Traceable Drug Delivery: Lighting The Way With Qdots

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Semiconductor nanocrystals, also known as quantum dots (Qdots), have become an indispensable tool in biomedical research, especially for multiplexed, quantitative, and long-term fluorescence imaging and detection.[1-4] The basic rationale of using Qdots arises from their unique and fascinating optical properties that are generally not available for individual molecules or bulk semiconductor solids. In comparison with conventional organic dyes and fluorescent proteins, Qdots 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 Qdots solved the problems of quantum yield decrease, chemical sensitivity, and short shelf-life previously encountered by the ligand exchange-based Qdot solublization method.[8] As a result, these particles, linked with bioaffinity molecules, have raised new opportunities for multicolor molecular imaging in living cells and animal models, as well as for traceable drug delivery.[5-7, 9-11]

Traceable drug delivery

Traceable drug delivery has the potential to elucidate the pharmacokinetics and pharmacodynamics of drug candidates and to provide the design principles for drug carrier engineering. Due to the concerns of long-term *in vivo* toxicity and degradation, Qdots are currently limited to cell and small animal uses. Nevertheless, traceable therapeutics in cells and animals still has paramount impact on life

science research, such as drug discovery, validation, and delivery. This is because cells and small animals are used extensively in 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 magnetic resonance imaging (MRI) and positron emission tomography (PET), optical imaging is highly sensitive, quantitative, capable of multiplexing, and is significantly cheaper, which will reduced the cost and shorten the time of new drug development substantially. Therefore, for nano-carrier development and optimization, Qdots can become an excellent 'prototype', from which biocompatible carriers of similar sizes and surface properties can be made for clinical uses.

For drug delivery research, the importance of Qdots' structural properties has just been realized. First, the size of Qdots 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 the renal filtration;[12] whereas bigger particles are more likely to be uptaken by the reticuloendothelial system (RES) before reaching the targeted disease sites. Additionally, larger particles have limited penetration depth into solid tissues. Recent advance in Qdot nanocrystal synthesis will allow scientists to systematically assess this size effect on delivery efficiency and specificity and identify the optimal dimensions of drug carriers. Secondly, owing to the high surface-to-volume ratio of nanomaterials, it is possible to link multiple functionalities on single Qdots while keeping the overall size in the optimal range. For example, the Qdot 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. 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 also emit detectable signals for real-time monitoring of its trajectory.

siRNA delivery using Qdots

RNA interference (RNAi) is emerging as one of the most powerful technologies for sequence-specific suppression of genes and has potential applications ranging from functional gene analysis to therapeutics. Due to the relatively low immunogenic and oncologic effects, the development of non-viral delivery methods *in vitro* and in organisms is of considerable current interest. In recent years, a number of strategies have been developed based on liposomes, gold and silica nanoparticles (NPs), cationic and biodegradable polymers, and peptides.[13-23] The delivery efficiency, however, remains low, especially under *in vivo* conditions. Another limitation shared by all the existing delivery technologies is the lack of an intrinsic signal for long term and real-time imaging of siRNA transport and release.

We recently developed a new technology by combining QDs with another class of nanomaterial, amphipol, to for traceable and efficient delivery of siRNA molecules. Amphipols are linear polymers with alternating hydrophilic and hydrophobic side chains. They are widely used for solubilizing integral membrane proteins and delivering them into cell lipid bilayers.[24-28] Unlike detergent-based micelles, amphipols belt around the transmembrane domain of membrane proteins and do not disrupt the integrity of cell membrane during delivery. To our surprise, however, when amphipols are mixed with nanoparticles coated with hydrophobic surface ligands, these two types of nanomaterials form stable complexes that are not only capable of carrying siRNA molecules into cytoplasm but also protecting them from enzymatic degradation. Compared with the classic siRNA carriers such as Lipofectamine™ and polyetheleneimine, this new class of nanocarrier works in both serum-free and complete cell culture media, which is advantageous over Lipofectamine. It also outperforms polyethyleneimine in

gene silencing under both conditions with significantly reduced toxicity. In addition, the QDs also provide a bright and stable fluorescent signal for intracellular siRNA imaging (Figure 1).

Conclusion and Perspective

As a powerful imaging probe, Qdots have already played an important role in fundamental biology, and *in vitro* disease diagnostics and prognostics. Their unique structural and surface properties such as tunable and uniform size, flexible drug linking and doping mechanism, large surface-to-volume ratio,

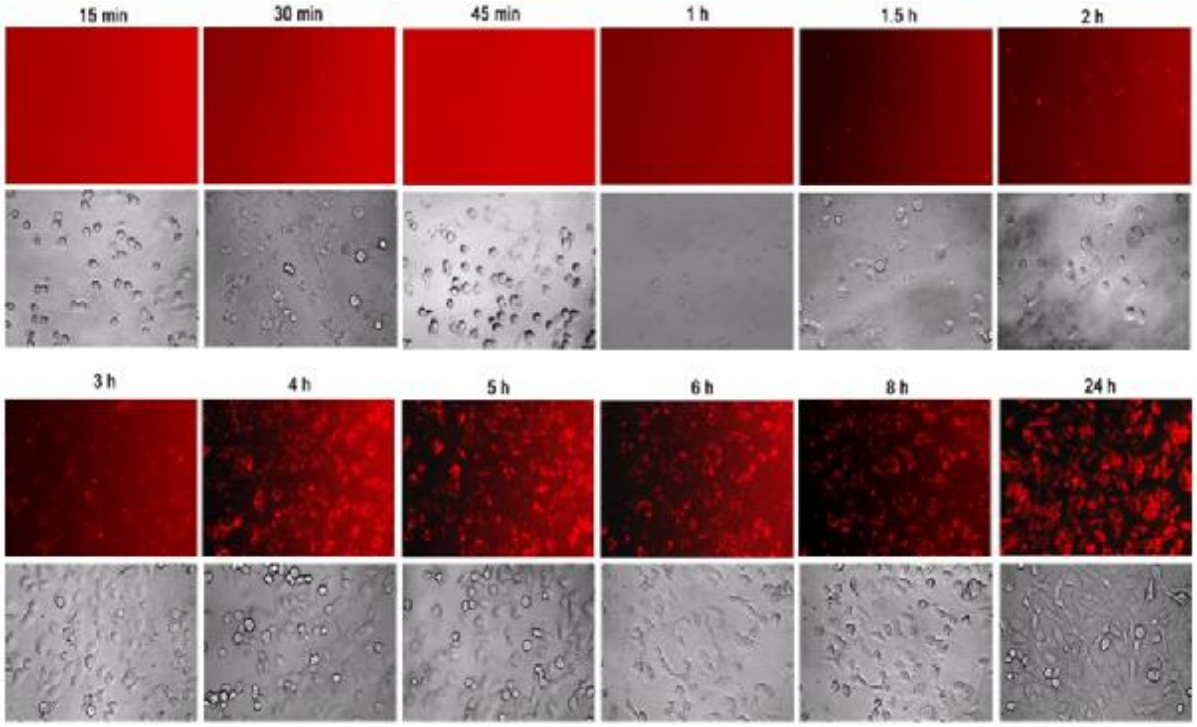

Figure 1. Time-dependent fluorescence imaging of QD-siRNA nanoparticle conjugates and their entry and transport in living cells. Images were obtained 15 minutes to 24 hours after the addition of QD-siRNA. Top panels are fluorescence images and bottom panels are the corresponding brightfield images.

and a wide spectrum of surface reactive groups have recently enable a new avenue of research, targeted and traceable drug delivery. However, high-quality Qdots (visible and near infrared dots with 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, Qdots have been applied to cells and

small animals as a drug carrier, which serves as an outstanding discovery tool for drug screening and validation, and as a prototype material for drug carrier engineering. If high-quality Qdots can be prepared from relatively nontoxic compounds (*e.g.*, silicon and carbon), or if the toxic components can be inertly protected from exposure and subsequently cleared from the body, then their clinical relevance could be foreseeable. Another 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 Qdots thus far, and requires systematic investigation. Ideally, the engineered Qdots should 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 also be covalently linked to Qdot surface via cleavable chemical bonds, so that the bioconjugates are initially large enough to avoid renal filtration, and later, after the ligands are cleaved, small enough to be cleared out of the body.

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