Research Focus

Semiconductor quantum dots as contrast agents for whole animal imaging

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Recent developments in quantum dot (QD) technology have paved the way for using QDs as optical contrast agents for *in vivo* imaging. Pioneering papers showed the use of QDs as luminescent contrast agents for imaging cancer and guiding cancer surgery. The possible future use of QDs for clinical applications is expected to have a significant impact, however many challenges in this field have yet to be overcome.

Advances in nanotechnology have led to the design and construction of structures at the nanometer scale, in a precisely controlled manner. Amongst these improved nanostructures, nanoprobes (particles <100 nm) have stimulated strong interest in the area of biological and clinical research. Unlike macroscale structures, nanoscale structures have optical and electronic properties that can be tuned by the structure's size, shape or material composition. These properties offer engineers, clinicians, and researchers an unlimited supply of precursor materials for the design of contrast agents for imaging applications, optical switches for triggering drug release, or for therapy. Previous work using nanoparticles made of gold, iron, semiconductors and various organic materials has already shown promising results for biomedical applications. Semiconductor nanocrystals or quantum dots (QDs) were used in cell labeling and immunoassays [1–3]; gold nanoparticles and carbon nanotube-based nanosensors can detect DNA, proteins, and other biomolecules [4–5]; and nanoparticles have been incorporated into a polymer matrix for controlled drug release [6]. *In vivo* applications of nanoparticles are starting to emerge. For example Ruoslahti and coworkers linked QDs to a peptide for labeling tumor vasculatures in live mice [7]; silica nanoparticles coated with gold nanoshells have photo-thermal capabilities for cancer treatment [8]; and iron magnetic particles were used to track progenitor cells *in vivo* using magnetic resonance imaging (MRI) [9]. Nie and coworkers reported the first simultaneous *in vivo* targeting and imaging of tumors in live animals using QDs tagged to antibodies [10].

QDs will probably be one of the first nanotechnologies to reach clinical applications. This is attributed to rapid progress in the synthesis of QDs, design of QD-surface coatings, and integration of QDs with biomolecules. In 1998, Alivisatos, Nie and coworkers demonstrated the first applications of QDs for biology [1–2]. Since those foundational papers, the use of QDs has been demonstrated in a host of biomedical applications as a substitute for fluorescent organic dye molecules. QDs are rapidly advancing as contrast agents for *in vivo* multiplexed molecular imaging, for which commercially available optical organic-based contrast agents are limited by their spectral properties [e.g. low quantum yield in the near-infrared (IR) emission region]. Work published by Ruoslahti, Frangioni, and Nie and their coworkers clearly showed the huge potential of QDs as contrast agents for *in vivo* cancer detection and imaging [7,10–12].

Design of QD-targeting probes

Overall design

Figure 1 shows the overall design of a QD-targeting probe. Single nanometer-sized QDs ($\sim$2–6 nm) are coated with targeting biomolecules (e.g. antibodies or peptides) and injected into animals. The targeting molecule directs the QDs to the diseased site. The accumulation of QD-target probes in the diseased site appears as a bright and distinguished stain (compared with the rest of the body) after illumination, pinpointing the location of the disease. Multi-QD labeling of the diseased site (by coating QDs with targeting molecules of different emission wavelengths) can provide more information on the state and evolution of the disease.

QDs for vivo *imaging*

Fluorescent probes that emit in the near-IR region (700–2000 nm) are ideal for *in vivo* imaging [13]. In this region of the electromagnetic spectrum there is low tissue scattering and absorption, yielding the greatest tissue penetration depth and optical signal. Biocompatible near-IR-emitting organic fluorophores suffer from low quantum yield, broad emission spectra, and inability to multiplex. QDs offer an excellent alternative to organic fluorophores for *in vivo* animal imaging. Near-IR QDs can potentially be designed to have high quantum yields and molar absorption coefficients, leading to a highly luminescent and useful *in vivo* contrast agent. In fact, high quantum yield near-IR organic soluble QDs have already been designed – a major hurdle is the preservation of the advantageous optical properties of organic-soluble near-IR QDs after surface modification with biocompatible coatings. Thus far the highest reported quantum yield for biocompatible near-IR-emitting QDs made from a cadmium–selenium/cadmium–tellurium (CdSe/CdTe) alloy

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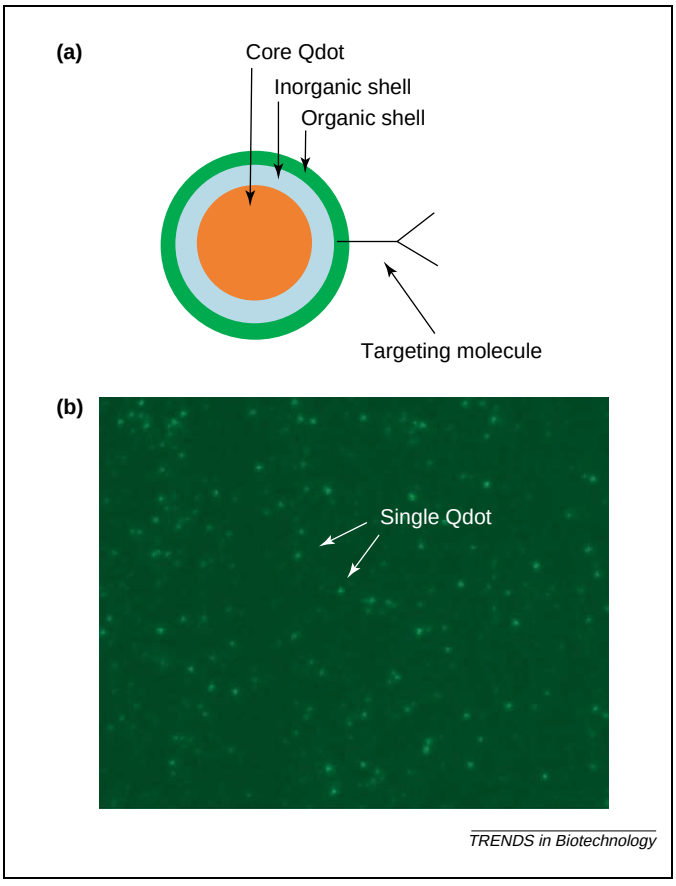

Figure 1. Schematic and optical image of biocompatible quantum dots (QDs). (**a**) Schematic depicting the general design of QD-target probes. The general design of current QD probes for bioimaging consists of the core semiconductor QD (e.g. CdSe) with an inorganic and organic shell (e.g. ZnS and amphiphilic copolymer, respectively). The inorganic shell improves the optical properties of the core QDs (e.g. increase in quantum yield) and minimizes the cytotoxicity induced by the core QD after photodegradation. The organic shell permits the QDs to interface with biological systems and provides reactive functional groups for conjugation to targeting molecules. The core is ~2–6 nm, the inorganic shell is ~1–4 nm, and the organic shell is ~1–30 nm. (**b**) Optical image of single biocompatible ZnS-capped CdSe QDs under ambient conditions. The QDs were spread on a coverslip and imaged with an oil immersion 100×objective (Olympus IX71, λ_{ex}=480+/−40 nm (100 W Hg lamp), λ_{em}=535+/−50 nm). Each QD is ~4.0 nm.

composition is 17% [12]. Other problems with current biocompatible near-IR QDs are broad emission spectra and lack of photostability compared with visible light-emitting QDs.

Although the design of high quality near-IR QDs is in its early stages, techniques for the synthesis, coating, and bioconjugation of visible light-emitting QDs are well developed and could be a model system for designing near-IR QDs for animal imaging. For example, studies by Nie *et al.* [10] and Ballou *et al.* [14] show that specific polymer coatings (e.g. polyethylene glycol) on QDs can enhance both circulation time and luminescence emission when injected into live animals and excited with a light source, respectively. These studies can be referred to when engineering the surface chemistry of near-IR QDs for animal imaging applications.

Targeting molecules for directing QDs

Directing QDs to a specific organ or diseased site *in vivo* requires the presence of targeting molecules that guide these nanoprobes. Antibodies have been used widely as recognition molecules for tumor imaging. Wu and coworkers used QD–immunoglobulin G conjugates to label human breast cancer cells that overexpress the receptor Her2 [3], Nie's group used prostate-specific membrane antigen (PSMA)-specific monoclonal antibodies to target QDs to prostate tumors *in vivo* [10]. Small peptides or antibody fragments are also available for targeting QDs. At present it is not clear whether antibody or peptide targeting is ideal for QD and nanoparticle targeting because each category of targeting molecule has advantages and disadvantages. Antibodies offer higher binding affinity values than peptides, but they will add size to nanoparticles (~5–30 nm). Furthermore, antibodies could limit the co-coating of other molecules (e.g. polymers) onto the surface of QDs. By contrast, peptides are smaller than antibodies – large libraries of targeting peptides for specific diseases can be identified using screening techniques, such as phage-display, and provide greater flexibility in QD surface engineering.

Integration of QDs with targeting molecules

Several techniques have been adapted toward the integration of QDs with targeting molecules [15]. When selecting a coating chemistry, QDs are generally modified with bifunctional or amphiphillic molecules (e.g. one end binding or interacting with the QD surface and the other, polar, end protruding from the surface). The protruding end has either a carboxylic acid or amine functional group. These functional groups can be linked to biomolecules using an organic catalyst (e.g. carbodiimide). These QD–biomolecule probes are then ready for use in bioimaging applications *in vitro* and *in vivo*.

Pharmacokinetics and toxicity studies

The possibility of using targeted QDs for biological applications and for animal imaging is exciting, however, data on the pharmacokinetics and toxicity of theses applications are lacking. QDs are composed of heavy toxic metal ions (e.g. Cd^{2+}); these metal ions can induce toxic effects *in vivo* (e.g. lung and kidney damage) [16]. But what are the side effects of QDs taking into account the fact that the metal ions are sequestered inside an organic shell and might not interact with biological tissues? Before the benefits of targeted QDs for imaging can be realized in clinical use, more information about their physiological effects must be gathered. Both Ruoshlahti [7] and Nie [15] and their coworkers showed some introductory pharmacokinetics studies of QDs in mouse models; while Derfus and coworkers showed the effect of QD-coating on cellular toxicity [17]. Furthermore, the doses of QDs needed for *in vivo* use are below the known toxicity levels for cadmium (one of the major components of the QDs used to date) [12]. However, cumulative effects have not been explored, nor have long-term effects, or how much cadmium release, if any, results from *in vivo* use of QDs with a coating intended to prevent oxidation. A detailed understanding of where nanoparticles (or their metabolites) go, at what rate, whether they are excreted or not and what influence their surface chemistry has, remain to be addressed. Before any QDs reach clinical application, these factors need to be thoroughly investigated.

Future considerations

The ability to design a wide variety of QDs and targeting molecules provides a new set of tools for engineering novel contrast probes for non-invasive imaging. As a research tool, these probes could have many uses, from monitoring tissue implants to studying the real-time dynamics of tumor metastasis. For clinical applications in humans, we envision the advancement of QDs as probes for non-invasive *in vivo* imaging of tumors or for visually guiding surgery. Although the initial groundwork of QDs for *in vivo* imaging has been laid out and demonstrated, there are many challenges ahead. These include: (i) design of high quality near-IR QDs, (ii) identification of targeting molecules; (iii) thorough understanding of the pharmacokinetics and toxicity of QD probes; and (iv) design of new instrumentation. Beyond QDs, the integration of QDs with other nanostructures for the construction of multifunctional nanosystems that can detect, image, and treat diseases could be possible in the future.

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The maturing of the human embryonic stem cell transcriptome profile

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Several recent reports used microarray, serial analysis of gene expression (SAGE), and expressed sequence tags (EST) strategies to characterize the human embryonic stem cell transcriptome and those of their differentiated derivatives. All three approaches yielded valuable data and highlight the fact that a large percentage of genes remain uncharacterized in these cells representative of early human development.

Realizing the full clinical potential of human embryonic stem cells (hESC) will undoubtedly benefit from a thorough cataloguing of gene expression in these cells and their differentiating derivatives (i.e. descendants of hESC representing transient cell types found in early development). Such a transcriptome characterization would provide insights into the genetic regulatory networks involved in maintaining pluripotency and directing differentiation. Until now the relatively recent establishment of hESC lines, the first in 1998 [1] and several others since then (http://www.isscr.org/science/sclines.htm) combined with the inaccessibility of human embryonic tissue has resulted in a lack of transcriptome information representative of the hESC and early human development particularly when compared with that of the mouse [2].

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