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Multifunctional Polymer Coated CdSe Quantum Dots Imaging and Drug Delivery Applications

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ABSTRACT

Quantum dots (QDs) are nanoparticles that have attracted widespread interest in medicine, drug delivery and imaging in living animals due to their unique electronic and optical properties. QDs with Bioconjugated have been introduced for imaging and targeting in living molecular cells, animal models and also in humans. Present efforts are focused on exploring the massive multiplexing capabilities of the QDs for Magnetic resonance imaging (MRI), drug delivery and in addition biocompatibility, bioconjugation and biotoxicity of QDs are also analyzed and discussed. These advances in the QD technology have unraveled a great deal of information about the molecular events in tumor cells and early diagnosis treatment.

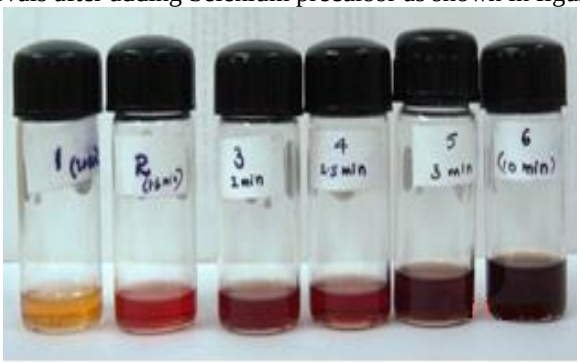
Introduction

Semiconductor quantum dots (QDs) have captivated scientists and engineers over the past two decades owing to their fascinating optical and electronic properties, which are not available from either isolated molecules or bulk solids [1]. Recent research has stimulated considerable interest in developing these QDs as fluorescent probes for biomedical applications [2]. Compared with organic fluorophores, these QDs have similar electronic and optical properties, such as tunable fluorescence emission from visible to Infrared wavelengths. The absorption coefficients of such (QDs) have a wide spectral range, strong photo stability and high brightness. Owing to their broad excitation profiles and narrow/symmetric emission spectra, high-quality QDs are also well suited for QDs as labels in biomedical imaging and drug delivery targeting [1, 2]. Nevertheless, due to the large research efforts dedicated to this work, a number of imaging applications has recently appeared and this is currently an expanding field of research. In this article, we discuss *in vivo* imaging techniques and use of multifuncutionalized QDs for molecular targeting and imaging in living animal models. In particular, the immobilization of nanocrystals in solid membranes and its use in combination with polymer platforms has seen some progress. Such arrangements are a key step towards the development of advanced drug delivery targeting and analytical imaging [3]. For this, QDs are very well suited because of their high photo stability and multiplexing ability.

Experiments And Discussions

Preparation of Colloidal CdSe quantum dots

CdSe nanocrystals are synthesized from CdO and elemental Se using a kinetic growth method where particle size depends on reaction time. A stock solution of Se precursor may be prepared ahead of time by combining 30 mg of Se and 5 mL of 1-octadecene (tech., 90%) in a 10-mL round-bottom flask clamped over a stirrer hot plate. A syringe is used to measure 0.4 mL of trioctylphosphine from its Sure-Seal bottle to the same 10- mL flask. A magnetic stir bar is added and the solution is stirred. It may be warmed as necessary to speed dissolution of the Se. The stock solution is stored at room temperature in a sealed container and has enough Se precursors for five preparations. The Cd precursor is prepared by adding 65 mg of CdO to a 250-mL round-bottom flask clamped in a heating mantle. To the same flask 3 mL of oleic acid and 50 mL of octadecene are added. A thermometer capable of measuring 225 ^{0} C is inserted, the temperature to which the flask is then heated. When the temperature reaches 225 ^{0} C, 5 mL of the room-temperature selenium solution is transferred to the 225 ^{0} C cadmium solution. Because the characteristics of the products depend on reaction time, one should begin timing when the selenium solution is added. A 9-inch Pasteur pipette is used to remove and quench approximately 1 mL samples at frequent time intervals, as quickly as possible in the beginning and when noticeable color change is detected at later times. Two sets of CdSe Quantum Dots were prepared with different time intervals after adding Selenium precursor as shown in figure 1.



Figure

CdSe Quantum Dots with different sizes

The second set preparation of six samples were pipette out within three minutes ,and other two samples pipette out by 5 minutes ,10 minutes respectively after adding selenium precursor as shown in the figure 2.

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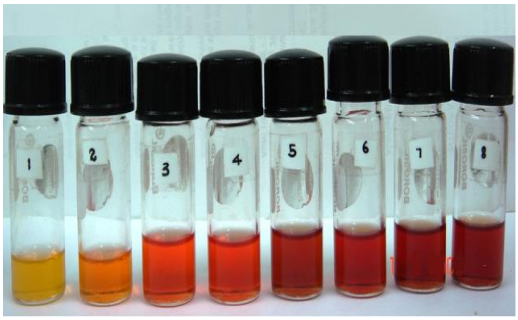

Figure 2. Colloidal suspensions of CdSe QDs of increasing size from Left to right

Preparation of polymer solutions

The polymer solutions were prepared on the weight basis. The 100 mg of PVA and PEG polymers were weighed in an electrical balance and added to different beakers containing 10 ml of distilled water each (1 wt %). Each beaker was added with a Magnetic Stirrer (bead) and was subjected to stirring in a Magnetic spinout. The PVA has been taken as hot water soluble, Hence the temperature of the spinout was set to 60 ˚C. The temperature was not allowed to exceed the set temperature as the cloud point of PVA is 85˚C beyond which the solution is bound to become turbid and evaporate due to the presence of water (Boiling Point: 100˚C). In a similar fashion, PEG polymer solutions were prepared [4].

Preparation of polymer functionalized CdSe QD's

The prepared polymer solutions with different weight % (0.2%, 0.4%, 0.6%, and 0.8%) were mixed with 2ml of freshly prepared CdSe colloidal solution. The solutions were stirred in a digital stirrer fitted with a three headed glass rod propeller at 500 rpm for 2hrs continuously. About 2ml of each sample of the solutions were taken in culture tubes to measure the size in the DLS Zetasizer.

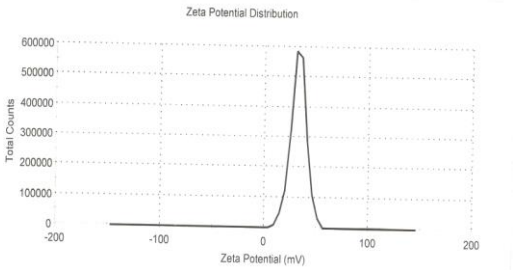

Figure 3. Zeta Potential Report for PEG-CdSe QD

Optical Properties

QDs can be made to emit fluorescent light in the UV to IR 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 [5-6]. The lifetime limited emission rates for single QDs are 5–10 times lower than those of single organic dyes, because of their longer excited state lifetimes (20–50 ns) [7].The rate of absorption is the main limiting factor of fluorescence emission because the fluorescence imaging usually operates under the absorption limited conditions. As the molar extinction coefficients of QDs are about 10-50 times larger than those of organic dyes (5_10_ 104 M _1cm_1), the QD absorption rates will be 10-50 times faster at the same excititation photon flux (1.e. the number of incident photos per unit area)[1, 8]. Fluorescence image of vials of monodisperse QDs with sizes ranging from 2.5 nm to 8.2 nm in diameter as shown in the figure 4.

Figure 4. Comparison of fluorescence light emission from the Organic dye Green and red QDs

According to the above statement, the figure has shown the comparison of fluorescence light emission with increased rate, individual QDs have been found to be 10-20 times brighter than organic dyes in the (first vial), green QDs (second vial) and red QDs (last vial) under normal root light illumination and at the same molar concentration (1.0 mM). Bright fluorescence emission is observed from the QDs but not from the dye, owing to the large absorption cross sections of QDs. In contrast with traditional dyes, which have broad emission spectra with a characteristic long red tail, nanocrystals present a symmetrical (approximately Gaussian) and relatively narrow emission profile and a very broad absorption spectrum. These two features combined allow obtaining a large equivalent Stokes shift, which can be tuned by setting excitation at any wavelength lower than the emission peak, facilitating the discrimination of the QDs' luminescent emission. Absorption spectra of the same four QD same figure shown in the figure 5a. Notice that the absorption spectra are very broad and wavelength for excitation.

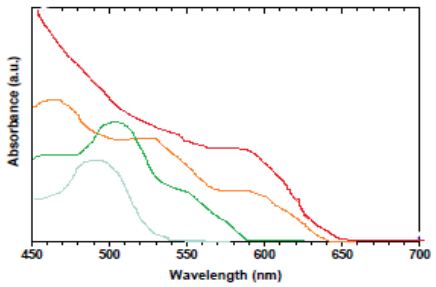

Figure 5a. Absorption spectra

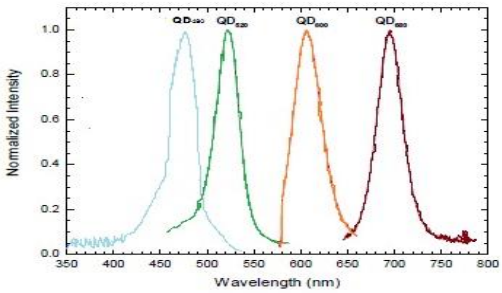

Figure 5b. Fluorescence spectra of the same QD Samples

In addition, it also introduces the possibility of exciting different QDs with the same optical source. This can be observed in Figure 5b where the emission of CdSe QDs Figure 5a. Absorption spectra Figure 5b .Fluorescence spectra of the same QD samples QDs with different emission wavelengths, which can be tuned from the UV, to the visible and near-IR spectra and also in the mid-IR. The figure 5b introduces the possibility of exciting CdSe QDs with the same optical source

of emission bands (24-27 nm FWHM of full-width half maximum) indicate narrow particle size distributions and more symmetric emission spectra. However QDs are macromolecules that are an order of magnitude larger than organic dyes, which may limit their use in applications in which the size of the fluorescent label must be minimized. Yet, this macromolecular structure allows the QD surface chemistry and biological functionality to be modified independently from its optical properties [7, 9]. Luminescence lifetimes can also give information about the recombination mechanism. So one can distinguish between fluorescence and a phosphorescence mechanism by means of the lifetimes. Fluorescence mechanisms have lifetimes in the nanosecond region, were as phosphorescence is in the millisecond region [9]. If decay is mono or multi exponential can give information if there is more than one electron population present in the particles. This reduces the signal to noise ratio and leads to difficulties to detect the fluorescence. So a good fluorophore is either one with a very intense emission compared to the background or with a long lifetime.

Photostability

In addition, QDs have narrow spectral line widths, very high levels of brightness, large absorption coefficients across a wide spectral range, high photostability and capability of multiplexed detection [10]. The figure 6 shows the QDs are several thousand times more stable against photostability than organic dyes under the same excitation conditions; hence it has been suitable for long period time in continuous tracking.

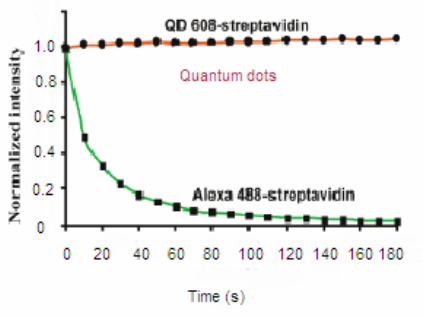

Figure 6. Photostability curve

QDs are very bright and stable even under complex in vivo conditions that make them suitable for advanced drug delivery, molecular and cellular imaging, and for highly sensitive bioassays and diagnostics .The large Stokes shifts of QDs measured by the distance between the excitation and emission peaks can also be used to further improve detection sensitivity. This factor becomes especially important for *in vivo* molecular imaging due to the high auto fluorescence background often seen in biomedical specimens [11]. As shown in Figure 5, the Stokes shifts of QDs can be as large as 30–40 nm, depending on the wavelength of the excitation light. This 'color contrast' is only available to QD probes, as the signals and background can be separated by wavelength-resolved or spectral imaging [13].

Tumour Targeting and Imaging

Female C3H/HeNCr MTV and athymic NCR NU/NU mice were used for experiments in this study. The mice were 7 –8 weeks old and weighed 20–30 g at the time of these studies. All experiments were carried out in compliance with the Guide for the Care and Use of Laboratory Animal Resources [14]. Figure 7 shows the Tumors were grown in C3H mice by administering a subcutaneous injection of 5 _ 105 murine squamous cell carcinoma (SCC) cells to the lateral aspect of the right hind leg (7a). Orange fluorescence light shows the *in vivo* imaging of initial growth stage of tumor with conjugated QD (7b). After in vivo imaging, histological and immune cytochemical results confirms from the underlying tumor from QD signals. From the visible light of certain limited penetration, QDs in were not detected in the deep organs. Tumor cells were approximately 12 mm in diameter (_500 mm3) was incolculated for seven days during the Imaging experiment. The C3H mouse was induced by anesthesia with 220 xylazine IP injection to test the Resolution of *in vivo* imaging.

A different image was collected in an hour by injecting A 125 _l bolus of the QD solution into the vein. Eventually, to maximize the resolution small aperture and long exposure was taken after five minutes of injecting another 125 _l bolus of QDs.

Figure 7(a). Tumors were grown in C3H mice

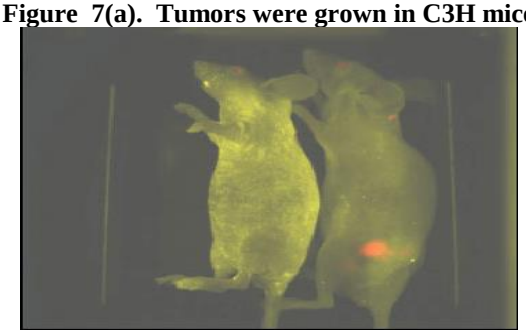

Figure 7(b). Initial growth stage of tumor

Toxicity

The CdSe QDs are highly toxic to cultured cells under UV illumination for extended periods of time [15]. This is because the ultraviolet irradiation energy and the covalent bonding energy were close to each other and it makes the QDs in a process known as photolysis (cadmium ions released into the cultured cells). Polymer coating QDs does not cause UV irradiation, such QDs were nontoxic. The QDs protected with polymer might be cleared the enzymatic or chemical degradation from the body by slow filtration and excretion. Besides cytotoxicity, the degradation and metabolism of nanocrystals in the body remains to be investigated and there are reports that injected nanocrystals can accumulate in kidney, liver and spleen [16]. Whether nanocrystals can ultimately be cleared from the body is not known. More research in this area must be completed before they can be used as probes for diagnostic applications. These applications should examine carefully before implementing to the human tumorimaging.

Conclusion

The polymer coating QDs had shown an innovative class of imaging agents in tumor detection and drug delivery. In conclusion, a simple, economic, environmental friendly, aqueous synthesis method has been developed to produce quantum dots in water directly at room temperature.

Without any toxic element, the aqueous CdSe QDs were prepared with various capping molecules, possessing outstanding features such as small particle size (~8 nm), biocompatibility, In particular, the strong luminescence and the resistance to photo bleaching make them ideal candidates for pharmacokinetic studies [17]. The present aqueous QDs have great potential for a wide range of imaging applications. This technique helps to identify the growth of tumors/lesions by correlating optical imaging with MRI (Magnetic Resonance Imaging) and to remove the diseased tissue and cells completely. Besides, this kind of multifunctional polymer coated QDs also addresses the issue of toxicity. Furthermore, advances in handling and tagging these materials should be directly transferable if new types of nanocrystals, made with less toxic materials, become available in the future [17,18]. So if the goal is to carry out quantum dots experiments on waveguides, it is worth to continue the research on this kind of material for biological systems and for biomedical applications.

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