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Mini Review

Water-soluble quantum dots for biomedical applications

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Abstract

Semiconductor nanocrystals are 1–10 nm inorganic particles with unique size-dependent optical and electrical properties due to quantum confinement (so they are also called quantum dots). Quantum dots are new types of fluorescent materials for biological labeling with high quantum efficiency, long-term photostability, narrow emission, and continuous absorption spectra. Here, we discuss the recent development in making water-soluble quantum dots and related cytotoxicity for biomedical applications.

Keywords: Water-soluble; Semiconductor nanocrystal; Quantum dot; Cytotoxicity

Quantum dots

Organic dyes have been widely used as fluorophores in biomedical imaging and detection. However, organic dyes are generally vulnerable to the physiological environment and are quickly photobleached under normal imaging conditions. They are also not good for multicolor imaging because of two inherent properties (Fig. 1): (a) organic dyes have relatively broad emission spectra and hence result in the signal overlap from different dyes; and (b) one organic dye can only be suitably excited by the lights within a certain narrow wavelength range and it thus needs nearly the same numbers of excitation light sources as the dyes used. On the other hand, inorganic quantum dots are usually bright (20–80% quantum efficiency) and stable under relative harsh environments [1,2]; the absorption spectra of quantum dots are continuous, and the emission spectra are narrow (typically 20–30 nm for FWHM, full width at half maximum of the emission spectrum) (Fig. 1). Excitation–emission matrix (EEM) reveals that quantum dots always emit the same lights no matter what excitation wavelength used (Fig. 2). Therefore, the entire different emission colors from quantum dots can be seen at the same time by only one laser excitation source. The emission intensity of quantum dots could also be used as a variant for imaging because of their excellent photostability. Theoretically, six colors with 10 intensity levels could determine $(10^6 - 1)$ nucleic acid or protein sequences [3]. The long-term multiplexed biomedical imaging has recently become one of the hottest research topics [3–8].

Biomedical applications require high-quality water-soluble quantum dots. Quantum dots could be made directly in water but often have narrow available size ranges and wide size distribution (leads to wide FWHM) [9–12]. On the contrary, quantum dots produced from high temperature organic solvent synthetic strategies are monodisperse (leads to narrow FWHM) with very wide emission color ranging from ultraviolet to near infrared (300–2500 nm) by simply changing the size, composition and/or structure [13–21]. However, these quantum dots synthesized in organic solvents are insoluble in water. Hence a challenge is how to make the high-quality hydrophobic quantum dots soluble in water and also active in bioconjugate reactions.

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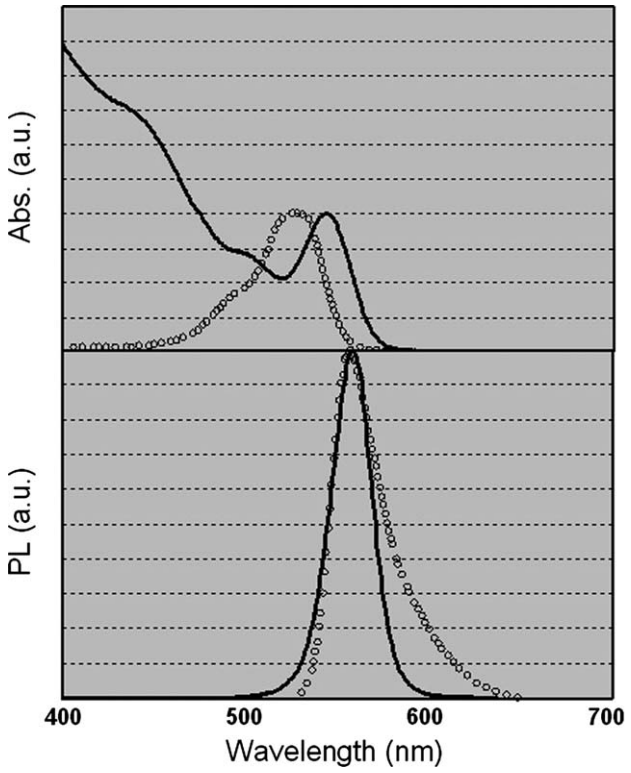

Fig. 1. Comparison of organic dye Rhodamine 640 (dot lines) and a CdSe quantum dot (solid lines) with the same emission peak position in absorption (top) and emission (bottom) spectra.

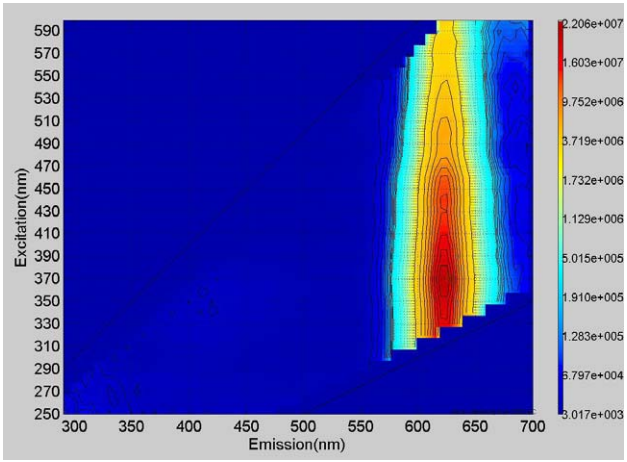

Fig. 2. Excitation–emission matrix (EEM) of quantum dots (emitting 621 nm light).

Water solubilization

Ligand exchange

Typically, the quantum dots synthesized in organic solvents have hydrophobic surface ligands such as trioctylphosphine oxide (TOPO), trioctylphosphine (TOP) [13,22,23], tetradecylphosphonic acid (TDPA) [15,24,25] or oleic acid [15,16,18]. These hydrophobic ligands could be replaced by some water-soluble bifunctional molecules in which one end connects to quantum dot surface atoms and the other end is hydrophilic and may also be reactive to biomolecules (Fig. 3). Examples of some water-soluble bifunctional molecules used are mercaptocarbonic acids [HS-(CH_2)*n*-COOH, $n = 1$–15] [1,26,27], 2-aminoethanethiol [27], dithiothreitol [28], dihydrolipoic acid [29], oligomeric phosphines [20,30], peptides [31], and cross-linked dendrons [2].

However, ligand exchange inevitably alters the chemical and physical states of the quantum dot surface atoms and in most cases dramatically decreases the quantum efficiency of the quantum dots [30,32]; thiol-based molecules (e.g. mercaptocarbonic acids) may form disulfides over time and come off from the quantum dot surface and finally the quantum dots aggregate and precipitate out of water [1]; the other water-soluble bifunctional molecules are expensive and instable, either; The cross-linking of dendrons needs low quantum dot concentration to avoid inter-particle reactions [2].

Forming micelle through hydrophobic interaction

Phospholipids such as 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)] or 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine have both hydrophobic and hydrophilic ends. They could encapsulate quantum dots in the core by forming oil-in-water micelles through hydrophobic interaction between their hydrophobic ends and the surface ligands of the quantum dots and provide water-solubility via hydrophilic exterior ends [33,34]. A more promising approach is to use long chain-length amphiphilic polymers to form micelle-like structures to transfer the hydrophobic quantum dots into water. In one case, poly(acrylic acid) was partially grafted with octylamine through EDC-coupling [EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride] to become amphiphilic and then formed micelle-like structures where hydrophobic quantum dots were encapsulated in the core and –COOH faced outward (Fig. 3) [6]. In order to increase the water-solubility as well as the stability of these structures, some carboxylic groups (–COOH) were further grafted by amino-terminated poly(ethylene glycol) (PEG-NH_2) molecules through EDC-coupling. Nie and coworkers reported the use of a triblock polymer containing segments of polybutylacrylate, polyethylacrylate and polymethacrylic, acid to transfer hydrophobic quantum dots into water, in which methacrylic acid segments were also partially derivatized with octylamine and PEG-NH_2 through a two-step EDC-coupling [35]. A most recent report used poly(maleic anhydride-*alt*-1-tetradecene) to transfer hydrophobic quantum dots into water; the stability was increased by cross-linking the polymers with bis(6-aminohexyl)amine [36].

This strategy of using amphiphilic polymers is generally superior to the ligand exchange, because (a) there is no direct interaction with the quantum dot surface atoms and therefore can preserve the original quantum efficiency

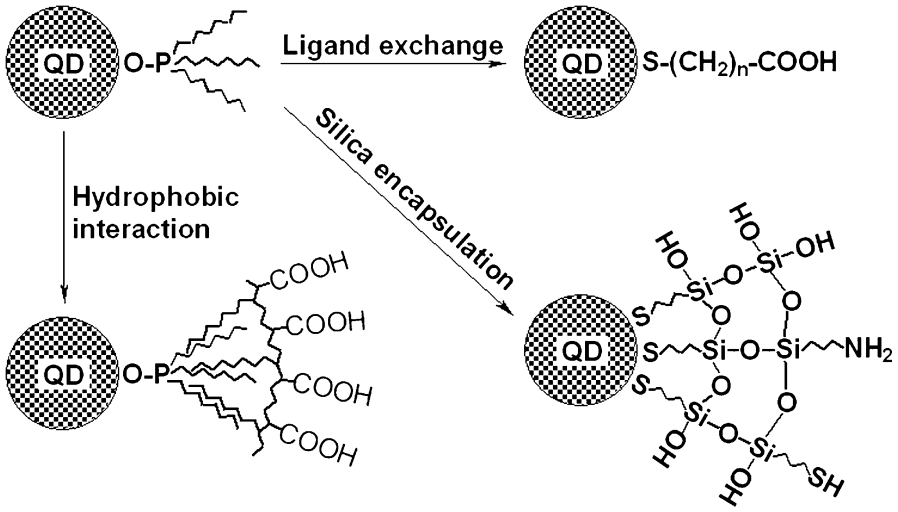

Fig. 3. Quantum dot (QD) water solubilization strategies.

to a highest extent; (b) the polymer's large number of hydrophobic side chains strengthens the hydrophobic interaction to form more steady structures and consequently more stable water-soluble quantum dots; and (c) these amphiphilic polymers are generally commercially available with low prices (PEG-NH_2 polymers are expensive) that make them better materials than other molecules such as peptides and phospholipids in large-scale preparation.

Silica encapsulation

Quantum dots can also be encapsulated by a layer of silica to become biocompatible (Fig. 3) [37–43]. Functional organosilicone molecules containing $–NH_2$ or –SH, are incorporated into the shell and provide surface functionalities for biomedical applications [37,44]. This method seems to stand in between the above two strategies but much closer to ligand exchange. The quantum dot surface situation changes once introducing organosilicone molecules and results in quantum efficiency decrease [43]. The procedures to make a controllable silica layer (coating) around hydrophobic quantum dots are relatively complicated comparing to the other strategies. Another limit is that silica coating needs to be carried out at dilute conditions, which is not suitable for large quantity production [37]. However, Ying and her co-workers has recently reported a simple way to encapsulate quantum dots with silica via reverse microemulsion; the quantum efficiency could be increased if the silica shell thickness, silica coating time were well controlled and/or different starting silanes (e.g. aminopropyl trimethoxysilane instead of mercaptopropyl trimethoxysilane) were used [45].

In comparison, silica encapsulation produces stable but larger quantum dot-in-silica particles with tens of nanometers to several micrometers; ligand exchange and micelle formation are suitable for large-scale production and do not change the whole particle size much; forming micelles can also preserve the original high quantum efficiency to the highest extent; introducing PEG molecules greatly increases the stability and circulating time (for effective targeting) of water-soluble quantum dots. Additionally, the quantum efficiency of the water solubilized quantum dots can be enhanced through light irradiation caused by quantum dot photoionization (photobrightening) [6,45,46]. All the three strategies render water-soluble quantum dots with diverse surface functionalities that can react with biomolecules such as peptides, antibodies and nucleic acids via generic biconjugate techniques (e.g. EDC-coupling) [47]. Both in vitro and in vivo biomedical applications of those water-soluble quantum dots have made many achievements in imaging and detection. A number of recently published review articles provided excellent overviews about the various biomedical applications of quantum dots [5,21,48–57]. Some new results are listed here for your convenience: quantitative 3D technique for the analysis of en face preparation of arterial walls using quantum dots [58]; self-illuminating quantum dots based on bioluminescence resonance energy transfer (BRET) for deep tissue imaging [59]; quantum dot nanobarcodes for multiplexed gene expression analysis [8].

Though quantum dots are superior to organic fluorophores in many features as discussed above (especially the high quantum efficiency in near infrared for deep tissue imaging [20,60–62]), they are inferior to organic fluorophores in several aspects. First, they have larger size (several nanometers to several micrometers) than organic dye molecules, which may be a problem in some applications. Second, water-soluble quantum dots usually have multiple functional groups and make them difficult to build 1:1 quantum dot-biomolecule conjugates; separation is needed to isolate the desired structures [63,64]. Third, there is very limited knowledge about the toxicity of water-soluble quantum dots.

Cytotoxicity

Quantum dots contain toxic components, such as cadmium (from cadmium chalcogenide-based quantum dots)

or lead (from lead chalcogenide-based quantum dots). Cd^{2+} and Pb^{2+} could be released from quantum dots and then kill the cells [65–67]. Thus a direct way to avoid the possible toxicity of quantum dots is to make them well coated to become biologically inert. The coating materials can be low or nontoxic organic molecules/polymers (e.g. PEG) or inorganic layers (e.g. ZnS and silica).

A few studies show direct, extracellular cytotoxicity of water-soluble CdSe and CdSe/ZnS quantum dots, because Cd^{2+} is released from the nanoparticles which is highly due to poor purifications and/or simple surface cappings/coatings [68,69]. Quantum dots coated by simple molecules, such as mercaptoacetic acid, mercaptopropionic acid, 11-mercaptoundecanoic acid, 2-aminoethanethiol, are more toxic than the ones coated with silica. The silica layer is different from the amphiphilic polymer layer or small molecular ligands; it can be very thick (up to several micrometers) and therefore reduce the possible leaking of interior toxic cadmium or lead under physiological environments. There is no clear toxic effect observed if additional PEG molecules were attached to the silica exterior surface [68–70].

In addition to the extracellular cytotoxicity studies, Chang et al. have found that quantum dots could enter the cells through endocytosis, and the cell death was highly related to the uptake quantity no matter what surface coating is [71]. Nonetheless, the surface coating did affect the uptake quantity and in turn influenced the intracellular cytotoxicity [72].

In fact, many biomedical imaging and detection applications of quantum dots encapsulated by complex molecules do not exhibit noticeable toxic effects [48,50,53,73]. It was reported that the tumor cells labeled with quantum dots survived the circulation and extravasated into tissues just as effectively as unlabeled cells; there was no obvious difference in their ability to form tumors in mice after 40 days; and quantum dots had no adverse effects on the physiology of the host animal or labeled cells [74]. Our recent study also disclosed that water-soluble quantum dots encapsulated by amphiphilic polymers (with PEG) were in very low toxicity to cells if the dosing level was lower than 100 nM (Fig. 4) [75].

Besides developing stable and robust coating strategies to minimize the toxicity of current available quantum dots (stabilizing quantum dot particles against bleaching, reducing cell uptake), another direction is to develop new high-quality quantum dot systems that do not contain very toxic components. Peng and his colleagues have synthesized Cu or Mn doped ZnSe quantum dots which emitted 470–600 nm lights with relatively narrow FWHM (45–65 nm) and acceptable quantum efficiency (10–30%) [76]. This will ultimately eliminate the extracellular and intracellular cytotoxicity of quantum dots from the toxic components.

However, quantum dots' toxicity may also come from their native properties no matter what components or coating strategies used. Quantum dots can transfer absorbed optical energy to nearby oxygen molecules to generate reactive oxygen species (ROS) such as free radicals (hydroxyl radical $^{\cdot}OH$ and superoxide $^{\cdot}O_2^-$ and singlet oxygen ($^1O_2$), and then result in cell damage and death. Reactive oxygen species have been found in several quantum dot systems, such as CdS, CdSe, CdSe/ZnS [77], and CdTe [78]. DNA nicking by free radicals generated from CdSe and CdSe/ZnS quantum dots has been reported even under dark conditions [79]. These reactive oxygen species on the contrary make quantum dots possible probes for photodynamic therapy (PDT) applications [80,81].

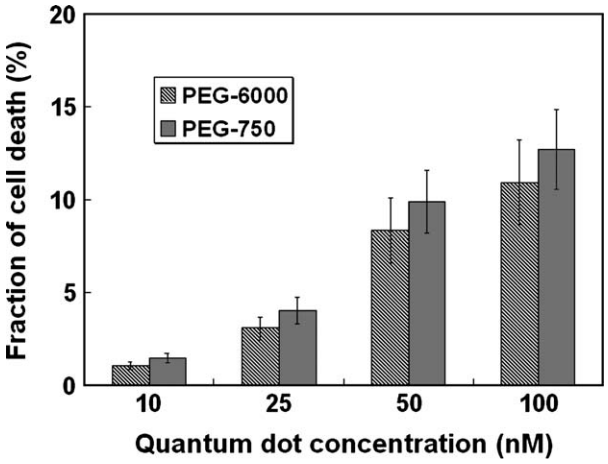

Fig. 4. In vitro cytotoxicity of water-soluble quantum dots with different PEG coatings (the numbers of 750 and 6000 are the molecular weights of PEG polymers) to SK-BR-3 human breast cells. Full growth medium of confluent SK-BR-3 cells were incubated with water-soluble quantum dots in the dark over 24 h. The cells which were then gently washed with PBS and stained using a commercial LIVE/DEAD stain assay (Invitrogen, CA). Images were collected using the Zeiss Axiovert 135 with a Nikon Coolpix 5000 digital camera and analyzed by ImagePro to attain statistical significance.

Last but not the least concern is the body clearance of these nanoparticles, which has not been carefully studied. Quantum dots have been found accumulated in mouse bone marrow, spleen, and liver for at least four months after administration [82]. Particles larger than 50 nm could not be easily cleared from kidney; and the typically negative charged quantum dots may further reduce their clearance size from kidney [83]. Large and stable nanoparticles may be cleared from animal and human bodies through liver [84–87].

Summary

With the fast development of new methods to make low-cost, low-toxicity, high-quality (monodisperse, high quantum efficiency, long-time photostability, versatile surface functionality) water-soluble quantum dots, the understanding of the toxicity mechanisms and the processes to clear nanoparticles from animal/human bodies, quantum dots for non-invasive biomedical multiplexed imaging, detection, as well as possible drug delivery and therapy could be established in the near future.

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