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Quantum Dots: A Novel Tool to Discovery

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Abstract: Quantum dots, namely semiconductor nanocrystals, have caught great interests of scientists in physics, chemistry, materials science, biology, in particular drug discovery in recent years. In this paper, we mainly review the characteristics of quantum dots, its significance and potential applications to drug discovery. In addition, biocompatibility, bioconjugation and biotoxicity of quantum dots are analyzed and discussed.

Key words: Quantum dots, drug discovery, CdSe, semiconductor nanocrystals

INTRODUCTION

Advancements of genomics, nanotechnology and biochemistry bring about new opportunities and challenges for drug discovery and development. Recent developments in nanotechnology have made it possible to obtain a new class of highly fluorescent homogeneous semiconductor nanocrystals termed ''quantum dots''(QDs) (Chan and Nie, 1998; Bruchez *et al*., 1998). Since 1998, quantum dots have been first demonstrated capable of labeling specific components of cells and tracing proteins within cells in biomedicine. Recently, the investigations of quantum dots in the biomedicine have developed rapidly. Quantum dots are very stable light emitters, and their emission can be broadly tuned through size variation. So they can offer significant advantages over organic fluorophores. In the past few years, it was reported that, as an alternative to organic fluorophores, bioconjugating quantum dots (Tran *et al*., 2002; Gerion *et al*., 2001; Parak *et al*., 2002) are used widely in diverse areas: cell labeling (Wu *et al*., 2003), cell tracking (Hoshino *et al*., 2004), *in vivo* imaging (Dubertret *et al*., 2002), DNA detection (Taylor *et al*., 2000; Xu *et al*., 2003), biomacromolecules labeled (Dahan *et al*., 2003), signal transduction (Lidke *et al*., 2004) and multiplexed beads encoded (Han *et al*., 2001).

Recently UV irradiation of quantum dots as photosensitizers has been used to kill cancer cells in the body (Bakalova *et al*., 2004). Quantum dots coated with functional molecular will lead to the evolution of novel and powerful "smart drug", even bring about the evolution of drug delivery and drug screening. Can quantum dots offer advantages for drug discovery and development? Will quantum dots have a tremendous prospect in drug discovery and development? In the review, the excellent properties and potential application of quantum dots in drug discovery will be summarized, furthermore, limitation and future opportunities of quantum dots will be discussed realistically.

CHARACTERISTICS OF QUANTUM DOTS

Quantum dots are a class of nanoparticles that are synthesized with II-VI or III-V column elements in the periodic table, as an example of CdSe. Quantum dots have attracted a great interest for fundamental research and industrial development due to its unique optical and electronic properties.

Optical properties: Quantum dots possess remarkable optical characteristic as a consequence of their nano-length scale. These quantum dots have advantages over conventional organic fluorophores in terms of bright, tunable and narrow fluorescence emission, broad absorption spectra, etc.

First, the excitation spectrum of quantum dots is broad, which facilitates a single excitation wavelength to excite quantum dots of different diameter. By contrast, organic dyes fluorophores can be optimally excited only by the light of a defined wavelength, which usually makes it necessary to use as many excitation sources as types of fluorophores.

Second, Quantum dots have symmetric and narrow emission spectrum bandwidth of less 40 nm full width at

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half maximal fluorescence which enables emission of pure color. Whereas organic fluorophores have a relatively broad emission spectra that vary between 50 and 100 nm and have a log-normal line shape with long tails extending to the red, which often cause the signals from different fluorophores to overlap. In addition, the size distribution of the core dictates the width of the emission spectrum. That is, the emission of quantum dots can be tuned by controlling the size of the CdSe core. An increase in the size of the core shifts the emission to the red end of the spectrum.

Third, the Stork's shift, i.e., the separation between the excitation and emission wavelength of quantum dots, is large which enable the whole emission spectra to be collected, resulting in improved sensitivity of detection.

These unique features of quantum dots make it easy to use more than one-size quantum dots at one time to tag different biological molecules simultaneously, which allow multiplexed cell assays and enable multiple molecular targets tracked simultaneously (Han *et al*., 2001; Jaiswal *et al*., 2003). And it is one of the main attraction to biologist and chemist (Chan and Nie, 1998; Bruchez *et al*.,1998; Han *et al*., 2001; Jaiswal *et al*., 2003; Leatherdale *et al*., 2002).

In addition, the high brightness is another advantage of the quantum dots. The dynamic range of intensity levels may be improved over organic fluorophores. Quantum dots can go through repeated cycles of excitation and fluorescence for hours at one time.

Stability: Quantum dots have very large molar extinction coefficients in the order of $0.5\text{-}5\times10^{6}M^{-1}cm^{-1}$ (Leatherdale *et al*., 2002), which makes them brighter probes under photon-limited *in vivo* where light intensities are severely attenuated by scattering and absorption. These values are 10-100 times lager than those of organic dyes. In addition, quantum dots are several thousand times more stable against photobleaching than organic dyes (Wu *et al*., 2003; Sukhanova *et al*., 2004). For example, when illuminated constantly with a light, quantum dots do not photobleaching even after 14 h, whereas organic dye photobleaching completely in less than 2 min. Organic fluorophores are susceptible to photodamage and to metabolic and chemical degradation, which making it difficult to label cell for long period. Whereas quantum dots, as inorganic materials, are resistant to photodamage, degradation by enzymes in live cell and chemical damage. Thus quantum dots are well-suited for continuous tracking studies over a long period of time. Resent the photostability of quantum dots has been used to study tumor cell migration and metastasis *in vitro* (Pellegrino *et al*., 2003; Voura *et al*., 2004).

APPLICATIONS IN DRUG DISCOVERY

Unique characteristics of quantum dots mentioned above and their applications in biomedicine create the potential for application in a wide range of drug discovery, such as drug delivery, drug screening and drug target identification and validation as follows.

Drug delivery: Drug Delivery Systems (DDS) such as lipid- or polymer-based nanoparticles can be designed to improve the pharmacological and therapeutic properties of drugs. Many of the early problems that hindered the clinical applications of particulate DDS have been overcome, with several DDS formulations of anticancer and antifungal drugs now approved for clinical use. Furthermore, there is considerable interest in exploiting the advantages of DDS for *in vivo* delivery of new drugs derived from proteomics or genomics research and for their use in ligand-targeted therapeutics. After surface modification and functionalization, drug delivery systems such as solid lipid particles (Muller *et al*., 2002), polymer nanosystems (Guzman *et al*., 1996), which have been used to transport a wide variety of drugs, posses particular target effect.

After quantum dots are bioconjugated protein or peptide, single-molecule movement in single living cell can be track in real time (Dahan *et al*., 2003). Lidke *et al*. (2004) study provided new insight into erbB/HER receptor-mediated signal transduction. This study demonstrated that EGF-QDs (quantum dots bearing epidermal growth factor) were highly specific and potent in the binding and activated of the receptor (erbB1), being rapidly internalized into endosomes that exhibit active trafficking and extensive fusion. Similarly, when drug molecules are linked to the surface of quantum dots, the kinetics and transport of drug molecules can be recorded and tracked for a longer period of time, which help to understand the mechanism of diffusion, particle fusion and internalization into cells. The movement of different drug molecules tagged with quantum dots of different colors can also be studied simultaneously. In addition, the elaborate DDS that consist of drug molecules, quantum dots and target molecules (e.g., antibody or peptide) can be designed.

After the DDS are transported into cancer cell guided by target molecules, under UV irradiation momently the photoluminescence of quantum dots trigger the DDS, and drug molecules are released into cancer cells and kill them (Fig. 1). Furthermore, under UV irradiation continuously, quantum dots behave photocatalysis of semiconductor nanocrystals. On the surface of quantum dots photochemical reactions take place resulting in a production of the cytotoxic singlet oxygen ($O_2^{\cdot}$), which causing biomembrane of cancer cell oxidation and

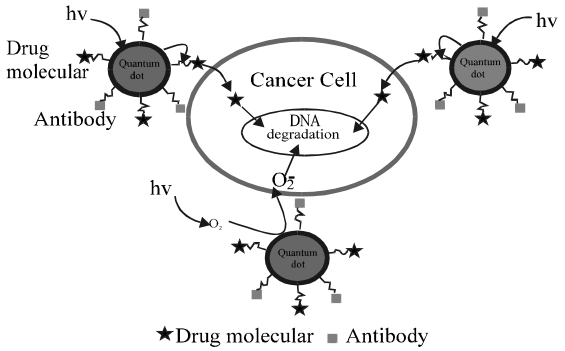

Fig. 1: Possible mechanisms of the elaborate DDS for cancer therapy

degradation (Bakalova *et al*., 2004). Using the surface functionalization properties of quantum dots, targeting capabilities can be added as well. Compared with their organic counterparts their interaction with their immediate environment *in vivo* can be minimal because of the inorganic nature of quantum dots as nanocarrier.

Drug screening: Within the last 5-10 years the demands of growing compound files have sparked a technical revolution within assay development and High Throughput Screening (HTS). Whereas initial developments focused on throughput, the cost of reagent production soon demanded effort in the miniaturization of assay formats. The demand for sensitive readouts in HTS has driven the introduction of fluorescence-based readouts. During the past decade, fluorescence assay technology has grown very fast in drug screening based on receptor-binding. As result of their superior optical properties, quantum dots can be adopted for screening candidate drugs as alternative to organic fluorophores.

A successful drug must be able to bind to several different molecular targets to achieve the desired effect, and steer clear of other targets to avoid side-effects. Testing could be made a simple matter by attaching quantum dots of different colors to the various targets. A good hit might be a drug that displaces blue, aqua and green nanocrystals where you want it to attach, but doesn't displace red, yellow and orange ones at proteins that indicate side-effects (Klarreich *et al*., 2001). Mattheakis *et al*. (2004) described a quantum dots system for drug screening and studying mixed cell populations, consisting of encoding (different types of cells are tagged with different-colored quantum dots), imaging and decoding single cells. QDs-cells can be used to potentially multiplex virtually any microscope-based cell assay with an optical readout. Typically HTS measures a single target and binary in its output (i.e., it shows if the unknown compound produces an effect or not). Using different-colored quantum dots to tag different target, compounds against multiple targets can be screened in parallel which is called Multi-target High-throughput Screening (MTHTS) (Palfreyman *et al*., 2002). Even if the effect on some target is not desired one, it could be of interest for another target. Most importantly, based on MTHTS, a lead with different effects, different leads with different effects or multiple leads with same effect can be gained from multiple screening models simultaneously in one screening. QDs-based multiplexing assay being used to drug high-throughput screening can enhance screening throughput, which can achieve bi-high-throughput even multi- high-throughput screening.

Drug target identification and validation: The human genome sequence shows that there are approximately 24000 proteins coding genes in the genome and that about 10% of all genes will be drug targets (Hopkins *et al*., 2002). To date, one of the major challenge facing the drug discovery community is drug target identification and validation through genetics. Establishing a causal relationship between a potential drug target and a disease in humans is a difficult process fraught with the complexity. For example, Single Nucleotide Polymorphisms (SNPs) that is the most common type of genetic variation among individuals can change receptors, transport proteins and drug metabolizing enzymes (Vogel, 2002). So the establishment of a link between an identified genetic polymorphism (particularly Single nucleotide polymorphisms, SNPs) and a disease phenotype remains a formidable challenge, particularly for multifactorial disease such as diabetes or cancer (Austen and Dohrmann, 2005). To expect success from genome technology, it is crucial to adopt a high-throughput approach assaying many genes or SNPs genotype simultaneously to identify and validate drug targets. Quantum dots technology have this property mentioned above. Recently Xu *et al*. (2003) described a new method for high-throughput and multiplexed SNPs genotyping for using the Qbead system that employs quantum dots to encode microspheres used as a platform for multiplexed assays. By combining mixtures of quantum dots with distinct emission wavelengths and intensities, unique spectral barcodes are created that enable the high levels of multiplexing required for complex genetic analyses. In theory, N intensity levels with m colors will produce N^m-1 unique codes. For example, a combination of three colors and ten intensity levels theoretically would produce 999 unique codes. In practice, however, fewer unique codes may be produced due to spectral

overlapping, fluorescence intensity variations and signal-to-noise requirements. Nonetheless, a realistic scheme using 5-6 colors with six intensity levels would be expected to yield at least 10000 to 40000 recognizable codes (Han *et al*., 2001). So the Qds-encoded bead technology is the potential encoding capacity that enables the high level of multiplexing necessary for genetic analysis. This technology will improve efficiency of drug target identification and validation. Gene analysis using QDs-encoded bead system is an ideal technology that has the powerful potential to accelerate the discovery of new targets and to improve the efficiency of the drug discovery process.

RESULT AND DISCUSSION

As mentioned above, quantum dots have received a great deal of attention for biomedicine and drug discovery in recent years. Despite their potential and their success in biological application, quantum dots also have limitations associated with their use.

Biocompatibility: Use of quantum dots has been limited by difficulties in obtaining nanocrystals that are biocompatible. Before nanocrystals can be widely used as biolabels, they must maintain four properties under aqueous biological conditions: water-solubility, efficient fluorescence, colloidal stability, and low nonspecific adsorption. Unfortunately, despite recent advances (Chan and Nie,1998; Bruchez *et al*.,1998; Gerion *et al*., 2001, Mattoussi *et al*., 2000; Aldana *et al*., 2001; Gerion *et al*., 2002), these conditions have not been simultaneously satisfied, limiting the development of *in vivo* applications of nonaggregated (or individual) semiconductor nanocrystals. Quantum dots are inherently hydrophobic in nature, which are synthesized in organic solution. The main challenge is that the quantum dots have hydrophobic organic ligands coating their surface (Gao *et al*., 2005). To make the quantum dots water soluble, these hydrophobic quantum dots can be modified by hydrophilic groups.

To address this, various methods have been developed including surface modification and Encapsulation. For example, Mercaptoacetic acid which is linked to the surface of quantum dots can provide carboxyl groups not only to make quantum dots hydrophilic, but also for further covalent modification (Chan and Nie, 1998). The bidentate" type of interaction of dihydrolipoic acid (DHLA) (Mattoussi *et al*., 2000) or dithiothreitol (Pathak *et al*., 2001) with the inorganic quantum dots' surface make quantum dots more water-stable. Several polymers have been reported including octylamine-modified low molecular weight polyacrylic acid, PEG-derivatized phospholipids, block copolymers and polyanhydrides (Gao *et al*., 2004; Pellegrino *et al*., 2004). A more serious concern is that these modification approaches still cause agglomeration of quantum dots, which can adversely influence the kinetics of their movement after their glomeration into larger clusters *in vitro* and *in vivo* applications (Ozkan *et al*., 2004). For example, when the drug molecules are attached to the surface of quantum dots for tracking their transport and uptake, due to quantum dots with drug molecules agglomerating into larger clusters, their inherent transport and uptake might be influenced adversely, therefore they might never be transported inside the cell or will take longer time to undergo endocytosis. It is reported that silica-coated quantum dots have been further modified with small monomers of poly (ethylene glycol) to reduce nonspecific adsorption and agglomeration (Gerion *et al*., 2002). Recently a potent method (Dubertret *et al*., 2002) that individual quantum dot could be encapsulated in the hydrophobic core of a micelle composed of a mixture of n-poly (ethylene glycol) phosphatidylethanolamine (PEG-PE) and phosphatidylcholine (PC) provides efficient fluorescence, a great reduction in photobleaching, colloidal stability in a variety of bioenvironments, and low nonspecific adsorption. Because the environment around each quantum dot cannot be regulated at will in live cell, there is need for continued improvement in synthesized so that quantum dots are impervious to environment changes.

Bioconjugation: In their applications, quantum dots are always conjugated with functional groups, drug molecules and biomacromolecules (e.g., antibody, avidin, enzyme, DNA) to provide specificity. Under UV irradiation, drug molecules conjugated with the surface of quantum dots can be gradually photocatalytically oxidized using CdSe nanocrystal as the photocatalyst. Then the bioconjugation is not stable, what is more, and drug molecules would be destroyed. In addition, the functional groups are usually decorated the entire surface of quantum dots, which could cause attachment of multiple drug molecules or target molecules to the same quantum dot (Ozkan *et al*., 2004). Because attachment of multiple drug molecules to a single quantum dot results in a large volume, their transport across the membrane will be more difficult than a single molecule. Similarly in the multiplexed HTS a single quantum dot can conjugate with the different receptors or different leads that will interfere with fluorescent assay. Recently, several methods have been reported to reduce the number of surface groups around a single dot in biomedicine (Akerman *et al*., 2002). But

these methods might be not desired in drug discovery, especially in studies related to kinetics and transport of drug molecules. Then it is worth paying our attention to how to reduce the numbers of molecules attached to a single quantum dot.

Biotoxicity: There has been a growing concern over the potential toxic effects of semiconductor crystals quantum dots. Recently it is reported that CdSe quantum dots are highly toxic to culture cells under UV illumination for extended periods of time (Derfus *et al*., 2004).When quantum dots have been exposed to UV-light excitation in the aqueous, the surface of quantum dots can be oxidated, which can lead to the release of cadmium ions in CdSe quantum dots. It is well known that Cd ions are highly toxicity. In the absence of UV irradiation, quantum dots with a large band-gap-semiconductor or stable polymer coating(or encapsulated) have been found to be essentially nontoxic to cells and animals which could create a barrier for oxygen diffusion (Gao *et al*., 2005; Larson *et al*., 2003). However a combined aqueous and UV-light excitation environment can still act as a catalyst and enhance the diffusion process (Jaiswal and Simon, 2004). Indeed the doses of quantum dots needed for *in vivo* use are below the known toxicity levels for cadmium. Whether or not, both the cellular toxicity and degradation mechanism of quantum dots need to be thoroughly investigated before any human application in *in vivo* imaging or drug delivery.

In addition, using organic based shell layer on the surface of quantum dots for improving their optical properties and reducing their toxicity can add to the final size of the quantum dots which might not desired in many cases. This can lead to the limitation of QDs application. Therefore how to improving the properties of quantum dots overcoming their shortcomings in the drug discovery application should be paid attention to, which will be our future effort.

CONCLUSIONS

The potential value of quantum dots in drug discovery is due to their bright fluorescence, narrow emission, broad UV excitation, tunable size and high photostability. However, as mentioned above there are some disadvantages yet to be resolved for the application of drug discovery, such as biocompatible, bioconjugation, toxicity, photooxidation, size variation. With the current interest and the concerted efforts of physicists, chemists and biologists, the advantages and disadvantages will be understood very well in the near future. Quantum dots, a novel tool, will become a standard tool in drug discovery.

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