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Review: therapeutic application of quantum dots (QD)

Sushant Tope ^{1*}, Shrikant Saudagar ², Niwedita Kale ³, Sagar khambayat ⁴, kiran bhise ⁵

1. Government College of Pharmacy Amravati, Maharashtra, 444604. *[Email: sushanttope@gmail.com]
2. Government College of Pharmacy Amravati, Maharashtra, 444604. [Email: s.saudagar2905@gmail.com]
3. Vidyabharti college of Pharmacy, Amravati, Maharashtra, 444604. [Email: kalenivedita7@gmail.com]
4. Government College of Pharmacy Amravati, Maharashtra, 444604. [Email: sagarkhambayat92@gmail.com.]
5. Government College of Pharmacy Amravati, Maharashtra, 444604. [Email: arbianboy456@gmail.com]

Quantum dots (QDs) are luminescent Nanocrystals with rich surface chemistry and unique optical properties that make them useful as probes or carriers for traceable targeted delivery and therapy applications. QDs have proven themselves as fluorescent probes, especially for long-term, multiplexed, and quantitative imaging and detection. QDs are nanoscale semiconductor crystals ranging typically between 1-10 nanometers and have capacity to glow or fluorescence brightly when excited by a light source such as a laser QDs are tiny bits of microscopic metal, thousand times smaller than width of a hair or semiconductor boxes such as cadmium selenide-zinc sulphide. The nature of this technology makes it suitable for application such as *in vivo* imaging including live cell and whole animal imaging, blood cancer assay, cancer detection and treatment. Multiplexed analysis such as DNA detection and cell sorting and tracking. Recent progress in the surface chemistry of quantum dots expanded their use in biological applications, reduced their cytotoxicity and rendered quantum dots a powerful tool for the investigation of distinct cellular processes, like uptake, receptor trafficking and intracellular delivery. QDs are luminescent Nanocrystals with rich surface chemistry and unique optical properties that make them useful as probes or carriers for traceable targeted delivery and therapy applications. QDs can be functionalized to target specific cells or tissues by conjugating them with targeting ligands. Recent advancement in making biocompatible QD formulations has made these Nanocrystals suitable for *in vivo* applications. QDs can be used as labels for the electrochemical detection of DNA or proteins.

Keyword: Quantum dots, bioanalysis, cell imaging Dyes, Analytics Nanocrystals, targeting, siRNA.

I. Introduction

Quantum dots are nanoscale semiconductor crystals ranging typically between 1-10 nanometer and have capacity to glow or fluorescence brightly when excited by a light source such as a laser and have unique photo physical properties due to quantum confinement effects. QDs have unique optical and electronic properties, such as larger absorption coefficients, size-tunable light emission, superior signal brightness, resistance to photobleaching and

simultaneous excitation of multiple fluorescence colors ^[1-6]. More energy is needed to excite the dot, because of greater the difference in energy between the highest valence band and the lowest conduction band concept behind this is the smaller the size of the crystal, the larger the band gap, concurrently, more energy is released when the crystal returns to its resting state. e.g. in fluorescent dye applications, this equates to higher frequencies of light emitted after excitation of the dot as the crystal size grows

smaller, resulting in a color shift from red to blue in the light emitted. Nanoparticles have a maximum surface: volume ratio, which makes it suitable for surface functionalization along with incorporation of good therapeutics load. Furthermore, due to their nano-size and tunable surface properties (enabling the synthesis of aqueous, injectable solutions and the development of passive or active targeted systems), nanoparticle potentially have better access to target sites as compared to conventional drug delivery carriers. Over the past few decades quantum dots (QDs) have been an area of intense research due to their unique physical properties. Quantum dots, sometimes called artificial atoms, are tiny Nanocrystals made of inorganic transition metal, that glow when stimulated by an external source such as ultraviolet (UV) light. How many atoms are included in the quantum dot determines their size and the size of the quantum dot determines the colour of light emitted. Gallium arsenide (GaAs) is a popular material out of which quantum dots can be made, because the effective mass of an electron and the shape of the crystal correlate at room temperature to form desirable properties. Other than GaAs they are made up of cadmium selenide (CdSe), cadmium

telluride (CdTe), indium phosphide (InP), and indium arsenide (InAs) as core elements inside a shell, usually zinc sulfide (ZnS). In comparison with conventional organic dyes and fluorescent proteins, QDs have distinctive characteristics such as size-tunable light emission, improved signal brightness, resistance against photo bleaching and simultaneous excitation of multiple fluorescence colors. Recent advances in nanoparticle surface chemistry have led to the development of polymer-encapsulated probes that are highly fluorescent and stable under complex biological condition. The success of using QDs in biological imaging, sensing and detection has encouraged scientists to further develop this technology for clinical and translational research's for nano-carrier development and optimization, QDs can become an excellent 'prototype,' from which biocompatible carriers of similar sizes and surface properties can be made for clinical uses. QDs have broad absorption spectra, allowing them to be excited by light of a wide range of wavelengths (Figure 3). This allows one to simultaneously excite QDs with different emission spectra for multiplex imaging using a single excitation source.

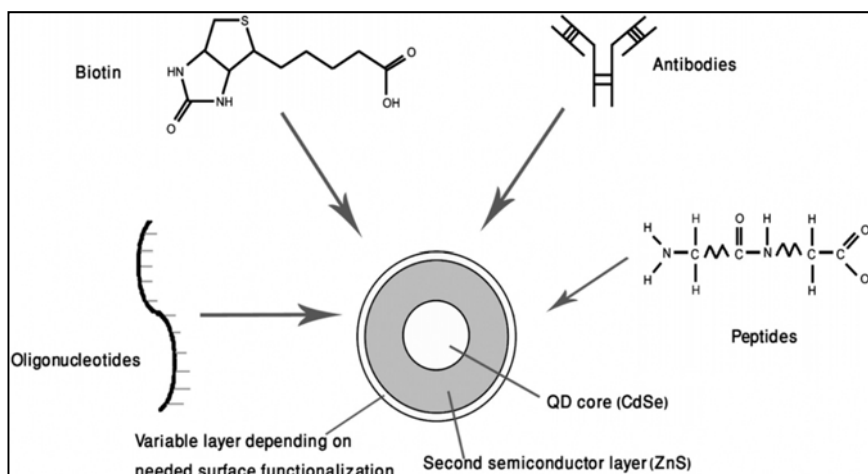


Fig 1: Assembly of Quantum dots.

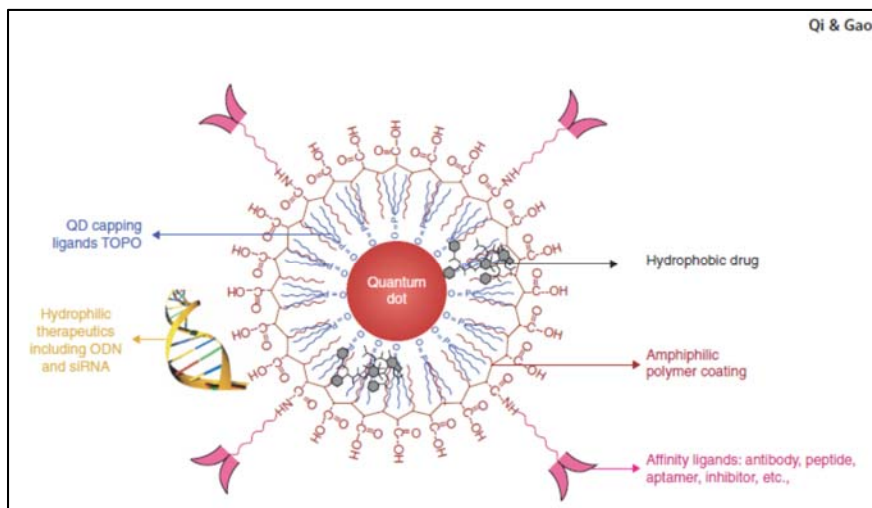


Fig 2: Schematic illustration of multifunctional Quantum dot coated with amphiphilic polymer.

I a) Properties of quantum dots:

1. Quantum dots “designer atoms” offer innumerable optical and electronic properties that can work around natural limits inherent in traditional semiconductors.
2. Quantum dots are made from tiny bits of metal about thousand times smaller than width of a hair.
3. Quantum dots can be molded into different shapes and coated with a variety of biomaterials.
4. Quantum dots luminescence under UV light, with the size of the dots controlling its colour. e.g. 2 nm Quantum dots luminescence bright green, 5 nm Quantum dots –luminescence red.
5. Fluorescent quantum dots are usually compounds from group II to VI and III to V e.g. Ag, Cd, Hg, Ln, P, Pb, Se, Te, and Zn etc.
6. As size of quantum dots decreases, the wavelength it emits turns shorter.
7. Quantum dots have a broad excitation range.
8. Quantum dots have precise emission wavelength, so the spectra doesn't overlap [7]

I b) Synthesis of quantum dots:

In large numbers, quantum dots may be synthesized by means of a colloidal synthesis. Colloidal synthesis is by far the cheapest and has the advantage of being able to occur at bench top conditions. It is acknowledged to be the least

toxic of all the different forms of synthesis. Highly ordered arrays of quantum dots may also be self-assembled by electrochemical techniques. A template is created by causing an ionic reaction at an electrolyte-metal interface which results in the spontaneous assembly of nanostructures, including quantum dots, on the metal which is then used as a mask for mesa-etching these nanostructures on a chosen substrate. Another method is pyrolytic synthesis, which produces large numbers of quantum dots that self-assemble into preferential crystal sizes [9].

Calcium Oxide + Selenium-----Quantum dots

In surfactant solution ↓
 (TOPO) Cooling the reaction after
 Reaching desired crystal size [8]

I c) Uptake of QDs: QD cellular uptake involves three major stages including endocytosis, sequestration in early endosomes, and translocation to later endosomes or lysosomes. The endocytosis was probably assisted by receptors specific to ligands with negative charges. These findings could be exploited to reduce non-specific targeting, thereby improving specific targeting of QDs in cancer diagnosis and treatment applications. The findings are also important in understanding the cytotoxicity of QDs and other nonmaterial's in general and in

emphasizing the importance of strict environmental control of nanoparticle^[11].

I d) Advantages of quantum dots:

1. Quantum dots are much more resistant to degradation than other optical imaging probes, allowing them to track cell processes for longer periods of time and shed new light on molecular interactions.
2. As Quantum dots are Nanocrystals they provide good contrast for imaging with an electron microscope as scattering increases.
3. Quantum dots have size-tunable emission (from UV to IR)
4. Fluorescence lasts for longer time as compared to conventional dyes.
5. Quantum dots have increased optical activity with innumerable avenues of applications in biotechnology and life sciences.
6. Anti-counterfeiting measure- their extremely small size gives them great versatility by allowing them to be injected into many environments, including liquid mixtures, fabrics, and polymer matrices^[10].

II) Optical Properties of Quantum Dots:

Most organic dyes display narrow absorption spectra and require specific excitation wavelengths to excite them^[12-13]. In contrast, QDs have broad absorption spectra, allowing them to be excited by light of a wide range of wavelengths^[15]. This allows one to simultaneously excite QDs with different emission spectra for multiplex imaging using a single excitation source^[14]. Organic dyes also have relatively broad emission spectra, resulting in the overlap of their fluorescence spectra, thus limiting their use for multiplex imaging^[16]. In contrast, QDs have narrow emission spectra, which can be manipulated by changing the core size and composition of the QDs. More importantly, the QDs can be tuned to emit emission ranging from UV to near-infrared region. The high photostability of QDs is another unique feature from QDs for fluorescence imaging applications^[17]. Unlike organic dyes, which may photo-bleach rapidly, QDs are stable and can withstand many cycles of excitation for

long periods of time with a high level of brightness^[18]. For example, dihydrolipoic acid-functionalized core/shell CdSe/ZnS QDs showed no change in the luminescence intensity after more than 10 hours of continuous excitation, and were 100 times as stable as rhodamine dye. In addition, QDs have a long luminescence lifetime after ex-citation and this can be an advantage for time-gated imaging. The fast fluorescence emission of organic dyes is similar to the short lifetime of the autofluorescence background from cells and tissues; thus, the signal-to-noise ratio is reduced. However, QDs generally emit light with a decay time of 30 to 100 ns, which is much slower than that of the autofluorescence background decay, while remaining fast enough to maintain a high photon turnover rate. In time-gated analysis, photons detected in the first few ns after pulsed excitation are disregarded to decrease background noise and increase sensitivity. This advantage has been utilized to produce images of 3T3 mouse fibroblasts with a high signal-to-background ratio and to monitor the dynamics of erbB1 and erbB3 receptors^[19] In this case, this technique can be used to differentiate the erbB3 receptors labeled with citrine and erbB1 receptors labeled with QDs. Therefore, owing to their high brightness, photostability, and long decay time, the dynamics of QDs can be optically traced *in vitro* and *in vivo*^[20].

III) QDs-As diagnostics in clinical applications:

The most important potential applications of Quantum dots (QDs) are for cancer diagnosis. Luminescent and stable QD bioconjugates enable visualization of cancer cells in living animals. QD scan be combined with fluorescence microscopy to follow cells at high resolution in living animals. QDs have been coated with a polyacrylate cap and covalently linked to antibodies for immunofluorescent labelling of breast cancer marker Her2 carbohydrate encapsulated QDs with detectable luminescent properties are useful for imaging of cancer. Another application of QDs is for viral diagnosis. Rapid and sensitive diagnosis of Respiratory

Syncytial Virus (RSV) is important for infection control and development of antiviral drugs. Antibody- conjugated nanoparticles rapidly and sensitively detect RSV and estimate relative levels of surface protein expression. A major development is the use of dual-colour QDs or fluorescence energy transfer nanobeads that can be simultaneously excited with a single light source. A QD system can detect the presence of particles of the RSV in a matter of hours. It is

also more sensitive, allowing detection of the virus earlier in the course of an infection. When an RSV virus infects lung cells, it leaves part of its coat containing F and G proteins on the cell's surface. QDs have been linked to antibodies keyed to structures unique to the RSV coat. As a result, when QDs come in contact with either viral particles or infected cells they stick to their surface [21]. (Table 1)

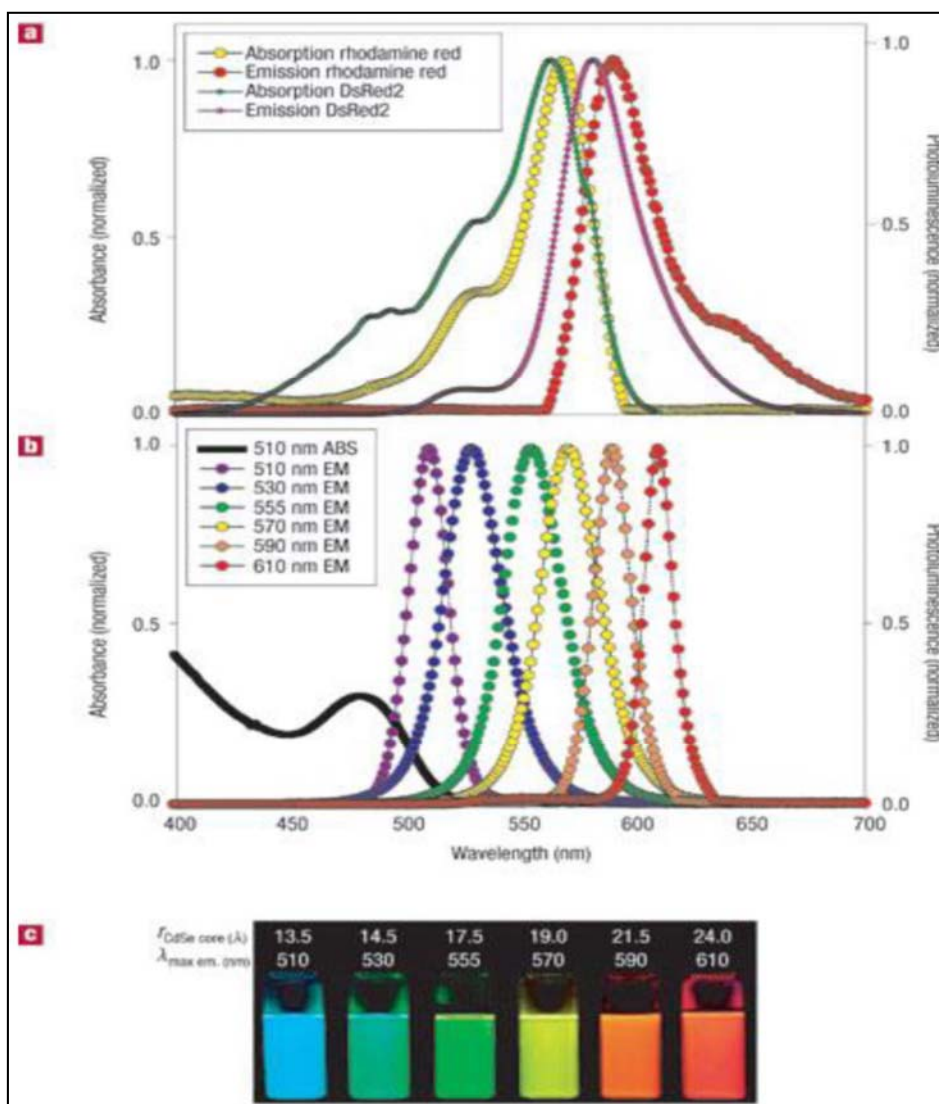


Fig 3(a): Absorption and emission of rhodamine red, a common organic dye, and genetically-encoded DsRed2 protein.

Fig 3(b): Absorption and emission of different QD dispersions. The black line shows the absorption of the 510-nm-emitting QDs.

Fig 3(c): Photo demonstrating the size-tunable luminescence properties and spectral range of the six QD dispersions plotted in b versus CdSe core size. All samples were excited at 365 nm. Reprinted by permission from Macmillan Publishers Ltd: Nature Materials (Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. Quantum dot bioconjugates for imaging, labelling and sensing. Nat Mater. 2005; 4: 435-46. <http://www.nature.com>), copyright 2005.

Table 1: Summary of application areas for nanoscale pharmaceuticals and medicine in diagnostics ^[22]

Material/Technique	Property	Applications	Timescale (To market launch)
Diagnostics Nanosized markers, i.e. the attachment of nanoparticles to molecules of interest.	Minute quantities of a substance can be detected, down to individual molecules	E.g. detection of cancer cells to allow early treatment	-----
'Lab-on-a-chip' technologies	Miniaturisation and speeding up of the analytical process	The creation of miniature, portable diagnostic laboratories for uses in the food, pharmaceutical and chemical industries; in disease prevention and control; and in environmental monitoring	Although chips currently cost over £125 (US\$ 2085) each to make, within three years the costs should fall dramatically, making these tools widely available
Quantum dots	Quantum dots can be tracked very precisely when molecules are 'bar coded' by their unique light spectrum.	Diagnosis.	In early stage of development, but there is enough interest here for some commercialization

III a) Quantum Dots/Drug Formulation for Targeted Delivery

The mechanism of delivery of QD/drug formulations to tumor cells is determined by the architecture and properties of the nanostructures. Several rules must be considered in preparing QD/drug nanoparticle formulations for targeted therapy *in vivo*: (i) the nanoparticle surface must be functionalized with targeting ligands for specific delivery to tumor cells and must allow the drug to be delivered together with the carrier; (ii) the size of the nanoparticle must be minimized to allow excretion from the body; (iii) the drug molecules must be confined within the nanoparticle delivery system to prevent any harmful effects to the normal tissue; however, the drug must be released at tumor cells after being triggered externally or by local environmental factors, and (iv) the surface of QDs must be passivated with a long lasting biocompatible polymer to prevent degradation or breakdown of the QDs upon encounter with the biological environment. In the next few paragraphs, we

discuss recent findings on the preparation of QD/drug nanoparticle formulation for targeted delivery and therapy ^[20].

III b) Quantum dots as carriers with integrated functionalities: -Current biomedical applications of QDs are focused on molecular imaging and sensing because of the aforementioned optical properties. The structural properties of QDs, which are perhaps equally as important, have just been realized in drug delivery research. First, the size of QDs can be continuously tuned from 2–10 nm, which, after polymer encapsulation, generally increases to 5–20 nm in diameter. Particles smaller than 5 nm are quickly cleared by renal filtration ^[12]; whereas bigger particles are more likely to be uptaken by the reticuloendothelial system before reaching the targeted disease sites. Additionally, larger particles have limited penetration depth into solid tissues. Recent advances in QD nanocrystal synthesis will allow scientists to systematically assess this size effect on delivery efficiency and

specificity, and enable them to identify the optimal dimensions of drug carriers. Second, owing to the high surface-to-volume ratio of nanomaterials, it is possible to link multiple functionalities on single QDs while keep in the overall size within the optimal range. For example, the QD core can serve as the structural scaffold, and the imaging contrast agent and small molecule hydrophobic drugs can be embedded between the inorganic core and the amphiphilic polymer coating layer (Figure 2). Hydrophilic therapeutic agents (including small interfering RNA [siRNA] and antisense oligodeoxynucleotide [ODN]) and targeting biomolecules (such as antibodies, peptides and aptamers), in turn, can be immobilized onto the hydrophilic side of the amphiphilic polymer via either covalent or non-covalent bonds. This fully integrated nanostructure may behave like a magic bullet that will not only identify, bind to and treat diseased cells, but will also emit detectable signals for real-time monitoring of its trajectory^[23].

Biomedicine: -Among various nonmaterial's, quantum dots (QDs) distinguish themselves in their far-reaching possibilities in many avenues of biomedicine. QDs are nanometer sized fluorescent semiconductor crystals with unique photochemical and photo physical properties. Their much greater brightness, rock-solid photostability and unique capabilities for multiplexing, combined with their intrinsic symmetric and narrow emission bands, have made them far better substitutes for organic dyes in existing diagnostic assays. These properties, combined with the development of ways to solubilize QDs in solution and to conjugate them with biological molecules, have led to an explosive growth in their biomedical applications. Bioconjugated QD fluorescent probes offer a promising and powerful imaging tool for cancer detection, diagnosis and treatment^[24].

Imaging: Quantum dots, which range from about 2 to 10 nanometers across (roughly equivalent to a medium-sized protein), have distinct advantages over conventional fluorescent dyes. It has been

estimated that quantum dots are 20 times brighter and 100 times more stable than traditional fluorescent reporters. By simply varying the crystal size, scientists can produce dots that emit light in a wide range of wavelengths, or colors, that are less prone to overlap than those of organic dyes. And whereas each organic dye must be excited with a specific wavelength of light, a single light source can excite quantum dots of many colors, so scientists can use the dots to label and detect multiple targets simultaneously. In addition to this "multiplexing" capability, quantum dots are much brighter than organic dyes and retain their glow much longer. Compared with the traditional organic fluorophores (e.g., organic dyes and fluorescent proteins), QDs have unique optical and electronic properties, such as larger absorption coefficients, size-tunable light emission, superior signal brightness, resistance to photobleaching and simultaneous excitation of multiple fluorescence colors. In addition, the large-surface area of QDs is beneficial to covalently link to biorecognition molecules, such as peptides, antibodies, nucleic acids or small-molecule ligands for further application as fluorescent probes. A recent advancement in QDs technology is the use of QDs for near infrared (NIR) imaging (700–1000 nm wave length range) as an imaging probe. The main advantage of NIR QDs over its counterpart, visible QDs, is that it increases the depth of tissue penetration, allowing for more accurate and sensitive detection of photons *in vivo*. Additionally, NIR QDs evade the problem of auto-fluorescence associated with optical imaging because of the naturally-occurring compounds present in animal tissue. The use of NIR QDs for *in vivo* imaging was demonstrated for lymphatic mapping in animal models, and for biological imaging, using InAs/ZnCdS as a core/shell. NIR QDs coated with PEG allowed imaging of tumor vasculature as deep as 200 μm , contrary to the visible QDs-generated images with very poor vascular contrast^[24].

III c) Quantum dots as tags for other drug carriers: The second type of QD application in traceable drug delivery is more straightforward –

labeling a conventional drug carrier with QDs, which serve as photostable fluorescent reporters. The majority of current drug carriers are made of polymers, such as poly (lactic-co-glycolic acid) and polyethyleneimine (PEI), and fewer are based on inorganic materials. A common limitation shared by these delivery vehicles is the lack of an intrinsic signal for long-term and real-time imaging of drug transport. This problem has been partially addressed by conjugation with organic fluorophores. However, the photobleaching problem associated with essentially all organic dyes (including fluorescent proteins) prevents

long-term tracking or imaging. In this context, QDs become a natural choice because of their unique spectral properties. Indeed, they have been used to label both organic and inorganic drug carriers and potentially even bacteria and viruses [25-28], with a burst of activity in the area of ODN and siRNA delivery. It was found that the intracellular QD fluorescence intensity correlates well with the degree of silencing, which is easy to understand because stronger fluorescence indicates a higher level of lipoplex uptake by cells at a fixed ratio of Lipofectamine and QD concentration.

QD–Apt (Dox) as a complex conjugate for targeted cancer imaging, therapy, and sensing:

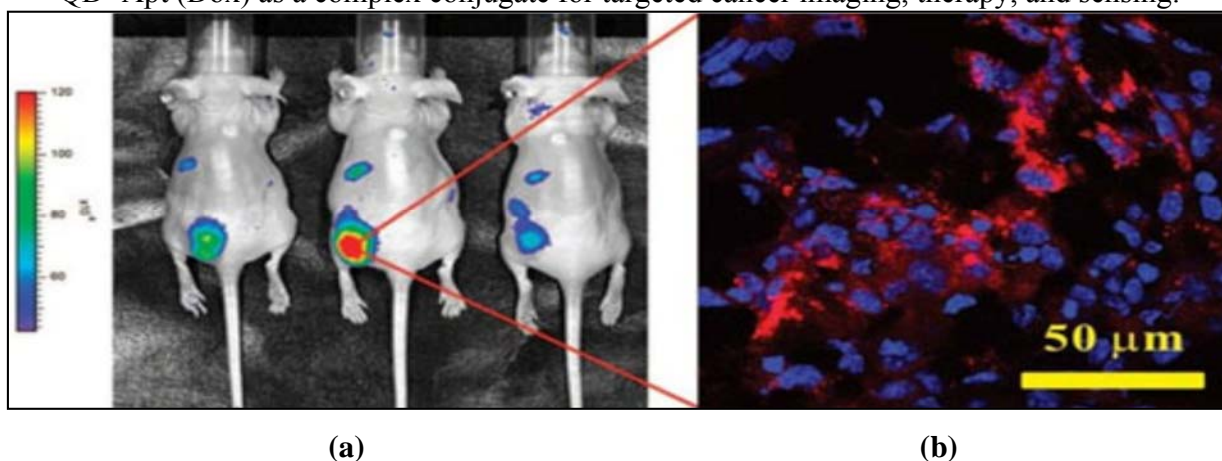


Fig 4: (a) Left panel: *In vivo* fluorescence imaging of three nude mice bearing MCF-7/HER2 xenografts implanted in the lower back 30 h after i.v. injection with anti-HER2 QD-ILs;

(b) Right panel: A 5 μm section cut from frozen tumor tissues harvested at 48 h postinjection and examined by confocal microscopy by a 63 \times oil immersion objective (image size, 146 μm \times 146 μm). The tumor section was examined in two-color scanning mode for nuclei stained by DAPI (blue) and QD-ILs (red). (Cited from Weng *et al.* [29].)

Basically, the QD surface was functionalized with an RNA aptamer that recognizes the extracellular domain of the prostate specific membrane antigen, enabling preferential targeting and imaging of prostate cancer cells. The anticancer drug, Dox, which is intercalated with the RNA aptamer, is released slowly from the QD system. The drug-release process was monitored using the Förster (fluorescence) resonance energy transfer (FRET) between QD and Dox. This system could deliver Dox to the targeted prostate cancer cells and sense the delivery of Dox by

activating the fluorescence of QDs that concurrently image the cancer cells. The specificity and sensitivity of this nanoparticle conjugate formulation as a cancer imaging therapy and sensing system were demonstrated *in vitro*. Chakravarthy *et al.* reported on the ability of nanoconjugates of CdSe/CdS/ZnS QD and doxorubicin to target alveolar macrophages, cells that play a critical role in the pathogenesis of inflammatory lung injuries. Confocal imaging showed the release of Dox from the QD-Dox nanoconjugate, as was evident by its

accumulation in the cell nucleus and induction of apoptosis, indicating that the drug retains its bio-activity after coupling to the nanoparticle. Inflammatory injury parameters (albumin leakage, proinflammatory cytokines, and neutrophil infiltration) were recorded after *in vivo*

administration of QD-Dox and Dox, indicating no significant effect after QD-Dox treatment compared with free Dox. These results show that nanoparticle platforms can provide targeted macrophage-selective therapy for the treatment of pulmonary disease.

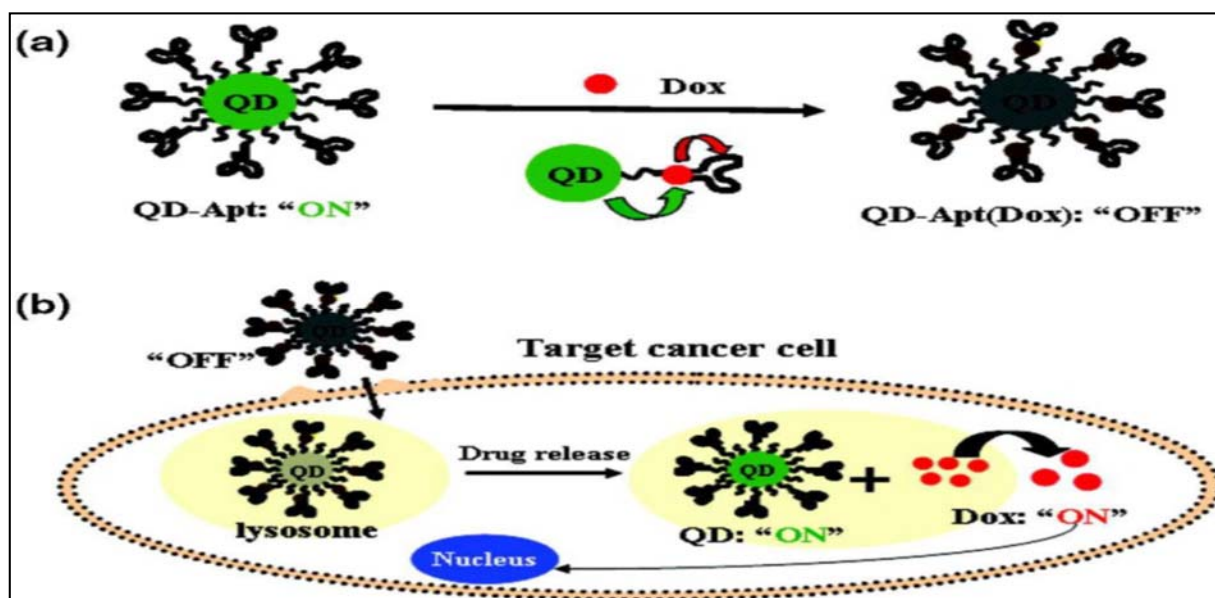


Fig 5: (A) Schematic illustration of QD–Apt (Dox) Bi-FRET system. In the first step, the QD are surface functionalized with the A10 PSMA aptamer. The intercalation of Dox within the A10 PSMA aptamer on the surface of QDs results in the formation of the QD–Apt (Dox) and quenching of both QD and Dox fluorescence through a Bi-FRET mechanism.

(B) Schematic illustration of specific uptake of QD–Apt (Dox) conjugates into target cancer cell through PSMA mediated endocytosis. The release of Dox from the QD–Apt (Dox) conjugates induces the recovery of fluorescence from both QD and Dox, thereby sensing the intracellular delivery of Dox and enabling the synchronous fluorescent localization and killing of cancer cells. Reprinted with permission from (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 Letters*. 2007; 7: 3065-70.).

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III d) Antiretroviral drug saquinavir and the biorecognition molecule transferrin (Tf) have been conjugated to carboxyl-terminated quantum dots using carbodiimide chemistry: - The aim of this study was to significantly enhance the transport of saquinavir into the brain, for the treatment of HIV-1 infected cells within the brain, via targeting the transferrin receptors (TfRs), which are overexpressed on the apical

surface of the blood brain barrier (BBB). Using an *in vitro* model of the BBB, they demonstrated that these targeted and drug-doped QDs can efficiently cross the BBB, and caused a marked decrease in viral replication in the HIV-1 infected peripheral blood mononuclear cells (PBMCs) within the brain. These results highlight the potential of this nanoformulation in the treatment

of Neuro-AIDS and other neurological disorders [20].

III e) Blue-emitting ZnO QDs were combined with biodegradable chitosan for tumor-targeted drug delivery: This showed that the chitosan-coated ZnO QDs could be loaded with anti-cancer drug molecules and could deliver anticancer drugs to the tumor. The presence of chitosan on the QDs surface enhanced the colloidal stability of the QDs due to the hydrophilicity and cationic charge of chitosan [20].

III f) QD-mucin1 aptamer doxorubicin conjugate for the chemotherapy of ovarian cancer: [30] Basically, the QDs were conjugated with a DNA aptamer specific for mutated MUC1 mucin that is over expressed in ovarian carcinoma. Doxorubicin was then attached to QD through a pH-sensitive hydrazone bond to provide long-term stability of the complex in systemic circulation and drug release in the acidic environment within tumor cells. The hydrazone bond is stable at neutral and slight basic pH and undergoes rapid hydrolysis at mildly acidic pH. *In vivo* studies showed that the QD conjugates had higher cytotoxicity than that of the free doxorubicin in cancer cells. More importantly, the QD conjugates were found to be preferably accumulated in the ovarian tumor. The study shows that the proposed QD conjugate has the potential for treating ovarian cancer *in vitro* and *in vivo*. [20]

III g) Use of water-dispersible CdTe QDs: Water-dispersible CdTe QDs coated with negatively charged 3-mercaptopropionic acid to enhance drug uptake into cancer cells. They reported that the MPA-coated CdTe QDs were able to facilitate the in-teraction of anticancer agent daunorubicin with leukemia cells and enhance the efficiency of biolabeling in cancer cells. Thus, that study demonstrated a potential method for simultaneous cellular inhibition and imaging of cancer cells [20]

III h) Enhance the efficient accumulation of anticancer drug daunorubicin in cancer cells through the combination with CdS QDs:

They reported that CdS QDs can readily bind with daunorubicin molecules on the membrane of the cells and promote the uptake of drug molecules in leukemia K562 cells. In addition, the competitive binding of both CdS QDs and anticancer drug to the membrane of leukemia K562 cells could efficiently prevent the drug release by leukemia cells and thus inhibit the possible multidrug resistance of cancer cells, which could be further utilized to improve the drug efficiency in respective tumor chemotherapies in the future [20].

III i) Delivery of more complex biomolecules: - Delivery of more complex biomolecules, such as short interfering RNA (siRNA) [84]. These short and double-stranded therapeutic RNA molecules function by blocking the expression of undesirable, disease-causing genes. However, in their free form they have high negative charge and are vulnerable to degradation in physiological fluids. Therefore, for optimal function *in vitro* and *vivo*, they must be delivered via electrostatic complexation with cationic nanocarriers Quantum dots/rods, appropriately surface functionalized with cationic moieties, are ideal siRNA carriers as they not only render these genetic drugs with physiological stability and target specificity, but also the whole complex (nanoplex) can be optically traced.

III j) Drug screening: A successful drug must be able to bind to several different molecular targets to achieve the desired effect, and steer clear of other targets to avoid side-effects. Testing could be made a simple matter by attaching quantum dots of different colors to the various targets. A good hit might be a drug that displaces blue, aqua and green Nanocrystals where you want it to attach, but doesn't displace red, yellow and orange ones at proteins that indicate side-effects. Described a quantum dots system for drug screening and studying mixed cell populations, consisting of encoding (different types of cells

are tagged with different-colored quantum dots), imaging and decoding single cells. QDs-cells can be used to potentially multiplex virtually any microscope-based cell assay with an optical readout. Typically HTS measures a single target and binary in its output (i.e., it shows if the unknown compound produces an effect or not). Using different-colored quantum dots to tag different target, compounds against multiple targets can be screened in parallel which is called Multi-target High-throughput Screening (MTHTS). Even if the effect on some target is not desired one, it could be of interest for another target. Most importantly, based on MTHTS, a lead with different effects, different leads with different effects or multiple leads with same effect can be gained from multiple screening models simultaneously in one screening. QDs-based multiplexing assay being used to drug high-throughput screening can enhance screening throughput, which can achieve bi-high-throughput multi- high throughput screening [31, 24].

IV) Drug delivery

1). ZnO QDs have also been evaluated as a platform for targeted and pH responsive Intracellular delivery of an anti-cancer drug. The cancer targeting feature is endowed by conjugating folic acid on to the surface of ZnO-NH₂ QDs *via* an amidation reaction. Doxorubicin (DOX) is then successfully loaded onto the folic acid functionalized ZnO QDs by capitalizing on its marked tendency towards the formation of metal complexes. Drug loaded ZnO-FA QDs remain stable at physiological pH but readily disintegrate in the mildly acidic intracellular environment of cancer cells as validated by a drug release profile, confocal microscopy and a cell-cytotoxicity assay. Compared to the conventional drug nanovector, ZnO-FA QDs themselves manifest a significant therapeutic activity after reaching their targeted site, therefore, combined DOX and ZnO QDs can be more efficacious than either alone. Hence, this approach provides a valuable ZnO QDs-based nanovector that can simultaneously realize targeting, diagnosis, and therapy of cancer cells.

2). Scientists in Switzerland studied that giving quantum dots an icing-like cap of certain sugars makes the nanoparticles accumulate in the liver but not other parts of the body. That selective targeting could be used to deliver anti-cancer drugs to one organ, without causing the body-wide side-effects that occur with existing cancer drugs, they suggest. They described development of a new type of quantum dot coated with certain sugar molecules that are attracted to receptors in specific tissues and organs. In a study with laboratory mice, the scientists coated quantum dots with either mannose or galactosamine, two sugars that accumulate selectively in the liver. The sugar-coated dots became three times more concentrated in the mice livers than the regular dots, demonstrating their higher specificity, the researchers say.

3). The potential of using a nanoparticle drug delivery approach provides a novel therapeutic strategy for treating lung diseases. The ability to target specific cells in the lung without exposing other pulmonary tissue or distant organs to detrimental actions of drugs is an exciting avenue to explore the ability to provide targeted therapeutic delivery in the lung would be a major advancement in pharmacological treatments for many pulmonary diseases. Critical issues for such successful delivery would require the ability to target specific cell types, minimize toxicity (e.g. inflammatory response), and deliver therapeutic levels of drugs. Our *in vitro* findings demonstrate that QD-Dox enhances intracellular uptake compared with free drug. We also demonstrate that Dox is released from the QD Dox formulation and migrates to the nucleus (site of bioactivity), whereas the QDs remain in the cytosol. QDs as a carrier system present distinct advantages of having both diagnostic and therapeutic benefits. The ease of formulation and uniformity of QDs make a more efficient approach to lung cell targeting.

4). Adeli *et al.* of Lorestan University and Tehran University of Medical Sciences, Iran, synthesized pseudo polyrotaxanes (Ps-PR) consisting of α -cyclodextrin rings, polyethylene glycol axes and end triazine groups. Dissociation of the α -cyclodextrin rings from the polyethylene glycol

axes was avoided by the host–guest relationship between its end triazine groups and β -cyclodextrins conjugated onto the surface of quantum dots (β -CD-*graft*-QDs), leading to a new type of the dynamic polyrotaxanes in which QDs play the role of stoppers noncovalently. Stability of the synthesized supramolecules was depended on the efficiency of the host–guest relationships between the end triazine groups of Ps-PR and β -CD-*graft*-QDs through which release of α -cyclodextrin rings from the polyethylene glycol axes was controlled.

5) After quantum dots are bioconjugated protein or peptide, single-molecule movement in single living cell can be tracked in real time. Another study provided new insight into erbB/HER receptor-mediated signal transduction. This study demonstrated that EGF-QDs (quantum dots bearing epidermal growth factor) were highly specific and potent in the binding and activation of the receptor (erbB1), being rapidly internalized into endosomes that exhibit active trafficking and extensive fusion. Similarly, when drug molecules are linked to the surface of quantum dots,

the kinetics and transport of drug molecules can be recorded and tracked for a longer period of time, which help to understand the mechanism of diffusion, particle fusion and internalization into cells. The movement of different drug molecules tagged with quantum dots of different colors can also be studied simultaneously. In addition, the elaborate DDS that consist of drug molecules, quantum dots and target molecules (e.g. antibody or peptide) can be designed. After the DDS are transported into cancer cell guided by target molecules, under UV irradiation momentarily the photoluminescence of quantum dots trigger the DDS, and drug molecules are released into cancer cells and kill them. Furthermore, under UV irradiation continuously, quantum dots behave photocatalysis of semiconductor nanocrystals. On the surface of quantum dots photochemical reactions take place resulting in a production of the cytotoxic singlet oxygen (O_2^-), which causing biomembrane of cancer cell oxidation and degradation [24].

Selected QD-based medical applications [32]:

S. No	Application area	Description	References
1	Diagnostics	<ul style="list-style-type: none"> • Detection of Her2 (hairy-related 2) on SK-BR-3 breast cancer cells by employing humanized anti-Her2 antibody, a biotinylated goat antihuman IgG, and streptavidin-coated QDs • Immunofluorescence labeling of mortalin using QDs showed different staining patterns between normal and cancer cells • Detection of ovarian cancer marker CA 125 in various specimens using streptavidin-conjugated QDs • Fluoroimmunoassay for the detection of prostate-specific antigen using streptavidin-coated QDs • QD-based FISH labeling was used to detect specific repeats in the Y chromosome in fixed human sperm cells • Antibody-conjugated QDs were used to detect prostate cancer cell marker PSA, the QD conjugates detected the tumor site in mice transplanted with human prostate cancer cells 	

Continued

2	Imaging	<ul style="list-style-type: none"> • Imaging skin and adipose tissues in mice by injection of water-soluble QDs • Mapping sentinel lymph nodes at 1 cm tissue depth using oligomeric phosphine-coated QDs that emit in the near-infrared region • Tracking diffusion dynamics of glycine receptors using QDs 	
3	Drug delivery and therapeutics	<ul style="list-style-type: none"> • Surface-modified CdS QDs were used as chemically removable caps to retain drug molecules and neurotransmitters inside a mesoporous silica nanosphere-based system • QDs showed potential in use as photosensitizers or to excite other photosensitizers in photodynamic therapy • Screening of siRNA sequences and monitoring RNAi delivery using QD–siRNA conjugates 	

V) Immunoassays

Härmä *et al.* [33] developed a fluoroimmunoassay for the detection of prostate-specific antigen (PSA). The assay employed 107 nm streptavidin-coated QDs consisting of β -diketones entrapping N30,000 europium molecules. The assay achieved a detection limit of 0.38 ng/L for biotinylated PSA and employed a time-resolved fluorometer for signal detection. PSA detection was achieved in both solid and liquid phases, and visualization of individual PSA molecules was also possible with the use of a fluorescence microscope.

VI) Other in vitro applications:

In a utilization of QDs' multiplexing potential, QDs of different sizes were embedded into polymeric microbeads [34, 35, 36, 37]. This allows creation of optical barcodes for analytes. Theoretically, the use of just ten intensity levels and six colors could result in coding for over a million nucleic acids or protein targets [37]. The optical bar-coding system utilizing QDs embedded in polymeric microbeads has an accuracy of 99.9% due to the high reproducibility in production of the beads [36, 37, 38]; up to 40,000 assays may be run simultaneously using the current capabilities of the QD technology [34, 36].

VII) Proteomic and genomic applications:

For applications requiring more than four readout fluorescence signals, where the use of conventional dyes becomes a significant challenge, QDs may offer the solution [39]. For example, in case of multiplex detection, the signals of conventional dyes may overlap due to their broad emission spectra, which is not the case with QDs that generate intense emission bands. The ability of QDs to undergo FRET was utilized to monitor telomere formation *in vitro* [40]. The QDs are conjugated to a DNA template which is then mixed with C,G, and A dNTPs, in addition to dUTPs labeled with Texas Red acceptor. As telomerization proceeds, the Texas Red acceptor is brought into a distance from the QDs where FRET is undergone, which can be monitored and reflects the telomerase activity. QDs can be used for profiling of microRNA (miRNA, single-stranded RNA molecules that have 21–23 nucleotides and play a role in gene expression). In one such study, miRNAs were labeled with biotin and hybridized with complementary oligo-DNA probes immobilized on glass slides. They were detected by measuring fluorescence of streptavidin-labeled QDs bound to miRNAs [41]. QDs conjugated to small interfering RNA (siRNA) can also be prepared

and utilized for monitoring delivery of siRNA sequences and the consequent RNA interference, giving more insight into gene silencing mechanisms [42, 43].

VIII) Quantum dots detect viral infections:

Quantum dots are multi-colored, microscopic fluorescent beads, which bind to molecular structures that are unique to the virus's coat and the cells that it infects. In a paper appearing in the June issue of the journal Nanoletters, the Vanderbilt researchers report that not only can a quantum dot system detect the presence of particles of the respiratory syncytial virus (RSV) in a matter of hours, rather than the two to five days required by current tests, but it is also more sensitive, allowing it to detect the virus earlier in the course of an infection [44].

IX) QDs Related Photodynamic Therapy for Cancer:

Presently, the conventional types of cancer treatment (chemotherapy and radiation therapy), work by destroying fast-growing cells, but other types of fast-growing healthy cells (such as blood and hair cells) also can be damaged along with cancer cells, causing adverse reactions, or side effects. These side effects can range from fatigue and flu-like symptoms to hair loss and blood

clotting problems. PDT developed in last century has become an FDA-approved therapy for different malignancies and with potential in other ailments such as coronary heart disease, AIDS and psoriasis. Exploration of the use of light-activated drugs known as photosensitizers (PS) has been one of the most active areas of photomedical research in recent years. PDT uses the combination of a photosensitizing drug and light in the presence of oxygen to cause selective damage to the targeting tissue. During PDT, reactive oxygen intermediates (ROI) is generated in the diseased cells by a simple and controllable light-activated process, which involves a photosensitizer that is capable of absorbing light appropriate wavelength and transfers energy or electron to oxygen or other molecules, and creates ROI such as singlet oxygen (1O_2), hydroxylradical (OH), super oxide anion(O_2^-) and hydrogen peroxide (H_2O_2). Then ROI will immediately react with vital biomolecules in cell organelles, leading to cell damage, mutation, death and photooxidation of cell constituents. Singlet oxygen (1O_2) is regarded as the main mediator of photo-induced cytotoxicity in PDT, which causes oxidation and degradation of cellular components, and ultimately cell apoptosis [45].

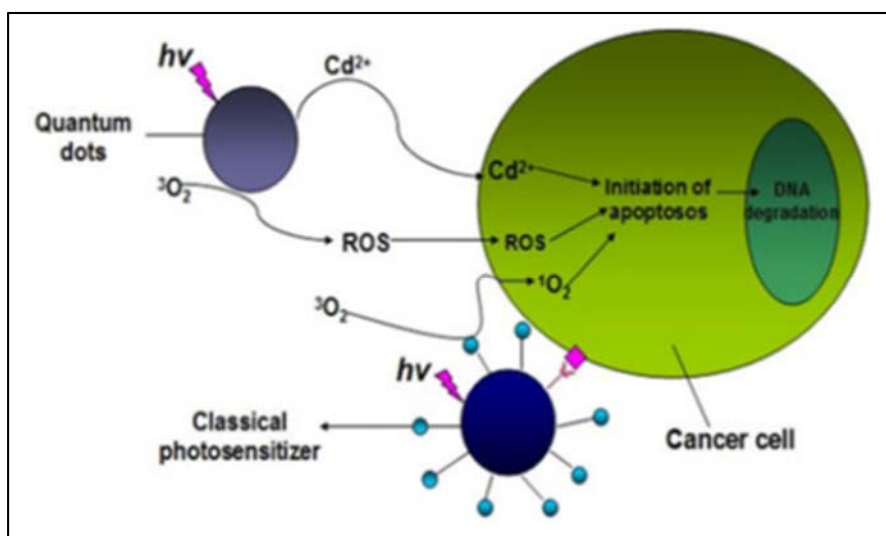


Fig 6: Schematic representation of possible mechanisms for induction of PDT processes by QDs and the classical photosensitizer.

X) Cytotoxicity

Cytotoxicity of QDs has been observed in a large number of in vitro studies, affecting cell growth and viability. The extent of cytotoxicity has been found to be dependent upon a number of factors including size, capping materials, colour, dose of QDs, surface chemistry, coating bioactivity and processing parameters. A number of mechanisms have been postulated to be responsible for QD cytotoxicity. These include desorption of free Cd (QD core degradation), free radical formation, and interaction of QDs with intracellular components. Examination of QD toxicity in a hepatocyte culture model showed that exposure of core CdSe to an oxidative environment causes decomposition and desorption of Cd ions. Such exposure during synthesis and processing played an important role in subsequent toxicity. Addition of a silica (SiO₂) and ZnS shell can reduce oxidation, but is unable to eliminate it, particularly under concomitant exposure to UV light. The addition of ligand shells has also been observed to reduce Cd desorption, but again is unable to eliminate it under oxidative conditions, and ligand addition brings its own attendant problems. The generation of free radicals, particularly reactive oxygen species has also been seen to contribute to toxicity. In addition to the effects of the QD core, ligands added to render the probe biologically active may have toxic effects on cells. Mercaptopropionic acid (MPA) and mercaptoacetic acid, which are commonly used for solubilisation, have both been shown to be

mildly cytotoxic. MUA, cysteamine and TOPO have all been shown to have the ability to damage DNA in the absence of the QD core. PEGylated QDs have been shown to have reduced cytotoxicity, but modification of these to produce PEG-amine for biological activity renders them cytotoxic once again. Unfortunately, interpretation of information on cytotoxicity is difficult as a result of differences in cellular handling of QDs and the possible contribution of unexpected factors to toxicity. The reduced cytotoxicity seen with QD-PEG compared with unmodified QDs has been found to be related to reduced uptake of these modified QDs, and not necessarily to an inherently reduced toxicity. The way in which QDs are handled by cells after uptake is also variable, and different intracellular fates are likely to contribute to different toxicity. With the limited data accumulated so far it is very difficult to estimate the true extent of QD cytotoxicity, which factors contribute, and the effects they may have. Groups III–V QDs may provide a more stable alternative to groups II–VI QDs due to the presence of a covalent, rather than an ionic, bond, and have been reported to have lower cytotoxicity. However these QDs are difficult to prepare on a competitive time scale, and tend to have much lower quantum efficiencies, meaning uptake has been slow. Data relating to cytotoxicity is understandably much more limited for these QDs, making it difficult to draw firm conclusions, and comment either way [46].

XI) Selected studies on QD toxicity [32]:

QD	Model used	QD concentration	Duration of exposure	Toxic effects	Toxicity/ Stability attribution	Reference
CdSe/ZnS -SSA	EL-4 lymphoma cells	0.1–0.4 mg/mL	0–24 h	Cytotoxic: 0.1 mg/mL altered cell growth. Most cells were non-viable at 0.4 mg/mL	QD capping material	

Continued

CdSe/ZnS -MUA	WTK1 cells a	1–2 μ M	12 h	2 μ M QD– COOH induced DNA damage at 2 h. DNA repair on prolonged incubation (12 h)	QD hydrophilic coating. It may be detached under acidic and oxidative conditions in the cell endosomes
CdTe	Rat pheochromocyto ma cells, murine microglial cells	0.01–100 μ g/mL	2–24 h	10 μ g/mL cytotoxic	QD core degradation and release of Cd ions
CdSe/ZnS – AMPcoate d QDs, and conjugated to methoxy terminated PEGamine (mPEGQD s)	Mice	Injections; ~180 nM QD, ~20 pmol QD/g animal weight	15-min cell incubation s, 1–133 days in vivo	No signs of localized necrosis at the sites of deposition	QD stability attributed to amphiphilic coating
Avidin- conjugated CdSe/ZnS QDs	HeLa cells	0.5 to 1.0 μ M	15 min	No effect on cell growth, development	—

XII) Removal of quantum dots toxicity:

The usefulness of quantum dots comes from their peak emission frequency's extreme sensitivity – quantum mechanical in nature - to both the dot's size and composition. QDs have been touted as possible replacements for organic dyes in the imaging of biological systems, due to their excellent fluorescent properties, good chemical stability, broad excitation ranges and high photobleaching thresholds. However, the main

drawback of QDs is their toxicity and therefore their application is problematic. If this toxicity problem could be addressed, QDs may one day be safely utilized in many areas. For instance, cadmium telluride (CdTe - which is toxic) QD based nanocomposites can be used as fluorescent probes for biological imaging, they can also be utilized to monitor targeted drug delivery and for controlled modification of structural and functional properties of intracellular components.

Scientists in Ireland have been using gelatin during the production of CdTe QDs thereby reducing the toxicity of the particles. Their approach could be useful for the development of other nanoparticle composites with low toxicity as well [47].

XIII) Evaluating multiple biomarkers

Quantum dots linked to biological molecules, such as antibodies, have shown promise as a new tool for detecting and quantifying a wide variety of cancer-associated molecules. In general, quantum dot preparation takes approximately one day. Clinical assays take an additional one to three days, depending on the number of biomarkers being assessed simultaneously. Because quantum dots come in a variety of colors, it is possible to use a uniquely colored quantum dot for each biomarker being assayed. Multiplexed imaging and computer aided analysis of the resulting fluorescence emitted by the quantum dots then provides quantitative results for each biomarker [48].

XIV) Quantum Dot Products [49]

1) EviDots® - Core & core-shell quantum dots
EviDots are available as core quantum dots in their fundamental state, or enhanced with our proprietary coating technologies as core-shell semiconductor nanocrystal quantum dots. EviDots are available in wavelengths ranging from 490nm - 2100nm.

2) EviComposites™ - Quantum dot composites.
EviComposites use the properties of Evident's proprietary EviDot quantum dots as well as common insulating polymer matrix materials.

3) EviTags™ - Water soluble quantum dots.
EviTags are conjugation-ready with a bio-active surface. Carboxyl or amine functionalized dots are available in wavelengths ranging from 490 nm – 680 nm.

4) EviFluors® - Water soluble quantum dots conjugated to antibodies and proteins. EviFluors are ready-to-use high quality, activated quantum dots coupled to secondary antibodies and

proteins. Goat anti-Mouse, Goat anti-Rabbit, Goat anti-Rat, Streptavidin, and Biotin conjugated quantum dots are available in wavelengths ranging from 520 nm -680 nm.

XV) Conclusion

QDs play an important role in fundamental biology and *in vitro* disease diagnostics and prognostics as potent imaging probes. QDs can make a worthy contribution to the development of new diagnostic and delivery systems due to their unique optical properties. Their unique structural and surface properties, such as their tunable and uniform size, flexible drug linking and doping mechanisms, large surface to- volume ratio and wide spectrum of surface reactive groups have enabled a new avenue of research to be opened: targeted and traceable drug delivery. QDs as a novel probe for *in vivo* analysis and clinic therapy such as PDT open an attractive new field with promising prospective in biomedicine. Throwing in the unresolved toxicity issues of QDs, it may be a couple of decades before we see any QD-based drug delivery or *in vivo* imaging systems on the market. Despite all their promise, QDs are still away from large scale use in nanomedicine, pending the resolution of toxicity concerns and regulatory and commercialization issues. These intelligent, multifunctional, low- or non-toxic nanomachines are only a few possible achievements for the future. With advances being made in the identification of new targeting ligands, the development of specialized nanoparticles and the discovery of elegant conjugation techniques, the QD-based bionanotechnology will be constantly expanding its list of amazing applications.

XVI) Future Outlook

In the near future, we envision that the QD/drug nanoparticle formulations will gain wide interest in many healthcare-related research areas. For example, the developed formulations can be used for early cancer detection and therapy. Also, the formulations can be systematically tailored for personalized drug treatment more importantly, additional modalities such as magnetic resonance imaging and positron emission tomography

contrast agents can be integrated into the QD/drug formulations, thus allowing one to use two or more imaging modalities to verify the bio distribution and efficacy of the drug *in vivo*. We believe that in the next few years there will be a tremendous growth in developing QD/drug nanoparticle formulations for therapeutic and diagnostic applications.

XVII. References

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