Reviews

Quantum Dots for Single Bio-Molecule Imaging

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The emerging nanomaterial, quantum dots or QDs, offers numerous potential applications in the biological area. As cell labeling probes, QDs become now an alternative of existing organic fluorescent dyes and fluorescent proteins. In this short review, we cover typical and successful applications of QDs as fluorescent probes in cell labeling and genomic diagnosis. As a future important application, biomolecular detection at a single molecule level utilizing QDs is also discussed.

(Received September 29, 2006; Accepted November 6, 2006; Published January 10, 2007)

1 Introduction

Recent advances in the development of fluorescent semiconductor nanocrystals so-called quantum dots or QDs, open up new possibilities especially for biomolecular and cellular imaging. Despite QDs' incompatibility with biomolecules, due to highly hydrophobic and toxic properties, their unique properties are worth more than current organic dyes and fluorescent proteins: broad excitation spectrum, size-tunable and narrow emission spectrum, and resistance against photobleaching. The first step towards biomolecular and cellular imaging is to functionalize their hydrophobic surfaces by amphiphilic ligands. Although their size must be larger than the original non-coated state (2 – 9.5 nm in diameter), notable phenomena that include serious binding kinetics or steric hinderance problems have not yet been reported. These coated-QDs have been found to be essentially nontoxic to cells; furthermore, some therapeutic applications have been reported.[1] In this paper, we briefly review biological applications of QDs as fluorescent probes for cell labeling, DNA diagnosis, and single biomolecule probing, and discuss their future applications for realizing single molecule analysis.

2 Properties of QDs

Many researches have been focused on the synthesis, solubilization and functionalization of QDs for applications in the biological field. QDs are single crystals of a few nanometers in diameter and are generally synthesized from various types of semiconductor materials, such as CdSe, CdTe, InP, or InAs. Different compositions and sizes of QDs provide different bulk band gap energies which characterize their emission wavelength; typically, the size range of QDs from 2 to 9.5 nm will correspond to the emission wavelengths from 400 to 1350 nm.[2,3] Recent developments of synthesis processes allowed precise control of QDs size by control of synthesis conditions. These synthesized core and core-shell QDs are only soluble in nonpolar organic solvents because of their hydrophobic surfaces. Therefore, for biological imaging applications, biocompatibility is the most important challenge to make them work as novel fluorescent probes. Over the past few years, various strategies to solubilize QDs in aqueous buffers have been used. Thiol (-SH)-containing molecules are often used to anchor functional groups on QDs surfaces[4,5] and the hydrophilic ends such as carboxyl (-COOH) groups make QDs water soluble.[6] Oligomeric phosphine,[7] dendrons,[8] and peptides[9] are alternative choice to change the surface properties. In contrast to these covalent modification approaches, coating or encapsulation by a layer of amphiphilic polymers such as diblock[10] and triblock copolymer,[11] phospholipid micelles,[12] and polysaccharides[13] have been reported. After the solubility and stability values of QDs in water were brought above a satisfactory level, the next requirements toward probing biological molecules and live cells involve functionalizing QDs surface with some sort of recognition molecules (*e.g.*, DNA oligonucleotides, RNA, peptide, antibody, *etc.*). Several

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successful approaches have been used to make a conjugate of biological molecules and QDs. Nonspecific adsorption of simple small molecules, such as oligonucleotides[14,15] and various serum albumins,[16] onto the surface of water-soluble QDs has been reported. Although these nonspecific attachments of biological molecules make it easy to produce bio-molecule conjugates, the surface state is easily influenced by surroundings such as ionic strength, pH, and temperature. Negatively charged QDs could be conjugated with the fusion protein which has a positively charged domain.[6] QDs which are modified by either an amine of a carboxyl group offer the possibility of specific and stable attachments of intended biological molecules by covalent bonding.[2,4,8,17-20] One of most frequently used modifications is streptavidin-coated QDs in combination with biotinylated oligonucleotides,[12,14,15,20,21] proteins,[21] and antibodies.[4,10,22-24] These functionality processes could be repeated so that QDs become able to work as multipotent fluorescent probes. Although the sizes of "dots" become larger according to the formation of functional layers, there is no evidence that the biological function of conjugated biological molecules is disturbed by QDs. Therefore, QDs can function as organic dyes despite their relatively large size.

3 QDs as Probes in Cell Biology

The most successful usage of QDs in cell biology is immunofluorescence labeling of cells and tissues. Researchers have performed a variety of immunofluorescence assays by QDs-based fluorescent probes; immunostaining of membrane proteins,[10,25-28] microtubles,[10] actin,[2,10] and nuclear antigens.[10] Because their signals are intrinsically brighter than those organic dyes and because they exhibit photostability against bleaching over long periods of time, QDs become ideal probes in this area. There are also some photophysical properties of QDs which are generally thought to be disadvantages. One of them is a phenomenon of blinking that is caused by random alteration of QDs between an emitting state and a non-emitting state. This discontinuous fluorescence from QDs limits the application of QDs especially in the area of single-molecule detection. In some cases, however, this discontinuous emission helps to distinguish a signal of a QD apart from artifacts in immunocytological experiments. Recently, Hohng *et al*. suggested that QDs blinking could be suppressed by passivating the QDs surface with thiol moieties.[29] Such anti-blinking studies would enable the long-term trajectory tracking of a single QD-conjugate and would expand its ability to facilitate the study of membrane proteins, gene trafficking to cell cytoplasm and nucleus, and so on. Michalet *et al*. demonstrated that the avidin receptors expressed in the cytoplasmic membrane of HeLa cells could be labeled with biotinylated peptide-coated QDs and the conjugates could be observed at a single-moleule level.[3] They observed their diffusion in the membrane of live cells and trafficking into the cytosol. This approach could reveal the relationship between glycosylphosphatidylinositol-anchored receptors and lipid rafts in the membrane. Another interesting application of a single QD-conjugate is in drug or gene delivery systems. Several pathways are known through which outer specimens with vectors enter the cell cytoplasm and reach the nucleus. QDs functionalized with the appropriate antenna like a targeting peptide sequence, in most cases, result in aggregation of QDs in the endosomes. Derfus *et al*. showed that microinjection allows the delivery of QDs containing functional peptide sequences to mitochondria and the cell nucleus.[30] Both intracellular delivery and escape from endosome to the nucleus still remain challenging areas. QDs approaches will bring insights to the pharmacological field.

4 Toxicity

With a rapid expansion of nanomaterials such as carbon nanotubes and fullerene, concerns about cytotoxicity of QDs have been raised especially in the study of live-cell or animal experiments because QDs may contain toxic heavy metals such as cadmium and selenium. This question is very complicated due to the diversity of materials, synthesis methods, solubilization, and functionalization protocols of QDs. Until now, most of reports found no acute and obvious toxicity of CdSe QDs on cell viability, morphology, function, and proliferation when they were properly coated by hydrophilic shells.[22,31] However, cytotoxicity could happen when the QDs surface coating has deficiency and Cd^{2+} was released by, for instance, exposing the CdSe to oxidization by air or UV damage.[31] Therefore, coating the QDs surface to prevent the oxidative damage is critical in live-cell and animal experiments. Further investigations are required to elucidate the safety of QDs conjugates, so as to proceed safely to the next stages of QDs applications such as diagnostic applications.

5 Genomic Analysis with QDs-DNA Probes

FISH, fluorescence *in situ* hybridization, is an important nucleic acid-based technique to visualize and map genetic materials in cells. The technique generally provides the location of specific genes or portions of genes, and quantitative information about gene copy numbers in tumor cells that have abnormal gene amplification. Commonly used organic dyes are rapidly photobleached before they give a perfect fluorescence image for quantification, and moreover, detection became more difficult when the number of targets is limited. The most serious problem is intrinsic autofluorescence of cells, which reduces the signal-to-noise ratio. These drawbacks could be resolved by QDs which have enough photostability over long-term exposure, a broad excitation and a narrow emission wavelength range. Human metaphase chromosomes from transformed lymphocyte cultures and breast cancer cell line SK-BR-3 were analyzed by FISH based on streptavidin-linked cadmium selenide QDs by Xiao *et al*.[32,33] Further, FISH of Y chromosome in human sperm cells was conducted by Pathak *et al*.[5] Not only for human chromosomes but also for E. coli, the QDs-probe with a sequence complementary to multiple clone sites in plasmid pUC18 was constructed.[34] QDs provide the possibility to evolve even such a well-developed technique for genomic analysis as a part of clinical diagnosis.

6 QDs for Single-molecule Probes

One of ultimate goals of analytical sciences is a single molecule analysis, which intends to read out various kinds of molecular information from only a single molecule. QDs are thought to be potential probes to realize single molecule analysis owing to their unique photophysical properties, that is, spectral range, brightness, and long lifetimes. These properties enable long-term tracking of QDs-probed single molecule, and thus, they could be applied to *in vivo* studies of protein dynamics and ligand trafficking. Their other properties, relatively large size and high electronic density, could be used as molecular tags

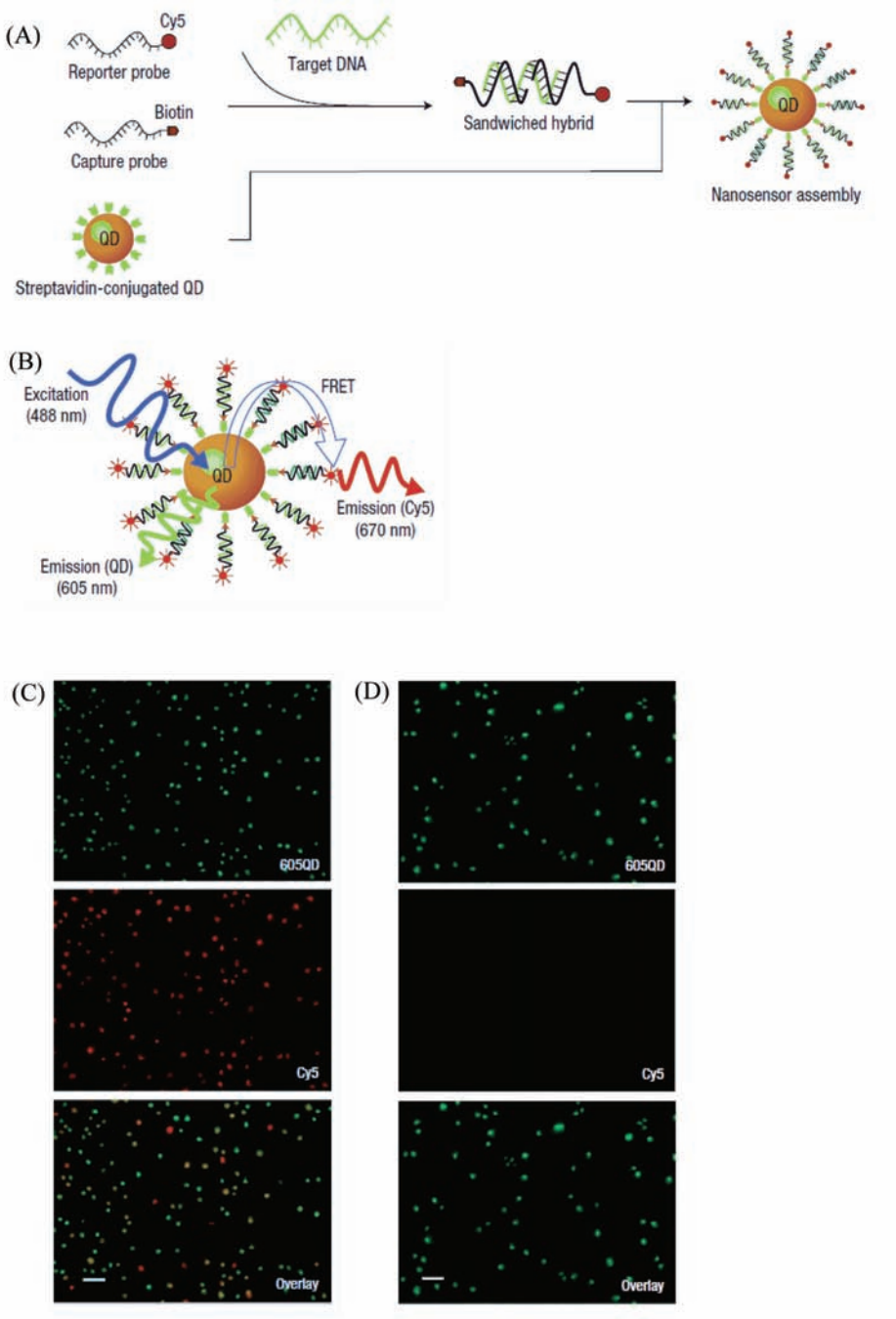

Fig. 1 Schematic concept of single-QD-based DNA probe for a detection of point mutation. (A) The assembly processes of target DNA with Cy5-labeled reporter probe and biotin-labeled capture probe on 605QD surface. (B) The principal of detection is based on FRET between Cy5 acceptors and QD donor. (C) Fluorescent images of QDs (top), Cy5 (middle) and merged colors (bottom) with complementary DNA target and (D) non-complementary DNA target. The figure is reproduced with permission from Ref. 39.

combined with their fluorescence, for instance, in experiments using atomic force microscopy and electron microscopy. Combined with these advantages, Dahan *et al*. applied single-QD tracking (SQDT) to study rapid lateral dynamics of glycine receptors (GlyR).[35,36] Bright and photostable QD enabled the observation of multiple exchanges between extrasynaptic and synaptic domains, in which a GlyR alternated between free and confined diffusion states, respectively. To quantify the observation results, researchers determined the instantaneous diffusion coefficients by SQDT. Moreover, by electron microscopy imaging, the entry of GlyR into the synapse by diffusion was observed and confirmed. Utilizing the diversity of surface functionalization and the narrow emission spectrum, multi-color QDs-modified DNA were constructed and applied in existing DNA diagnosis techniques. SNP typing,[37] point mutation detection as shown in Fig. 1,[38-40] and DNA-protein interaction study based on molecular combing were demonstrated at a single DNA level.[41] Since the human genome sequence was already revealed, it became important to see when and which protein binds to where on DNA and to regulate the

expression. This could be assessed by using fluorescence study or a combination of fluorescence and static imaging technique such as AFM or electron microscopy. Unlike conventional organic dyes, when QDs are labeled at the ends of DNA, purely dynamic study of DNA–protein interaction could be observed. Considering their relatively high density globule and large surface area, we expect many interesting applications.

7 Conclusion and Perspectives

In summary, we discussed the properties of QDs and biological applications as a novel type of fluorescent probe. Although some parts of QDs-staining methods in cell biology seem now to be established, the application for single molecule analysis as a single molecule probe are still challenging. Although their unique photophysical and morphological properties will provide an evolving approach in biology, they will not replace the conventional organic dyes or fluorescent proteins. They will give insights in biology and provide new applications in clinical diagnosis.

8 References

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