Expert Opinion

Emerging application of quantum dots for drug delivery and therapy

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Quantum dots have proven themselves as powerful fluorescent probes, especially for long-term, multiplexed, and quantitative imaging and detection. Newly engineered quantum dots with integrated targeting, imaging and therapeutic functionalities have become excellent material to study drug delivery in cells and small animals. This fluorescent 'prototype' will provide important information in the rational design of biocompatible drug carriers and will serve as a superior alternative to magnetic and radioactive imaging contrast agents in preclinical drug screening, validation and delivery research. This *Editorial* article is not intended to offer a comprehensive review on drug delivery, but to highlight the breakthroughs in the emerging applications of quantum dots in this field and to provide our perspective on future research.

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1. Introduction

Semiconductor nanocrystals, also known as quantum dots (QDs), have become an indispensable tool in biomedical research, especially for multiplexed, quantitative and long-term fluorescence imaging and detection [1-4]. The basic rationale for using QDs arises from their unique and fascinating optical properties that are not generally available for individual molecules or bulk semiconductor solids. In comparison with conventional organic dyes and fluorescent proteins, QDs have distinctive characteristics such as size-tunable light emission, improved signal brightness, resistance against photobleaching 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 conditions [5-7]. This new generation of water-soluble QDs solved the problems of quantum yield decrease, chemical sensitivity and short shelf-life previously encountered by the ligand exchange-based QD solublization method [8]. As a result, these particles, linked with bioaffinity molecules, have raised new opportunities for ultrasensitive and multicolor imaging of molecular targets in living cells and animal models [5-7,9-11].

The success of using QDs in biological imaging, sensing and detection has encouraged scientists to further develop this technology for clinical and translational research. One of the most important emerging applications of QDs appears to be *traceable* drug delivery, because it has the potential to elucidate the pharmacokinetics and pharmacodynamics of drug candidates and to provide the design principles for drug carrier engineering. Due to concerns about long-term *in vivo* toxicity and degradation, QDs are currently limited to cell and small animal uses. Nevertheless, traceable delivery of therapeutics in cells and animals still has a big impact on life science research, such as drug discovery, validation

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and delivery. This is because cells and small animals are used extensively in the testing of drug candidates. Following drug molecules or drug carriers non-invasively and in real time in live organisms requires specialized imaging techniques. Compared with traditional imaging modalities such as MRI and positron emission tomography, optical imaging is highly sensitive, quantitative, capable of multiplexing and is significantly cheaper, which will reduce the cost and shorten the time involved in new drug development substantially. Therefore, for nano-carrier development and optimization, QDs can become an excellent 'prototype', from which bio-compatible carriers of similar sizes and surface properties can be made for clinical uses. Current applications of QDs in drug delivery are focused on two major areas: using QDs as carriers and labeling therapeutics or drug carriers with QDs.

2. 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 keeping 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 1**). 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.

Towards this ambitious goal, a few reports have appeared recently. Yamomoto *et al.* have conjugated captopril, an antihypertensive drug, to the QD surface and studied its pharmacodynamics and pharmacokinetics in stroke-prone spontaneously hypertensive rats [13]. The results show that the administered QD–captopril conjugates are capable of decreasing rat blood pressure to the same extent as the captopril alone in the first 30 min, but the therapeutic effect of QD–captopril disappears after 60 min. This short therapeutic time window was suggested to be as a result of non-specific uptake by macrophages and endothelial cells. Additionally, it is unclear whether the therapeutic effect results from the QD–captopril conjugates or captopril molecules detached from the QD surface. Further comparative experiments using covalently linked captopril molecules should provide a definitive answer. Another piece of interesting work was reported by Baqalkot *et al.* [14], wherein a targeting functionality was added to QDs by linking them with RNA aptamers (A10) that specifically bind to prostate specific membrane antigen (PSMA). Doxorubicin, a DNA-interacting drug widely used in chemotherapy, was immobilized onto QDs by intercalation within the A10 RNA aptamer. It was of interest that the resulting nano-complexes were non-fluorescent because of a 'Bi-FRET' mechanism – the QD fluorescence is quenched by the Doxorubicin molecules and subsequently the energy is relayed to the A10 aptamer. When the nano-complexes are uptaken by PSMA-positive cells, the slow release of Doxorubicin results in recovery of both QD fluorescence and Doxorubicin fluorescence, which can be monitored by confocal microscopy. Evaluation of the therapeutic effect on PSMA-positive LNCaP cells and PSMA-negative PC3 cells treated with the nano-complex showed a 30% difference in cell viability, which could potentially be improved by controlling the kinetics of Doxorubicin release. This design not only allows targeted delivery of chemotherapy drugs, but also provides a sensing mechanism for drug release.

Bhatia *et al.* recently reported on siRNA delivery using QDs as delivery vehicles [15]. Targeting peptides and siRNAs were conjugated to QDs in a 'parallel' manner. That is, the targeting peptide and siRNA were synthesized separately and simultaneously linked to the QD surface. We envision that alternative 'serial' attachment (e.g., siRNA molecule and targeting aptamer are synthesized as a chimera and linked to QD surface) may also work well in light of the results reported by McNamara *et al.* [16]. As a proof-of-principle in the Bhatia report, siRNA sequence targeting eGFP expression and a tumor-homing peptide (F3 peptide) targeting cell surface nucleolin were conjugated to the surface of QDs. A key finding was that the siRNA molecules should be linked to the QD surface via *cleavable* chemical bonds for the siRNA molecules to function effectively (siRNA must be released from the nanoparticle first), which is a different finding from the results previously reported by Medarova *et al.* using iron oxide nanoparticles with covalently linked non-cleavable siRNA [17]. These contradictory results require further systematic investigation. It is important to note the size similarity between QDs and siRNA

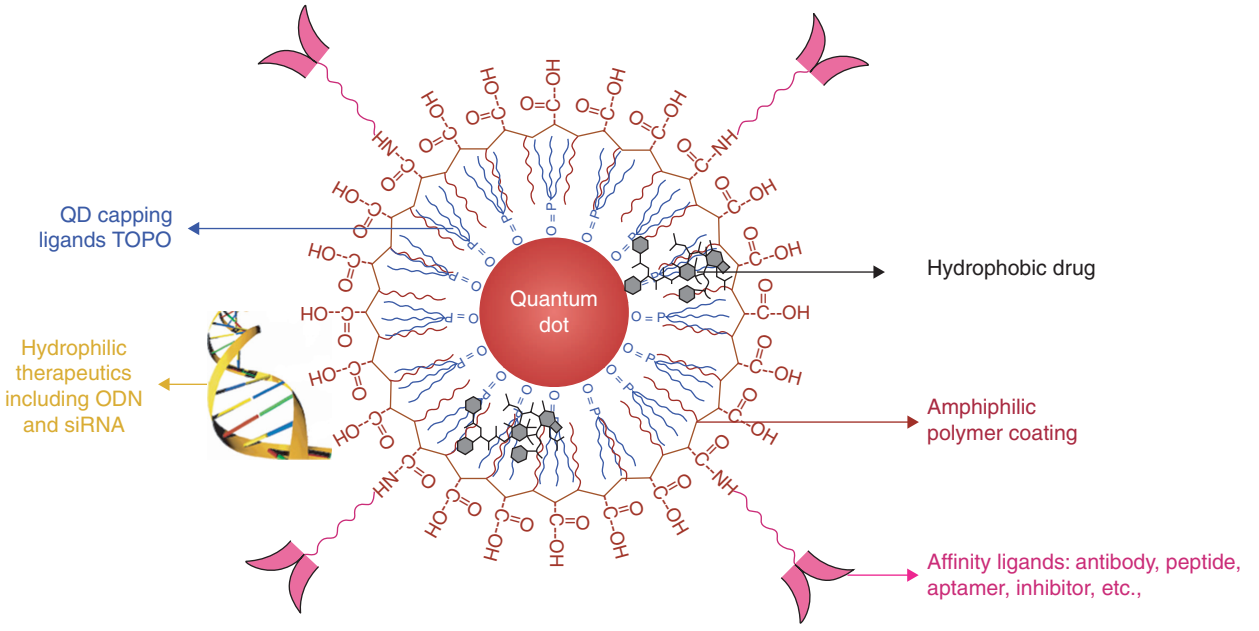

Figure 1. Schematic illustration of a multifunctional quantum dot coated with amphiphilic polymer. Hydrophobic therapeutics can be trapped between the hydrophobic nanoparticle core and amphiphilic polymer coating layer, and hydrophilic targeting molecules and therapeutic compounds can be immobilized on the outer surface.
ODN: Oligodeoxynucleotide; QD: Quantum dot; siRNA: Small interfering RNA; TOPO: Tri-n-octylphosphine oxide.

molecules, which is one of the determining factors for successful delivery of siRNA *in vitro* and *in vivo*. Larger biomolecules, such as plasmid DNA, will probably require many QDs to work in synergy for efficient transfection [18].

3. 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 [19-22], with a burst of activity in the area of ODN and siRNA delivery.

Chen *et al.* have reported a siRNA delivery approach by co-transfection of siRNA and QDs with Lipofectamine™ 2000 [19]. Small interfering RNA was first condensed on the cationic membrane following standard cell transfection protocol, and the lipoplex was further incubated with fluorescent QDs. 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 concentrations. Western blotting data suggested that the correlation between optimal fluorescence and gene silencing for the least amount of QDs occurs at a 1:1 ratio (in mass) between QDs and Lipofectamine. Lower concentrations of QDs fail to provide detectable fluorescence, whereas excess QDs reduce the silencing efficiency. The investigators speculated that the excess QDs, which are also negatively charged similar to the siRNA molecules, compete with siRNA in binding to Lipofectamine. Since it has been proposed that oligonucleotides are probably entrapped between lipid bilayers of multilamellar vesicles [23], it is also possible that the excess QDs simply neutralize the positive charge on Lipofectamine and consequently reduce its capability to bind to the cell surface. Similarly, Zhang *et al.* have synthesized highly uniform QD-doped chitosan nanobeads for traceable siRNA delivery [20], and most recently Jia *et al.* have combined PEI-coated carbon nanotubes with QDs for antisense ODN delivery [21]. These innovative approaches have opened up exciting opportunities in targeted DNA and RNA delivery. For example, after being treated with QD–oligonucleotides, cells with differential expression levels of the protein of interest, which correlates with QD fluorescence, can be isolated using fluorescence-activated cell sorting;

and, if multicolor QDs are used, it will allow the screening of siRNA sequences and the monitoring of downstream cell behaviors in a multiplexed manner.

4. Expert opinion

As powerful imaging probes, QDs have already played an important role in fundamental biology and *in vitro* disease diagnostics and prognostics. 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. However, high-quality QDs (visible and near infrared dots with a narrow emission profile and high quantum yield) are mainly made with heavy metals whose long-term toxicity are largely unknown at the current time. Despite this limitation, QDs have been applied to cells and small animals as drug carriers, serving as an outstanding discovery tool for drug screening and validation, and as prototype materials for drug carrier engineering. If high-quality QDs can be prepared from relatively non-toxic compounds (e.g., silicon and carbon), or if the toxic components can be inertly protected from exposure and subsequently cleared from the body, then the clinical relevance of QDs could be foreseeable. One primary challenge of drug delivery is maintaining a useful concentration of the drug in the targeted tissue while preventing toxicity. How to achieve this therapeutic window has not been studied with QDs thus far, and requires systematic investigation. Ideally, the engineered QDs would be able to stabilize therapeutic compounds, increase their plasma circulation time while reducing the concentration of free drug to minimize unwanted side effects, and to release the drug with a well-controlled profile. In addition, the targeting and therapeutic compounds may be covalently linked to the QD surface via cleavable chemical bonds, so that the bioconjugates are initially large enough to avoid renal filtration, and subsequently, after the ligands are cleaved, small enough to be cleared out of the body [12]. These intelligent, multi-functional, 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.

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