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Progress in the toxicological researches for quantum dots

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Quantum dots (QDs) have received more and more attention as a novel example of nanomaterials. Due to their unique fluorescent characteristics, quantum dots have been successfully applied in biotechnology and medicine applications. Recently, the toxicity and the potential environmental effects of QDs have become a research hotspot. In this paper, toxicological effects of QDs are reviewed, and the prospects and research directions are given based on the analysis of this research field.

quantum dot, toxicology, nanomaterial

1 Introduction

With the industrialization of nanotechnology, nanomaterials have been widely applied in diagnostics, material modification, degradation of pollutants and biological techniques, etc. Inevitably, the latent damage brought by these hyperfine particles to the environment has gradually caused concerns in the past years[1,2]. Quantum dots (QDs), which are also known as semiconductor nanocrystals, are generally composed of group II-VI or group III-V elements. Because of QDs' small particle size (ca. 2—10 nm), electrons and holes are restricted, thereby continuous energy bands become discrete energy levels of molecular characteristic structures. As a result, QDs have unique optical properties. At present, the most promising application of QDs is its usage as fluorescent markers in the field of biology and medicine[3−6].

As a new type of fluorescent probes, QDs are more superior to traditional organic dyes (such as Rhodamine 6G) due to their narrow emission range, broad UV excitation range, bright fluorescence, and high photostability. However, for bare QDs, the quantum yields are often very low. QDs are also liable to release the poisonous element cadmium. Therefore, the core/shell structure consisting of a metalloid crystalline core (CdSe or CdTe) and a shell (ZnS or ZnSe) renders QDs' bioavailability. The structure can ensure the higher fluorescent efficiency and better stability of photochemistry[7,8], and can prevent cadmium ions from being released. Subsequently, how to solve the problems of the dispersibility and solubility of QDs, and how to decrease nonspecific combination with other media have come to be new challenges for the application of QDs in life sciences. In 1998, Alivisatos et al.[9] and Nie et al.[10] used QDs as fluorescent probes in the living cells. This study preliminarily solved the difficulties in the solubility and conjugation of QDs with biological macromolecules by surface functional groups. Successively, all kinds of biocompatible QDs have been applied to cell recognition and identification, biological macromolecular location, subcellular distribution, endocellular ingredients' transportation and signal transmission. However, the real

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application of QDs in biology has not been achieved until 2001. Nie et al.[11] transplanted QDs of different numbers and different fluorescent characteristics into macromolecular spheres and consequently produced corpuscles with the spectral coding function. They discovered that the QDs corpuscles, comprising the particles with five or six different colors and particles with six different fluorescent intensities, could produce $1 \times 10^4 - 4 \times 10^4$ identifiable code signals. These coding corpuscles succeeded in the model experiment of DNA segments' detection and recognition. Hitherto, QDs have been successfully utilized in single molecule detection, single cell tracing and *in vivo* imaging, etc.[12−15].

Undoubtedly, the contact between QDs and biology is becoming closer with the in-depth researches. Thus, new problems have come forth during the processes of absorption, transportation and metabolism, what effects and responses will the QDs bring to organisms? Whether will the application cause the potential toxicity? How to evaluate the effects? These series of problems have promoted the demands of the evaluation of toxicity for this kind of nanomaterials. Currently, the diversity of QDs and the paucity of toxicological information make assessment of the adverse effects of these artificial nanomaterials on biologic systems difficult.

2 Recent advances in toxicological effects on QDs

Heretofore, most investigations indicated that toxicity of QDs depends on multiple factors arose from inherent physicochemical properties, such as QD size, stability, dispersibility, surface charge, surface coating, oxidative, concentration, species, and exposure time. Thus, both the intrinsic natures of QDs and the external environmental conditions should be considered when evaluating QD toxicity.

2.1 Effect of physicochemical properties of QDs on cytotoxicity

From 2004, the relationships between the QD structure and toxicity have been discussed by some researchers (Table 1). Shiohara et al.[16] probed into the effect of the QD (CdSe/ZnS) size on cell viability. Sheep serum albumin (SSA) was conjugated to three kinds of CdSe/ZnS with different dimensions (fluorescent emission wavelengths were 520, 570, 640 nm, respectively), and then the cell viability was investigated in Vero cells, Hela cells and primary human hepatocytes. By using MTT assay, it was found that cell viability noticeably decreased even in a rather low concentration (0.1 mg/mL). On the other hand, the effects of QD520 and QD570 on the cell viability were greater than that of QD640. Previous study showed that the size of QDs determined the mobility of QDs inside the cell, and this result may also explain the phenomenon that smaller particles cause cell damage more easily. With respect to the cell acute cytotoxicity, the death rate of Vero cells was studied after their exposure to various concentrations of QD520. The results demonstrated that the cell death rate had obvious dose-dependent and time-dependent effects. The consistent results were also obtained about the cytotoxicity of CdTe with different sizes to HepG2 cell in Zhang et al.'s[17] study.

Lovric et al.[18] examined the subcellular distribution and toxicity of CdTe QDs with different particle sizes and surface charges. The results demonstrated that the localization of CdTe in PC12 and N9 cells mainly depend on the QD size. Furthermore, the confocal fluorescent micrographs showed that CdTe with red emission light (Red type, diameter: 5.2±0.1 nm) were mostly distributed throughout the cytoplasm. In contrast, CdTe with green emission light (Green type, diameter: 2.2±0.1 nm) were predominantly localized in the nuclear compartment. Besides, both of CdTe QDs exhibited remarkable cytotoxicity at 10 μg/mL. While at high concentration exposure (100 μg/mL), red QD and green QD caused different decreases in cell metabolic activity with 46.8±2.3% and 68.8±1.4%, respectively. It indicated that smaller QD had greater potential toxicity. On the other hand, cationic QDs (amido-modified) and anionic QDs (carboxyl-modified) showed differential toxicity in inducing the morphological changes of the nuclear (chromatin condensation and membrane blebbing) and decreasing the cell metabolic activity.

Most studies suggested that the physicochemical properties of QDs' surface coatings were the dominant factors to affect QD toxicity. Currently, a lot of research groups are carrying on work on surface modification of QDs. Hoshino et al.[19] investigated the genotoxicity of ZnS/CdSe modified by several functional groups to WTK1 cells. Synthesized QDs were coated with MUA (QD—COOH), cysteamine (QD—NH_2), or thioglycerol (QD—OH), and equal molar quantities of thioglycerol and MUA or cysteamine and thioglycerol were used to

Table1 Effect of physicochemical properties of QDs on toxicity

QDs type	Model animal	Exposure concentration	The toxicological effect	Reference
CdSe/ZnS-MUA	Vero cell Hela cell human primary hepatic cell	0－0.4 mg/mL	cytotoxic at 0.1－0.2 mg/mL; CdSe/ZnS with smaller size was more toxic.	[16]
CdTe	HepG2 cell; Rat	2 nmol/kg	cytotoxic at 2 nmol/kg; smaller CdTe was more toxic.	[17]
CdTe Red: 5.2±0.1 nm Green: 2.2±0.1 nm	PC12 cell N9 cell	0.01－100 μg/mL	cytotoxic at 10 μg/mL; chromatin condensation and membrane blebbing	[18]
－COOH, $-NH_2$, －OH, －OH/COOH, $-NH_2$/OH Conjugated CdSe/ZnS	WTK1 cell	1－2 μmol/L	QD-COOH: DNA damage; others were weakly or negative genotoxic.	[19]
PEG-SiO_2/ZnS-CdSe PEG-SiO_2/CdSe	Cos7, NIH3T3, HepG2 cell	0－100 μg/mL	100 μg/mL: 50% cell viability. the toxicity was smaller than those of MAA/QD and PA/QD.	[20]
CdSe-Fluronic 68 CdSe-CTAB CdSe-SDS	HepG2 cell	0－400 μg/mL	fluronic 68-CdSe QD was much less toxic than CTAB-QD and SDS-QD	[21]
MPA-CdTe Cys-CdTe NAC-CdTe, Cys-CdSe/ZnS	MCF-7 cell	10 μg/mL	Cys-CdSe/ZnS was non-toxic; MPA-CdTe, Cys-CdTe and NAC -CdTe showed significant toxicity.	[22]
CdSe/ZnS-MPA, PEG, silane: Red (24 nm) Green (13 nm)	NRK fiber primary cell MDA-MB-435S cell CHO cell, RBL cell	2－10 nmol/L	MPA-polymer and polymer-silane QDs were uptaken in a similar way, while PEG-silane QDs were reversed.	[23]
PEG-CdSe/CdS: 750-PEG-QD 6000-PEG-QD	SK-BR-3 cell	10－150 nmol/L	cytotoxicity in the order: bare QD>(750)-QD>(6000)-QD	[24]
PEG-CdSe/ZnS: 750-PEG-QD 5000-PEG-QD	mice	20 pmol QD/g b.w.	different accumulation and clearance; no obvious localized necrosis in target organs.	[25]
QD-LM QD-LM-BSA	rats	16 pmol QD/g b.w.	different pharmacokinetics; no obvious pathological changes in target organs.	[26]
PEG-CdTe/ZnS: 5000-PEG-QD	mice	1.12 pmol QD/g b.w.	no signs of apparent pathological alteration; long persistence in the blood.	[27]

introduce two functional groups (QD－OH/COOH, and QD－NH_2/OH, respectively). These five hydrophilic QDs varied on a certain extent in the fluorescent intensity and the maximum fluorescent emission wavelength. By comet assay, the genotoxic potential of different QDs was compared. It was found that QD－COOH exhibited distinct toxicity after 2 h of treatment at 2 μmol/L. However, QDs modified by other functional groups exhibited low or no genotoxicity at the same condition. To validate the origin of toxicity, three ingredients of QDs (MUA, cysteamine, and thioglycerol) were tested. The results showed cysteamine and thioglycerol were weakly or not toxic correspondingly, while MUA was highly toxic, which could be the explanation of severe cytotoxicity caused by QD－COOH. Moreover, the results provided evidence that some compound-coated QDs are responsible for the genotoxicity of QD. Selvan et al.[20] used the reverse microemulsion technique successfully to synthesize SiO_2-coated QD (SiO_2/ZnS-CdSe and SiO_2/CdSe), which had better stability and a higher quantum yield (17%~20%). Subsequently, comparison of cytotoxicity between SiO_2-coated QD and other two water-soluble QDs (MAA-coated QD and PA-coated QD) was conducted using Cos7, NIH 3T3 and HepG2 cells. The cytotoxicity tests indicated that SiO_2-coated QDs were much less toxic than the MAA-coated QD and the PA-coated QD at the same conditions, which could be attributed to the effective prevention of QD disintegration. Guo et al.[21] chose Fluronic 68 (F-68), cetyltrimethyl ammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) as the surface polymeric shells of QD CdSe, and then the cytotoxicity of the three surface modified QDs were compared in HepG2 cells using MTT assay. The results showed that QD modified with F-68 was much less toxic than QD modified with CTAB and SDS. Cho et al.[22] compared cytotoxicity toward human breast cancer (MCF-7) cells of four QD samples differing in terms of chemical composition and surface modification (MPA－CdTe, Cys－CdTe, NAC－CdTe and Cys－CdSe/ZnS). The results indicated that at the

exposure concentration of 10 μg/mL for 1 h, core-shell CdSe/ZnS QD (Cys-CdSe/ZnS) presented little damaging effect to cells, while all of CdTe QDs capped with small organic ligands (MPA, Cys and NAC) were cytotoxic. Kirchner et al.[23] investigated cytotoxicity of CdSe and CdSe/ZnS QDs for different surface modifications such as coating with MPA, silane, and polymer. The confocal fluorescent micrographs showed that MPA-polymer and polymer-silane coated particles were uptaken by MDA-MB-435S breast cancer cells in a very similar way: smaller green-fluorescent QDs (~13 nm) could be detected inside the cells, while bigger red-fluorescent QDs (~24 nm) were barely observed inside the cells. However, absolutely contrary behavior was found for PEG-silane coated QDs. It indicated that the different lipophilicify of QDs may lead to discrepant absorption ways, which could subsequently induce disparate toxicity. Chang et al.[24] made use of QDs modified by PEG of different molecular weights to evaluate the endocytosis and toxicity to the human breast cancer cell line SK-BR-3. It was found that cytotoxicity of QDs increased in the following order: bare QDs > (750)-PEG-QD > (6000)-PEG-QD, which was consistent with the cellular uptake by endocytosis reported previously.

2.2 Pharmacokinetics of quantum dots in vivo

Pharmacokinetics plays an important role in the toxicological research. To date, very limited comprehensive study on the *in vivo* toxicity of QDs exists. Ballou et al.[25] prepared QDs coated with amphiphilic polyacrylic acid and simultaneously conjugated to PEG with different molecular weights (750 and 5000). The prepared QDs were injected into the tail veins of mice by 20 pmol QD/g body weight. The noninvasive imaging revealed that significant liver uptake was visible even at 1 min using (750)-PEG-QD, but completely cleared away after 1 h, while (5000)-PEG-QD was absorbed by liver visibly in 1-3 h postinjection, which illustrated that uptake of QDs in mice depended on the surface modification. Fischer et al.[26] coated ZnS/CdSe with mercaptoundecanoic acid (MUA)/ lysine (Lys) to form QD-LM (about 25 nm) or coated with bovine serum albumin (BSA) to form QD-LM-BSA (about 80 nm). Synthesized QDs were given an injection of 16 pmol/g b.w. dose of QDs into the jugular vein of the rat *in vivo*. It was found that QDs could be detected in the liver, spleen, lung, kidney, colon and bone marrow, and the liver was the main target organ. By comparing the kinetic behaviors of the two modified QDs, the researchers found that the absorption percentages of QD-LM and QD-LM-BSA were evidently different in the liver with ratios of 36.4% and 99.5%, respectively. In the aspect of elimination, QD-LM was cleared from plasma with a clearance speed of 0.59±0.16 $mL \cdot min^{-1} \cdot kg^{-1}$, which was lower than that of QD-LM-BSA (1.23±0.22 $mL \cdot min^{-1} \cdot kg^{-1}$), and the half-life for QD-LM and QD-LM-BSA were 58.5±17.0 min and 38.7±3.5 min correspondingly. In the feces and urine, QD was not detected. Similarly, Yang et al.[27] conducted kinetic study in mice on a commercially available quantum dot, (5000)-PEG-ZnS/CdTe (QD705，about 13 nm). After single intravenous (iv) injection at the dose of 40 pmol (about 1.12 pmol QD/g b.w.) for up to 28 days, they demonstrated that the liver and spleen were the main accumulative organs. The clearance rate from plasma was 2.3 $mL \cdot h^{-1} \cdot kg^{-1}$ and the half-life $t_{1/2}$ was 18.5 h, which was far longer than the results reported by Ballou et al.[25] ($t_{1/2}$ of QD630, QD645 and QD655: 12－70 min) and Fischer [26] ($t_{1/2}$ < 60 min). Similar to the results reported by Fischer et al.[26], QDs were not detected in either urine or feces after the single administration for 28 days. But Ballou et al.[25] deduced that QDs could be excreted by feces according to the phenomenon of fluorescence observed in intestinal canal. In Zhang's[17] study, QD CdTe was administrated into rats by intravenous injection at a dose of 2 nmol/kg, and no significant changes of physiological parameters in blood and urine were observed. Although the literature published above did not give any evidence of pathologic changes in the target tissues, the discrepancy of pharmacokinetics probably resulted in the differences of QDs toxicity.

In order to improve the bioavailability of QDs effectively, many researchers have been doing a large number of studies on the structural modification and toxicity of QDs. There is no doubt that the structural amelioration not only can promote the fluorescent stability, intensity and lifespan of QDs, but also can influence the physicochemical behavior of QDs in organisms simultaneously. Hence, it is important to explore the mechanism if we want to find out the unknown chemical process.

2.3 Researches on the mechanism of QDs toxicity

In recent years, some research groups have started discussing the mechanism of QDs toxicity from different aspects. Derfus et al.[28] prepared QD CdSe coated with

tri-*n*-octylphosphine oxide (TOPO) and water-solubilized with mercaptoacetic acid (MAA). The succedent toxicity test showed that cellular viability decreased dramatically from 98% to 21% when TOPO-coated QDs were initially exposed in the air for 30 min. Thus, it was suspectable that the O_2 in the air probably oxidized the QDs' surface, resulting in the cytotoxicity. To validate the hypothesis, the investigator utilized ultraviolet radiation to accelerate the oxidation process. The results indicated that cytotoxicity increased with exposure time and was time-dependent, which was induced by ultraviolet light radiation. By determining the free Cd^{2+} concentration in QD samples at the same condition, low levels were found in QDs solution without any oxidation (6 $\mu g \cdot mL^{-1}$), while comparatively high levels of Cd^{2+} were found both in the air-oxidized (126 $\mu g \cdot mL^{-1}$) and UV-exposed samples (82 $\mu g \cdot mL^{-1}$). Based on the data, Derfus et al.[28] proposed the mechanism of QDs toxicity: the surface of QD CdSe was oxidized to release free Cd^{2+}, see Figure 1. Since hydrogen peroxide (H_2O_2) exists in the environment, the QD response to 1 mmol/L H_2O_2 for 24 h *in vitro* was investigated, and the freely dissolved concentration of Cd^{2+} was 24 $\mu g \cdot mL^{-1}$. According to the data in the published literatures, even low levels of cadmium ions (11—44 $\mu g \cdot mL^{-1}$) could lead to significant cell death[29,30], which illustrated the potential toxicity of QDs in the environment.

Understanding the different polymers may alter absorption ways of QD by cells, Kirchner et al.[23] further studied the connection between the release of Cd^{2+} and the toxicity of CdSe and CdSe/ZnS. The cell viability was tested and the free Cd^{2+} in the QD system by ICP-OES was determined synchronously. The results showed that the cell viability decreased significantly with the increase of the free Cd^{2+} concentration, which were coincident with Derfus et al.'s[28] report. Interestingly, polymer-coated Au nanoparticles showed the same effects as polymer-coated QDs under the same condition. This fully illuminated that the release of Cd^{2+} was a crucial but not the only possible factor to cause cell damage, thus the mechanism else of QDs toxicity should be further explored.

In virtue of the coatings of QDs may be oxidized and degraded, yielding bare QDs after accumulation in the body for a long period of time, it is informative to research the toxicity of QDs without any modifications. The Lovric group[31] found that the naked CdTe could induce MCF-7 cell damage to the plasma membrane, mitochondrion, and nucleus, leading to the release of cytochrome C and cell apoptosis. The other finding was that the cytotoxicity could be inhibited by the addition of some antioxidants. Therefore, the researches suggested that this kind of cell damage was possibly produced by the mediation of reactive oxygen species (ROS). They also proposed that proper design and control of the shell/core structure of QDs could prevent QDs from degradation. However, in the previous study also by Lovric et al.[18], it was observed that two different antioxidants (NAC, Trolox) exerted completely different effects on PC12 cells: 2 mmol/L of NAC inhibited cytotoxicity induced by QDs, whereas Trolox failed to prevent cell death under the same condition. The results indicated that free radicals are not the exclusive contributors to the QD-induced cell death.

On the basis of the summings-up to previous studies, Tsay et al.[32] provided the mechanism comprehensively from the aspect of free radical micromolecules and the poisonous element Cd: the shell/core QDs may undergo several oxidation processes in the cell resulting in the degradation of both layer and core (Figure 2). Consequently, generated ROS and released Cd^{2+} jointly trigger a series of radical chain reactions, cause lipid peroxidation, and lead to cell apoptosis. In the succedent study, Cho et al.[22] verified Tsay's standpoint by *in vitro* experiments. They exposed different QD samples on MCF-7 cells at the concentration of 10 μg/mL, and related the results of the cytotoxicity to the corresponding intracellular Cd^{2+} concentrations. A good negative correlation ($R^2 = 0.868$) between the intracellular Cd^{2+} con-

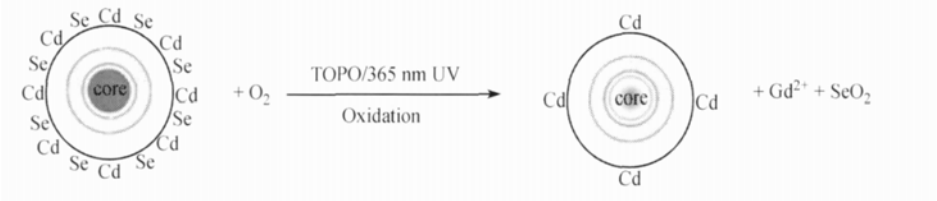

Figure 1 Mechanism of Cd release from the QD surface via oxidation[28]

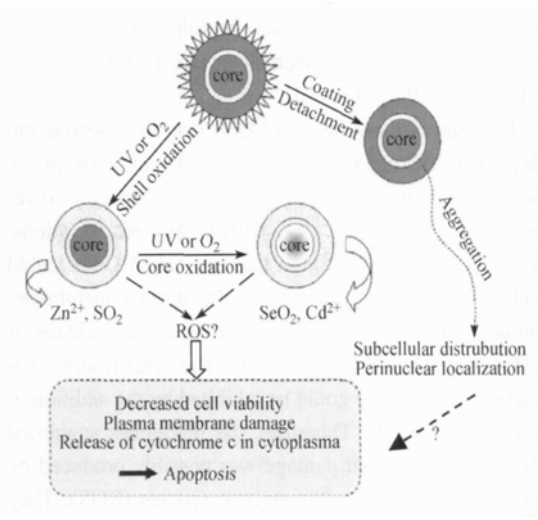

Figure 2 Schematic of the mechanism resulting in QDs cytotoxicity[32]

centration and cell viability was observed in the aqueous $CdCl_2$-treated group but not in all of the QDs-treated groups. The result, together with the inspection of tectological changes of lysosome and mitochondrion suggested that QD toxicity was induced both by free Cd^{2+} and ROS in the state of oxidative stress. Green et al.[33] demonstrated that quantum dots CdSe/ZnS could nick DNA: supercoiled DNA after UV excitation displayed less than 5% damage; DNA incubated with QDs in the dark showed about 29% damage; DNA concurrently exposed to QDs and UV revealed 56% damage. Therefore, they attributed the effect to free radicals generated via photocatalyse and photodegradation of the QD surface. Ipe et al.[34] compared the radical formation via photoirradiation by different QDs (CdS, CdSe and CdSe/ZnS) and reported that the type and quantity of radicals generated depended on the QD materials. Apparently, CdS QDs could generate hydroxyl and superoxide radicals, and CdSe QDs exclusively generated hydroxyl radicals. Contrarily, CdSe/ZnS QDs with the core/shell structure could not produce any free radicals under the same conditions. It indicated that QDs modified by appropriate capping ligands could partially or completely inhibit the production of free radicals.

In conclusion, the mechanisms of QDs toxicity were focused on the release of heavy metal ion Cd^{2+} and the generation of ROS in the state of oxidative stress. In order to eliminate or weaken the adverse effects caused by the mechanisms, the pervasive thoughtway was to simplify the toxicological effect of QDs by appropriate choices of QDs coating materials and modified techniques. One of the intentions is to render the QDs surface subordinate or completely ineffective on the QDs toxicity. The other one is to design proper capping materials through the chemical binding method to prevent the disintegration of QDs. Presently, relevant researches are still under investigation.

2.4 Discussions of other relevant researches about biological effects of QDs

Besides the factors above, there are a great deal of factors to affect QDs biological effects and toxicity. The minimal effective dose and the maximal noneffective dose are two of very crucial parameters, which should be taken into account in the application of QDs in bio-imaging. Larson et al.[35] carried out the QDs tracing in mice using two-photon excited fluorescent imaging, and observed no noticeable toxicity in mice injected 1 μmol/L solutions of CdSe/ZnS QDs, which were in agreement with the results reported by Voura et al.[36]. Hanaki et al.[37] exposed Vero cells to 0.24 mg/mL CdSe/ZnS QDs capped with MUA and coated with SSA for 2 h and did not find effects of QDs on cell viability. Chen et al.[38] observed that peptide-coated CdSe/ZnS at approximate 10 nmol/L had minimal impact on the survival of HeLa cells.

Kinetics research on ZnS/CdSe QD in aquatic animals (loach) was examined in our group. The results attested that the liver and kidney were the main accumulative organs. In addition, we investigated the estrogenic effect of PEG-ZnS/CdSe QD on the vitellogenin (Vtg) induced by 17β-estradiol (E2) in male loaches. It was observed that both CdSe and $CdCl_2$ (calculated by Cd) at the same concentration could inhibit Vtg levels induced by E2. Available data indicated that Cd, as a new type of environmental endocrine disrupting chemical, could bind to the estrogen receptor (ER) with high affinity[39], and inhibit the specific binding of E2 and ER, which affected the inducement of Vtg by changing the ER configuration[40]. Together with the proposed mechanism of QDs toxicity, we speculated that the inhibition mechanism of QDs was basically consistent with that of free Cd^{2+}. However, because QDs nanoparticles have large specific surface areas, there exists the possibility that non-specific adsorption of E2 by QDs decreases the uptake of E2 by fish.

3 Conclusions and perspectives

For the purpose of the integrated toxicology-based in-

formation to meet more biological applications, relevant researches can be done from the aspects mentioned hereinafter:

(1) The synthesis of QDs is determined by multiple factors including the raw materials, synthetical route, modification, etc. Hence, it is crucial that how to appropriately control the reactions to avoid the adverse effects produced by organic toxicants (like ligand and solvent effects).

(2) From the aspect of the component of QDs, the toxicity of ingredients (Cd, Se, Te, S, Zn) and further comparison with the reported results should be examined[41−44].

(3) Different exposure routes and diverse biomarkers from the views of neurotoxicity, reproduction toxicity, immune toxicity and pharmacokinetics need to be considered for the aim of making a comprehensive evaluation of QDs toxicity.

(4) Even though the amount of QDs used in bioimaging may be much less than that of used in toxicological experiments, the property of high adsorption of QDs would bring forth the adverse supra-accumulation. Therefore, it is significant to make comprehensive investigations on absorption, distribution, metabolism and excretion (ADME) of QDs.

(5) So far, there have been many in vitro experiments (cellular level), but comparatively much less *in vivo* (individual level) and bio-macromolecular researches (molecular level) can be found. Undoubtedly，by the association of these three levels, it would be helpful to achieve more convincible toxicological information.

(6) QDs have been incorporated into various nano-sized spherical supports (SiO_2, polymers, phospholipids) for the achievement of multifunctional nanocomposite probes and applications in biology and medicine. It is because this kind of multifunctional nano-probes might be one of the main probes utilized in biologic coding and functional identification that the researches on the effects of QDs toxicity would be instructional to further biological applications. In addition, based on the toxicological information of the nanocomposites, it would be helpful to assess QDs' toxicity of their own and explore various green environmental nano-probes.

(7) Although other types of QDs (for instance: SrSe, SrTe, BaSe) have not been widely applied in biology, it would be cogent to provide powerful evidence by assessing the similarity and dissimilarity of the other QDs' toxicity.

(8) From the view of research techniques, physicochemical properties of QDs should be controllable when carrying out the investigation of QDs toxicity. Thus, the corresponding rigorous criterion would be required for the preparation and characterization of QDs in order to ensure toxicologic studies of QDs.

As mentioned above, it is necessary to point out that as a powerful tool in life and medical sciences, the applications of QDs are greatly dependent on not only good water-solubility, high photostability and fluorescent effects but also low toxicity, which will require enormous and long-term investigation in this new field.

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