Small Is Now Big: Nano Technology for Electronics

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Abstract:

Semiconductor quantum dots (QDs) are nanoparticles that have attracted widespread interest in biology and medicine due to their unique optical and electronic properties. These properties, especially their reduced tendency to photobleach and the dependence of their fluorescence wavelength on their size, make them suitable for fluorescent probing applications to detect cancer biomarkers in vitro and in vivo in cells/tissues/whole body. There is considerable interest among researchers due to the recent developments in QD technology. QDs have been encapsulated in amphiphilic polymers and bound to tumor-targeting ligands and drug delivery vesicles for targeting, imaging and treating tumor cells. Present efforts are focussed on exploring the massive multiplexing capabilities of the QDs for the simultaneous detection of multiple cancer biomarkers in blood assays and cancer tissue biopsies. These advances in the QD technology have unravelled a great deal of information about the molecular events in tumor cells and early diagnosis of cancer.

I. Introduction to Quantum Dots:

QDs are inorganic semiconductor nanocrystals having typical diameter between 2-8 nm that possess unique luminescent properties. They are generally composed of atoms from groups II and VI elements (e.g. CdSe and CdTe) or groups III and V elements (e.g. InP and InAs) of the periodic table. Their physical dimensions are smaller than the exciton Bohr radius [1] that leads to quantum confinement effect, which is responsible for their unique optical and electronic properties.

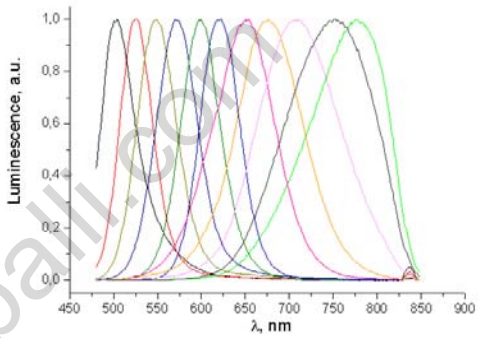

Figure 1: Fluorescence emission spectra of CdTe quantum dots of different sizes.

II. Quantum Dots in early diagnosis of Cancer:

Early screening of cancer is desirable as most tumors are detectable only when they reach a certain size when they contain millions of cells that may already have metastasized. Currently employed diagnostic techniques such as medical imaging, tissue biopsy and bioanalytical assay of body fluids by enzyme linked immunosorbent assay (ELISA) are insufficiently sensitive and specific to detect most types of early-stage cancers. Moreover, these assays are labour intensive, time consuming, expensive and don't have multiplexing capability. On the other hand, QD based detection is rapid, easy and economical enabling quick point-of-care screening of cancer markers. QDs have got unique properties which make them ideal for detecting tumors. These include intense and stable fluorescence for a longer time; resistance to photobleaching [1-5], large molar extinction coefficients, and highly sensitive detection due to their ability to absorb and emit light very

efficiently. Due to their large surface area-to-volume ratio, a single QD can be conjugated to various molecules, thus making QDs appealing for employment in designing more complex multifunctional nanostructures. Various types of biomarkers such as proteins, specific DNA or mRNA sequences and circulating tumor cells have been identified for cancer diagnosis from serum samples. Therefore, QD based multiplexed approach [1] for the simultaneous identification of many biomarkers would lead to more effective diagnosis of cancer. QDs have been covalently linked to various biomolecules such as antibodies, peptides, nucleic acids and other ligands for fluorescence probing applications [6-19]. Some of the applications of QDs in biology [20-32] along with their tremendous potential for in vivo molecular imaging [33-37] have already been explored.

Advantages of Inorganic Quantum Dots over Organic Fluorophores:

Compared to traditional organic fluorophores used for fluorescence labeling in biological experiments, inorganic QDs have wider applications due to their high resistance to photobleaching, which enables visualization of the biological material for a longer time. Fluorophores are highly sensitive to their local environment and can undergo photobleaching, an irreversible photooxidation process which makes them non-fluorescent. This is the main limitation for all studies in which the fluorophore labelled structure has to be observed over extended periods of time. Fluorophores can be optically excited only within a narrow range of wavelengths and fluorescent emission is also restricted to a certain range of wavelengths. Whereas QDs can be excited with a single light source having wavelength shorter than the wavelength of fluorescence. The fluorescence spectra of QDs are narrow, symmetric and have no red tail as observed in fluorophores. Various colors can be observed and distinguished without any spectral overlap. Therefore, multicolor labeling of different structures with QDs of different colors became possible. This multiplexed approach [3, 38-40] is of great interest in wide ranging applications such as disease diagnosis and drug delivery.

The field of QDs is of multidisciplinary as persons from different scientific disciplines i.e. chemistry, physics, biology and medicine are working together to harness their potential. Their employment for the detection and treatment of cancer is one such application which is of paramount importance.

Synthesis of Quantum Dots:

High quality QDs have been synthesized by various approaches [41-43]. But usually their synthesis is carried out in organic solvents such as toluene or chloroform at higher temperatures in the presence of surfactants. But the surfactant-coated particles are not soluble in water as they have polar surfactant head group attached to the inorganic core of QD and the hydrophobic chain protruding into the organic solvent. Usually, all experiments with cells involve water-soluble materials. Therefore, various strategies have been developed to make them water-soluble, where either the surfactant layer is replaced or coated with additional layer such as hydrophilic or amphipathic polymers [44-45]. The hydrophobic coating of surfactant is replaced by ligand molecules carrying functional groups at one end that bind to the QD surface, and hydrophilic groups at other end that make the QDs water soluble. The employment of amphiphilic polymers as an additional coating on QD surface has also been reported [38, 46-48]. The hydrophobic tail of the polymer reacts with the hydrophobic surfactant layer on QD surface whereas the hydrophilic groups of the polymer on the outer end impart water solubility. QDs have also been encapsulated in phospholipid micelles [8] to make them water soluble.

Properties and Applications of Quantum Dots:

The most commonly used QD system is the inner semiconductor core of CdSe coated with the outer shell of ZnS. The ZnS shell is responsible for the chemical and optical stability of the CdSe core. QDs can be made to emit fluorescent light in the ultraviolet to infrared spectrum just by varying their size. The wavelength of fluorescence of the QD depends on its energy gap (i.e. the difference between the excited and the ground state) which is determined by the size of the QD [49-52]. QDs have narrow spectral line widths, very high levels of brightness, large absorption coefficients across a wide spectral range, high photo stability and capability of multiplexed detection. They are very bright and stable even under complex in vivo conditions

that make them suitable for advanced molecular and cellular imaging, drug delivery and for highly sensitive bioassays and diagnostics [53-54]. Highly sensitive real-time imaging with greater resolution and tracking of single receptor molecules on the surface of living cells have been made possible by QD bioconjugates [13, 55]. Various applications of quantum dots are stated in figure 1. In most of the cases, functional QD conjugates for cancer detection are composed of a semiconductor core (CdSe, CdTe); an additional shell such as ZnS in the case of CdSe QDs having a higher band gap than CdSe to increase quantum yield; a water soluble hydrophilic coating; and, functionalized antibodies or other biomolecules complementary to the target cancer markers at the tumor sites.

Blinking Behavior of Quantum Dots:

Nirmal et al. [65] discovered for the first time that QDs shows a blinking behavior i.e. intermittent on–off emission upon continuous excitation, which was attributed to Auger ionization [65-66]. The principle of this behavior is not well understood even today. But it is a concern only when a signal from individual QD is required during the analysis such as flow cytometry applications. In such cases, it may be possible that the emission from the individual QD might be off due to 'blinking' thus leading to the missing of signal at the detector. But generally in most of the applications such as in cell-based assays, there are more than one QD involved and even if some QDs are blinking, others are giving signal for the final detection and thus, no signal will be missed by the detector. One way of counteracting the reduced quantum yield due to blinking is to grow a shell of a few atomic layers of a material with a larger band gap on top of the QD core.

Effect of Surface Functionalization on the Optical Properties of Quantum Dots:

Fundamental studies have revealed that luminescence of QD is very much sensitive to the surface Functionalization procedures as the interactions of the molecule with the QD's surface change the surface charges on the QD [67]. But many of the QD based probing applications are based on the change in fluorescence of QD after the interaction of the target analyte molecules with the biomolecules functionalized on the QD surface. It has been well reported that the surface functionalization of QDs improves their solubility. But it could reduce their quantum efficiency as well. This has demonstrated in the case of meracptoacetic acid-treated QDs where the quantum efficiency was reduced drastically [7, 63]. But protein functionalized quantum dots tend to retain their quantum efficiency and offer longer shelf life. They can also be further functionalized with multiple functional groups [7] without decreasing their quantum efficiency.

Measurement System for Observing and Tracking Quantum dots:

Single QDs can be observed and tracked for greater time duration up to a few hours with confocal microscopy, total internal reflection microscopy or epifluorescence microscopy. The scheme of the fluorescent imaging employing QDs as labels and its measurement has been described by Gao et al. [68] and So et al [69]. Gao et al. employed a whole-body macro-illumination system with wavelength-resolved spectral imaging, which allows high sensitivity detection of molecular targets in vivo. So et al. also employed the wavelength-resolved spectral imaging system having software that separated autofluorescence from quantum dot signals.

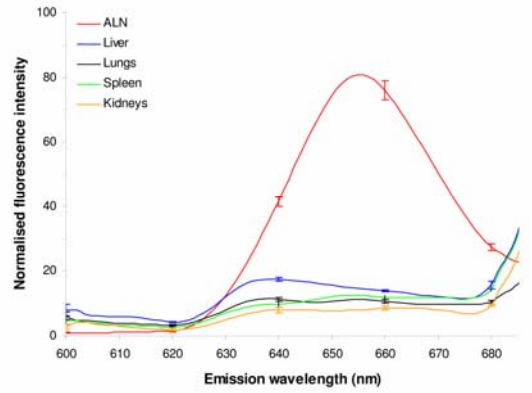

Figure 2: Normalised fluorescent intensity.

Active and Passive Quantum Dot Targeting Mechanisms:

QD bioconjugates can be delivered to tumors in vivo by both active and passive targeting mechanisms although the passive targeting is much slower and less efficient than active targeting. In the passive targeting mechanism, QD bioconjugates accumulate preferentially at

tumor sites due to enhanced permeability and retention effect [70-72]. This effect can be attributed to the facts that angiogenic tumors (i) produce vascular endothelial growth factors, which are responsible for enhanced permeability, (ii) lack an effective lymphatic drainage system, which results in QD bioconjugates accumulation. On the other hand, in the active targeting mechanism, antibody-conjugated QDs are employed where the antibody gets attached to their specific tumor biomarkers such as prostate specific membrane antigen present on the tumor cells at the target site.

Deep Tissue Imaging Requirements:

It has been shown that deep tissue imaging requires the use of far-red and near-infrared light [73]. This necessitates the employment of near-infrared-emitting QDs to increase the tumor imaging sensitivity as the major absorption peaks of blood and water [74] would not interfere in this region.

Removal of Quantum Dots from Living Cells:

The clearance of QDs from the living animals and their metabolism demands careful attention and in-depth study before the technology can be used in humans for the diagnosis and treatment of cancer. The only way of clearance of protected QDs from the body is by slow filtration and excretion through the kidney as chemical or enzymatic breakdown is highly unlikely.

Potential Applications of Quantum Dots in Disease Diagnosis and Treatment:

Near future will see many potential applications of QDs in the field of disease diagnosis and treatment based on the recent advances in the QD technology and the tremendous interest among researchers.

Advances in Quantum Dot Technology for the Diagnosis of Cancer:

In the early stages, QDs were employed for several imaging applications in place of organic dyes. But the tremendous potential of these materials was realized when it was observed that they kept on emitting intense fluorescent light for weeks. This was a major technological advancement for microscopic imaging, which helped in unfolding many cellular processes. In the subsequent stages of development, researchers developed a keen interest in the QD technology and started exploring their applications in different fields. Different QDs composed of the same material but of different sizes had been made, which can generate different colors after activation by light of a single wavelength. It was then demonstrated that QDs tagged with biomolecules such as antibodies, peptides etc. can be employed to detect specific molecules on the cell surface or inside the cell.

Quantum Dot-Peptide Conjugates Shown to Target Tumour Cells:

The use of QD-peptide conjugates to target tumor vasculatures in vivo was reported by Akerman and co-workers [58]. They employed ZnS-capped CdSe QDs and showed the targeting capabilities of QDs coated with different peptides. QDs coated with a lung-targeting peptide accumulated in the lungs of the mice after intravenous injection. The peptide got bound to membrane dipeptidase on the endothelial cells in lung blood vessels. In the second case, QDs coated with a targeting peptide got bound to blood vessels and tumor cells in certain tumors. In the third case, QDs coated with a targeting peptide got bound to lymphatic vessels and tumor cells. The group also showed that adding PEG to the outer coating of the QDs prevented nonselective accumulation of QDs in reticuloendothelial tissues.

Quantum Dot Based Drug Delivery System to Target Cancer:

Shuming Nie and co-workers [35] modified the original CdSe QD with an impermeable coating of polymer that prevented the leaking out of highly toxic cadmium ions from the QD conjugate and provided a means to chemically attach tumor-targeting molecules and drug delivery functionality to the QD conjugate. The group is working on the development of a drug delivery system targeted to the cancer cells. It is developing QDs conjugated to peptides or antibodies to target human tumor cells growing in mice. QDs would be tuned to radiate in the infrared region to prevent tissue damage from the QDs energy emissions. QDs conjugated to

peptide/antibodies specific against the cancer marker on the surface of the target cancer cells would be made to release the drug only when hit with laser light. This would allow control of the cells that will receive the toxin, thus minimizing side effects. There are also on-going efforts by the group to extend the wavelength of fluorescence of the QDs above 900 nm since there are hardly any biomolecules which emit above this wavelength.

The Current State of Play:

Today with the help of QD technology, cancer researchers are capable of observing the fundamental molecular events occurring in the tumor cells. This has been made possible by tracking the QDs of different sizes and thus different colors, tagged to multiple different biomolecules, in vivo by fluorescent microscopy. QD technology holds a great potential for applications such as in nanobiotechnology and medical diagnostics where QDs could be used as labels. But still the use of QDs in humans requires extensive research to determine the long-term effects of administering QDs.

Future Applications of Quantum Dots in Cancer Diagnosis and Treatment:

Researchers have started the exploration of QDs just from the last two decades. The field is still in its infancy but it has captivated scientists and engineers due to the unique optical and electronic properties of QDs. QDs have revolutionized the field of molecular imaging. The forthcoming years would see their potential applications in different fields. One of the major areas of impact is surely the intracellular imaging of live cells. The technology will provide new insights in understanding the pathophysiology of cancer, and in imaging and screening tumors. QDs will definitely be one of the components of the envisioned multifunctional nanodevices that can detect diseased tissue, provide treatment and report progress in real time.

III. Conclusion:

The biological examples show that even after a billion-year arms race, molecular machines have maintained successful defenses against molecular replicators. Failures have been common too, but the successes do indicate that defense is possible. These successes suggest that we can indeed use nanomachines to defend against nanomachines. Though assemblers will bring many advances, there seems no reason why they should permanently tip the balance against defense.

IV. References:

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