Research Focus

Molecular profiling of single cells and tissue specimens with quantum dots

Xiaohu Gao and Shuming Nie

Departments of Biomedical Engineering, Chemistry, Hematology and Oncology, Emory University and Georgia Institute of Technology, 1639 Pierce Drive, Suite 2001, Atlanta, GA 30322, USA

Quantum dots are tiny light-emitting particles on the nanometer scale. They are emerging as a new class of biological label with properties and applications that are not available with traditional organic dyes and fluorescent proteins. Recent advances, as reported in *Science* and *Nature Biotechnology*, have led to quantum dot bioconjugates that are highly luminescent and stable. These bioconjugates raise new possibilities for studying genes, proteins and drug targets in single cells, tissue specimens and even in living animals.

The integration of nanotechnology with biology and medicine is expected to produce major advances in medical diagnostics, therapeutics and bioengineering [1]. Recent research has demonstrated that functional nanoparticles (electronic, optical, magnetic or structural) can be linked to biological molecules, such as peptides, proteins or nucleic acids [2]. Because of their size-dependent properties and their dimensional similarity to biomacromolecules, these bioconjugates are well suited as optical or magnetic contrast agents for molecular imaging and profiling. Here we focus on new developments in semiconductor quantum dots (QDs) and their potential implications in molecular profiling of single cells and tissue specimens.

QDs possess many unique optical properties in comparison with traditional organic dyes, including size- and composition-tunable emission, large absorption cross sections, narrow emission spectra, wide absorption profiles and excellent photostability [3,4]. These properties have attracted considerable interest in the past 20 years, but most studies focused on the quantum confinement effect and potential applications in optoelectronics, QD lasers and high-density memory. In 1998, two breakthrough papers, one by Nie's group [5] and the other by Alivisatos's group [6], demonstrated that highly luminescent QDs could be made water soluble and biocompatible by surface modification and bioconjugation. Recently, Peng's group developed a procedure for preparing large quantities of QDs with fluorescence quantum yields as high as 85% at room temperature [7,8], nearly twice as bright as earlier QDs with 40–45% quantum yields. However, the biological applications of QDs were still limited by problems in surface chemistry and by the lack of widely (commercially) available materials. In the past six months, several independent papers have reported major improvements in surface chemistry and new results in biolabeling [9–12]. In another significant development, several companies have made high-quality QDs available in large quantities.

Surface chemistry

A key development is the use of amphiphilic compounds to coat the surface of hydrophobic QDs. In one method, QDs are solubilized with an octylamine-modified polyacrylic polymer [9]. The hydrophobic alkyl side chains strongly interact with tri-*n*-octylphosphine oxide (TOPO) on the QD surface, and the hydrophilic carboxylic acid groups face outward and render QDs water soluble. In another method, QDs are coated with a polyethylene glycol (PEG)–lipid layer, which has an amphiphilic surfactant structure [10]. The key feature shared by these two methods is that QDs are solubilized in aqueous solution without replacing the coordinating ligand TOPO, which is important for maintaining the optical properties of QDs and for shielding the core from contact with the outside environment. A different approach was described by Jaiswal *et al.* [12], in which QDs are linked to biomolecules through electrostatic intereactions; this technique was first developed by Mattoussi *et al.*, using genetically engineered recombinant proteins [13].

Labeling single cells

Cellular labeling using organic dyes and fluorescent proteins has had great success, and state-of-the-art instrumentation currently allows simultaneous measurement of up to 13 parameters on individual cells [14]. Nevertheless, traditional fluorophores suffer from several problems, such as photobleaching, spectral cross-talking and narrow excitation. QDs have the potential to overcome these problems. As demonstrated by Wu *et al.* [9], the QD-labeled cells are brighter and more resistant to photobleaching. In fact, organic dyes are often photo bleached and fade by >90% in less than one minute, whereas the QDs are stable for more than 30 minutes under identical experimental conditions. This result suggests that multicolor QDs could be used to determine the quantitative profiles of molecular targets for single normal or diseased cells.The improved brightness and photostability are also

Corresponding author: Shuming Nie (snie@emory.edu).

important for detection and identification of low-abundance antigens that are present in only 10–100 copies per cell.

…traditional fluorophores suffer from several problems, such as photobleaching, spectral cross-talking and narrow excitation. QDs have the potential to overcome these problems.

Clinical tissue specimens

A further application of QDs is in multiplexed labeling and molecular analysis of pathological tissue specimens. In comparison with single cells, clinical tissue specimens are often highly heterogeneous (containing different cell populations in various microenvironments) and are therefore more difficult to analyze [15,16]. QDs, coupled with spectroscopy and spectral imaging, could have an important role in mapping out the true molecular profiles associated with different diseases or different subtypes of common diseases. A new feature is that tissue morphological structures can be correlated with quantitative spectroscopic measurements. The recent development of near-infrared-emitting QDs and QD-encoded beads [17,18] should allow molecular profiling of a large number of genes, proteins and other biomolecules on a single tissue section [19].

The recent development of near-infrared-emitting QDs and QD-encoded beads [17,18] should allow molecular profiling of a large number of genes, proteins and other biomolecules on a single tissue section [19].

In vivo applications

Ruoslahti and co-workers first reported the use of QD–peptide conjugates to target tumor vasculature *in vivo* [11]. Histological staining revealed that QDs homed to tumor vessels guided by the peptides and were able to escape the reticuloendothelial system. They also showed that multiple peptide molecules could be conjugated to a single dot, achieving enhanced binding affinities and exquisite specificities through a multivalency effect. This is particularly important for small-molecule ligands and peptides, which usually have weaker interactions with targets than antibodies. In addition, Dubertret and colleagues [10] used lipid coated QDs to monitor frog embryogenesis, taking advantage of their biocompatibility and photostability. The use of QDs should enable biologists to track cell, tissue and organ developments over extended periods of time, a task not possible with photobleaching organic dyes.

We have pushed *in vivo* QD studies one step further by directly imaging QD-labeled cancer cells in live animals. QDs were derivatized with a synthetic peptide for efficient delivery into living cells. Similar studies using magnetic nanoparticles have been reported by Weissleder and colleagues for *in vivo* monitoring of cell migration and integration [20]. A surprising finding is that QD loading does not appear to affect cell viability and growth. In fact, the implantation of QD-tagged cancer cells leads to usual tumor growth in animal models. Quantitative measurements indicate that as few as 100 cancer cells can be detected with long-wavelength QDs.

It is worth noting that the extraordinary sensitivity achieved for *in vivo* imaging is a result of the novel optical properties of QDs. First, their large Stokes shifts allow the target signals to be separated clearly from the background fluorescence, a task not possible with conventional organic dyes and fluorescent proteins [21]. Second, under photon-limited *in vivo* conditions (where light intensities are severely attenuated by scattering and absorption), the large absorption coefficients of QDs (in the order of 10^6 cm^{-1} M^{-1}, ~10–20 times larger than those of common organic dyes) allow more efficient probe excitation. Third, multi-wavelength optical imaging with QDs should allow intensity ratioing, spatial colocalization and quantitative target measurements at single metastasized tumor sites and for single anatomical structures. Theoretical modeling studies by Bawendi and colleagues [22] indicate that there are multiple spectral windows for *in vivo* QD-imaging, and that the highest tissues penetration depths might be achieved at emission wavelengths longer than 1000nmnm.

In conclusion, the concept of QDs as biological labels has become a reality, and bioconjugated QDs should find widespread applications in medical diagnostics, molecular imaging, molecular profiling, pharmacogenomics and drug discovery. We also envision QDs being integrated with mutifunctional or 'smart' nanostructures for non-invasive sensing, imaging and treatment of cancer and other diseases.

Note added in proof

The recent 'burst' of activities on biological applications of QDs should also include a paper by Bruchez and colleagues [23], which demonstrated the use of QDs for multiphoton fluorescence imaging of small vasculatures *in vivo*, and a paper by Aida and colleagues [24], which showed that ATP molecules could trigger the release of QDs from the cavities of chaperonin proteins.

Acknowledgements

The authors are grateful to Dominic Ansari and Dr. Zhongxing Liang for technical help, and to Professors Leland Chung, Fray Marshall, John Petros, Hyunsuk Shim, Jonathan Simons, and Lily Yang for stimulating discussions on cancer research. They also acknowledge financial support from the National Institute of Health (R01 GM60562) and the Coulter Translational Research Program at Georgia Tech and Emory University.

References

1 Chan, W.C.W. *et al.* (2002) Luminescent QDs for multiplexed biological detection and imaging. *Curr. Opin. Biotechnol.* 13, 40–46
2 Niemeyer, C.M. (2001) Nanoparticles, proteins, and nucleic acids: biotechnology meets materials science. *Angew. Chem. Int. Ed.* 40, 4128–4158
3 Murray, C.B. *et al.* (2000) Synthesis and characterization of monodisperse nanocrystals and close-packed nanocrystal assemblies. *Annu. Rev. Mater. Sci.* 30, 545–610
4 Alivisatos, A.P. (1996) Semiconductor clusters, nanocrystals, and QDs. *Science* 271, 933–937
5 Chan, W.C.W. *et al.* (1998) Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science* 281, 2016–2018
6 Bruchez, M. Jr *et al.* (1998) Semiconductor nanocrystals as fluorescent biological labels. *Science* 281, 2013–2015
7 Peng, Z.A. *et al.* (2001) Formation of high-quality CdTe. *CdSe, and CdS nanocrystals using CdO as precursor. J. Am. Chem. Soc.* 123, 183–184
8 Qu, L.H. *et al.* (2001) Alternative routes toward high quality CdSe nanocrystals. *Nano Lett.* 1, 333–337
9 Wu, X.Y. *et al.* (2003) Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor QDs. *Nat. Biotechnol.* 21, 41–46
10 Dubertret, B. *et al.* (2002) *In vivo* imaging of QDs encapsulated in phospholipid micelles. *Science* 298, 1759–1762
11 Akerman, M.E. *et al.* (2002) Nanocrystal targeting *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 99, 12617–12621
12 Jaiswal, J.K. *et al.* (2003) Long-term multiple color imaging of live cells using quantum dot bioconjugates. *Nat. Biotechnol.* 21, 47–51
13 Mattoussi, H. *et al.* (2000) Self-assembly of CdSe–ZnS QDs bioconjugates using an engineered recombinant protein. *J. Am. Chem. Soc.* 122, 12142–12150
14 Stephen, C.D. *et al.* (2001) 11-color, 13-parameter flow cytometry: identification of human naive T cells by phenotype, function and T-cell receptor diversity. *Nat. Med.* 7, 245–248
15 Emmert-Buck, M.R. *et al.* (1996) Laser capture microdissection. *Science* 274, 998–1001
16 Bonner, R.F. *et al.* (1997) Cell sampling – laser capture microdissection: molecular analysis of tissue. *Science* 278, 1481–1483
17 Bailey, R.E. and Nie, S. Alloyed semiconductor QDs: tuning the optical properties without changing the particle size. *J. Am. Chem. Soc.* (in press)
18 Han, M.Y. *et al.* (2001) Quantum dot-tagged microbeads for multiplexed optical coding of biomolecules. *Nat. Biotechnol.* 19, 631–635
19 Liotta, L. and Petricoin, E. (2000) Molecular profiling of human cancer. *Nat. Rev. Genet.* 1, 48–56
20 Maite, L. *et al.* (2000) Tat peptide-derivatized magnetic nanoparticles allow *in vivo* tracking and recovery of progenitor cells. *Nat. Biotechol.* 18, 410–414
21 Yang, M. *et al.* (2000) Whole-body optical imaging of green fluorescent protein-expressing tumors and metastases. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1206–1211
22 Lim, Y.T. *et al.* (2003) Selection of quantum dot wavelengths for biomedical assays and imaging. *Molecular Imaging* 2, 50–64
23 Larson, D.R. *et al.* (2003) Water-soluble quantum dots for multiphoton fluorescence imaging in vivo. *Science* 300, 1434–1436
24 Ishii, D. *et al.* (2003) Chaperonin-mediated stabilization and ATP-triggered release of semiconductor nanoparticles. *Nature* 423, 628–632

0167-7799/$ - see front matter
doi:10.1016/S0167-7799(03)00209-9

Broad virus resistance in transgenic plants

Marcel Prins

Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands

Viruses are significant threats to agricultural crops worldwide and the limited sources of natural resistance warrant the development of novel resistance sources. Several methods of transgenic protection have been successfully applied, including protein- and RNA-mediated approaches. Increased understanding of the molecular biology of virus infection is starting to bear fruit, enabling specific strategies to be designed for virus resistance in crops.

Over the years, resistance genes present in wild varieties of cultivated crops have been the main source of antiviral activity in breeding programs, but few have withstood the fast evolution of pathogens over an extended period of time in the field. Although direct interactions have not been found between resistance genes and their elicitors, a single amino acid mutation is often sufficient to change a pathogen from avirulent to virulent. Resistance in the field under high pathogen pressure allows rapidly mutating pathogens such as viruses to select such single amino acid mutants quickly. Moreover, plant RNA viruses in the field exist as swarms of point mutants (so-called quasi-species), which further enhances their ability to overcome resistance. Durability of plant virus resistance seems mostly to be dependent on the loss of fitness accompanying the required point mutation in the pathogen. The use of natural resistance genes as transgenes in other plant species has been demonstrated for some resistance genes, but other transgenes of a different nature give more options for transgenic virus resistance. Here some of the latest advances in breeding for resistance in transgenic crop plants are reviewed.

Application of pathogen-derived resistance

A variety of ways of generating transgenic virus resistance have been developed over the past decade. Most strategies originated from the pathogen-derived resistance (PDR) concept [1]. This involves the untimely expression of dysfunctional pathogen-derived products. Shortly after the concept was introduced, coat-protein-mediated-resistance (CPMR) against tobacco mosaic virus (TMV) was reported [2], followed by reports of protein-mediated resistance against a number of other economically important viruses. CPMR was soon succeeded by alternative PDR

Corresponding author: Marcel Prins (Marcel.prins@wur.nl).