use with organic molecules, in the immunoenzymatic and immunocytochemistry immunoassay, the work by Korzhevsky et al. [51] reveals a number of drawbacks of QDs conjugation with streptavidin: its less stability at long-term storage in comparison with organic fluorochrome conjugates, poor aliquot reagents preservation, impossibility of long preservation of fluorescence of stained preparations at storage, incompatibility with a number of the commercial media intended for keeping of drugs.

QDs use in protein and DNA-biochips allow not only to improve significantly the sensitivity of diagnostic test systems (for example, protein biochips are more sensitive two orders of magnitude than a traditional solid-phase enzyme multiplied immunoassay), but also to perform breakthrough functional studies in the field of

genomics and proteomics. Thus, using of protein biochips it is possible to find and identify proteins and peptides in a proteome, carry out functional analysis of proteins and determine profiles of their expression and the level of their phosphorylation. Biochips are used for determining biochemical activity of a proteome, including interactions of protein-protein, protein-nucleic acids, protein-phospholipids, protein-drug substances. Additional intensity of fluorescence is achieved by the use of adapters [52].

Relatively large total area of the surface, combined with the universal controlled chemistry makes QDs the best decision in the field of development of selectively acting nanodrugs. QDs physical and chemical properties, including size, form, surface charge and type of covering, play an important role in definition and forecasting variants of cellular internalization, pharmacokinetics and biodistribution of certain QDs kinds. Knowing main mechanisms of internalization and tracing of nanoparticles, it is possible to evaluate their biological compatibility and safety [53, 54].

Nabiev et al. [55] revealed dependence of QDs endocytosis and intracellular tracing on their size. QDs CdSe were quickly absorbed by macrophages and depending on their size accumulated in different cellular compartments. The smallest green QDs were localized mainly in nuclei and nucleolus, and the process of absorption was multistage, including endocytosis, active cytoplasmic transport and entering the nucleus through nuclear pores. Red QDs concentrated in cell cytoplasm. In the study of CdTe QDs during their incubation with N9 microglial cells, Lovrić et al. [56] revealed a similar dependence of CdTe QDs distribution on their size: small-sized QDs with green fluorescence (2.2 ± 0.1 nm in diameter) concentrated in the nucleus and near it after 1 h of incubation, and large-sized QDs with red fluorescence (5.2 ± 0.1 nm in diameter) were found in the cytosol and did not enter the nucleus. QDs of the small size can easier enter into the nuclei and other compartments of cells, than a nanoparticle of the larger size. To test this hypothesis, QDs with green fluorescence were covered with BSA. As a result, QDs size increased, and they no longer entered into the nuclei. In other studies it was shown that, the size of nanoparticles significantly influence the binding and activation of membrane receptors, and the subsequent protein expression [57].

The possible explanation of the fact that nanoparticle internalization kinetics depends on the size of nanoparticles is that multivalent cation-active QD interactions with cells are more easily available to particles with the larger surface of contact, than to the particles with a smaller one. Champion and Mitragotri [54] showed by an example of alveolar macrophages that phagocytosis of polystyrene particles is influenced by a form, but not by the size. However, it is known that particle size primarily influences on phagocytosis completeness by cells.

Equally important characteristic of QDs is the charge on their surface, because at the initial stage of their contact with a negatively charged cell membrane electrostatic forces arise, which can prevent internalization of QDs by the cells. Shan et al. [58] showed that with QD surface covered with carboxyl groups, no endocytosis was observed. This allowed suggesting that positively charged QDs are uptake due to electrostatic interactions with a negatively charged HeLa cell membrane. Using the method of atomic force microscopy they showed that for absorption of one QD by a cell 0.4 s are enough [58]. However, in work by Hoshino et al. [59] it was shown that QDs carboxylated on a surface uptake the mouse EL-4 cells by endocytosis. Besides, Jaiswal et al. [60] revealed that negatively charged QDs CdSe/ZnS-dihydrolipoic acid are easily absorbed by mammal cells. Nabiev et al. [55] not only discovered endocytosis of the negatively charged CdTe-thioglycolic acid QDs by cells, but also demonstrated that after absorption intracellular QD transport does not stop in lysosomes. This QDs type enters into cytoplasm and accumulates in perinuclear area. Cationic particles can bind with negatively charged groups on cell surfaces (for example, by sialic acid) and to travel through the plasma membrane, unlike low level of interaction and internalization by the cells of neutral and negatively charged particles. Harush-Frenkel et al. [61] studied endocytosis mechanisms of the charged particles. Their results

showed that negatively charged nanoparticles are less effectively uptake by the cells whereas positively charged particles are uptake quickly. Besides, at inhibition of clathrin-dependent way of absorption in cells, internalization of positively charged particles proceeds on compensation ways with high speed.

Size, form, and charge of nanomaterials contribute significantly to their interaction with the cells. However, functional groups on QDs surface determine many important properties of nanomaterials, such as solubility and ability to interact with cell surface. As a rule, nanomaterial incubation in the cells media leads to plasma and/or proteins adsorption on nanoparticles surface [62]. As a result, QDs endocytosis can proceed by a receptor-mediated way. However, protein adsorption on the surface can lead to agglomeration of nanoparticles and their removal into reticuloendothelial system. Nonspecific interactions can also lead to nanoparticles binding with a cellular membrane that will make marking and detection inefficient. To avoid such problems, QDs can be covered with neutral ligands (for example, PEG) which do not interact with proteins. When comparing QDs covered and not covered with PEG it was found that in the latter endocytosis was higher [63].

Thus, development of new approaches to QDs synthesis and covering will promote not only their use as fluorescent markers in diagnostic test systems and experimental biology, but also will allow use them in therapeutic systems *in vivo*.

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