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Leading opinion

The cytotoxicity of cadmium-based quantum dots☆

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ABSTRACT

Semiconductor Quantum dots (QDs) have raised great attention because of their superior optical properties and wide utilization in biological and biomedical studies. More recently, there have been intense concerns on cytotoxicity assessment of QDs. Most QDs are made of heavy metal ions (e.g., Cd^{2+}), which may result in potential *in vitro* toxicity that hampers their practical applications. In this article, we aim to summarize recent progress on mechanistic studies of cytotoxicity of II–IV QDs. We have studied the cytotoxicity of a series of aqueous synthesized QDs (aqQDs), i.e. CdTe, CdTe/CdS core-shell structured and CdTe/CdS/ZnS core-shell-shell structured aqQDs. Our results suggested that released cadmium ions are responsible for the observed cytotoxicity of cadmium-based QDs. The fact that CdTe/CdS/ZnS core-shell-shell structured QDs are nearly nontoxic to cells further confirmed the role of released cadmium ions on cytotoxicity, and the effective protection of the ZnS shell. However, intracellular level of Cd^{2+} ions cannot be the only reason since the comparison with $CdCl_2$-treated cells suggests there are other factors contributed to the cytotoxicity of aqQDs. Our studies on genome-wide gene expression profiling and subcellular localization of aqQDs with synchrotron-based scanning transmission X-ray microscopy (STXM) further suggest that the cytotoxicity of CdTe QDs not only comes from the release of Cd^{2+} ions but also intracellular distribution of QD nanoparticles in cells and the associated nanoscale effects.

1. Synthesis of quantum dots

The utilization of functional nanomaterials in biology and biomedicine has been extensively explored, and become one of the fast moving and exciting research directions [1,2]. To date, a variety of nanomaterials (carbon nanotubes, silicon nanowires, gold/silver nanoparticles, quantum dots, etc.) have been studied and utilized in widespread biological applications [3–6]. Among them, fluorescent II–IV Quantum dots (QDs), as a type of high-performance bioprobes, are at the forefront of nano-biotechnology research. QDs, also referred as nanocrystals, are single crystals with several nanometers in diameter. With comparison to conventional fluorescent bioprobes, i.e., organic dyes and fluorescent proteins, II-IV QDs feature many attractive optical properties, such as high photoluminescence quantum yield (PLQY), broad absorption coupled with narrow emission, and strong photostability [3,7,8]. Consequently, several kinds of QDs (e.g., CdSe/ZnS core-shell QDs, CdTe/CdS/ZnS core-shell-shell QDs) haven been fabricated and utilized for applications including biosensing, bioimaging, disease diagnosis [9,10].

QDs are prepared primarily via two approaches, i.e., organometallic synthesis and aqueous synthesis. The organometallic route has been well established for synthesis of QDs with excellent optical properties [11,12]. For instance, CdSe QDs with PLQY as high as 85% were successfully prepared via the organometallic route [13]. However, such organic synthesized QDs (orQDs) are of hydrophobic nature and cannot be directly used in bioapplications. Posttreatment with hydrophilic ligands exchange and polymer or silica coating is thus required to render the orQDs with aqueous dispersibility [14,15]. In addition to relatively complicated manipulations, such posttreatment may have adverse effects on optical/physical/chemical properties of QDs [16,17]. For examples, such

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posttreatment may lead to significant size increase of QDs and significant decrease of PLQY, which hampers their bioapplications since nanoparticles with small hydrodynamic diameters (typically < 10 nm) and high PLQY are most favorable for *in vivo* and *in vitro* applications [18]. Compared to the organometallic methods, aqueous synthetic strategies are simpler, cheaper, and more environmentally friendly. More importantly, the aqueous synthesized QDs (aqQDs) are naturally water-dispersed without any posttreatment due to the presence of a large amount of hydrophilic ligand molecules (e.g., 3-Mercaptopropionic acid, thioglycolic acid, etc.) on their surfaces. As a result, aqQDs possess much smaller hydrodynamic diameter (typically < 5.0 nm) as compared with orQDs. However, aqQDs prepared via conventional aqueous methods often possess poor optical properties (e.g., PLQY<10%) [19]. Several strategies have been developed to improve the spectral properties of aqQDs. Particularly, CdTe aqQDs with PLQY ~50% were prepared through a hydrothermal method [20]; a variety of highly luminescent (PLQY: ~50–80%) aqQDs (e.g., CdTe/CdS core-shell QDs, CdTe/CdS/ZnS core-shell-shell QDs) were successfully achieved via microwave-assisted methods [21–24]. As a result, in the past several years, such highly luminescent aqQDs have been widely used as fluorescent nanoprobes for various bioapplications, such as bioimaging and protein chips [25–28].

2. Relationship between cytotoxicity and surface properties of QDs

Systematic cytotoxicity assessment of QDs is of critical importance for their practical biological and biomedical applications. To date, a large amount of studies on cytotoxicity of QDs have been carried out for this purpose [29–34]. Bhatia et al. showed that surface oxidation of QDs led to the formation of reduced Cd on the QD surface and release of free cadmium ions, which correlated with cell death [29]. Yamamoto et al. found that the cytotoxicity of QDs was not only caused by the nanocrystalline particle itself, but also by the surface-covering molecules of QDs, i.e., surface-covered functional groups (e.g., −NH2 and −COOH) covering on the surface of QDs [30]. Parak et al. further demonstrated that, in addition to the release of Cd^{2+} ions from the surface of QDs, precipitation of QDs on the cell surface could also impair cells. They further suggested that when QDs were only present in the medium surrounding the cells, they result in much small cytotoxic effects as compared to QDs ingested by cells [34]. These studies are useful for understanding the *in vitro* toxicity of QDs, and for systematic assessment of cytotoxicity of QDs. Given the progress, it is worth pointing out that these previously studied QDs all belong to orQDs that are prepared via organometallic routes.

aqQDs possess distinctly different surface properties as compared to orQDs. The surface of aqQDs are covered with a large amount of hydrophilic molecules (e.g., 3-mercaptopropionic acid, MPA), which are in contrast to the presence of hydrophobic ligand molecules (e.g., trioctyl phosphine/trioctyl phosphine oxide, TOP/TOPO) on the surface of orQDs. Hence, aqQDs are inherently water-dispersible without any posttreatment. In contrast, orQDs have to be subjected to additional surface modification to improve water-dispersibility of orQDs (See Fig. 1). This posttreatment usually significantly increases hydrodynamic diameter of QDs as determined by dynamic-light scattering (DLS). Consequently, while aqQDs and orQDs are of similar “dry” sizes (as determined with transmission electronic microscopy, TEM [35]), aqQDs typically possess small hydrodynamic diameter (<5.0 nm) while hydrodynamic diameter of posttreated orQDs are larger than 5.0 nm [35–37] (Fig. 1). As expected, the difference of surface properties between aqQDs and orQDs may lead to distinct cytotoxicity and *in vivo* behaviors.

We previously performed a systematic cytotoxicity assessment of a series of aqQDs, i.e., thiols-stabilized CdTe, CdTe/CdS core-shell structured and CdTe/CdS/ZnS core-shell-shell structured QDs. We demonstrated that CdTe aqQDs were highly toxic for different cell lines, which is consistent with previous reports on orQDs [38]. The cytotoxicity could be mitigated via epitaxial growth of a CdS layer that reduced the release of Cd^{2+} ions. A further modification with a ZnS outlayer that effectively prevented the Cd^{2+} release rendered aqQDs essentially compatible to cells, as evidenced by minimal variations of cellular viability [38]. Subsequent studies with ICP-MS revealed that the intracellular Cd^{2+} concentration of CdTe QDs were

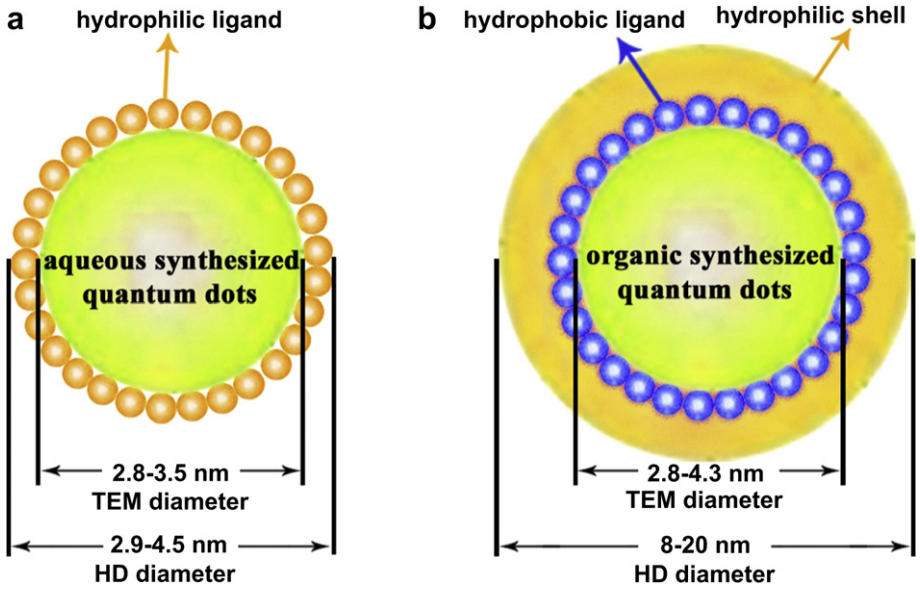

Fig. 1. Schematic structures of aqueous synthesized QDs (aqQDs) and organic synthesized QDs (orQDs). Hydrophilic ligand indicates thiol- and carboxyl-modified short chain organic molecules with hydrophilic property (e.g., 3-mercaptopropionic acid and thioglycolic acid). Hydrophobic ligand indicates long hydrophobic chain organic molecules often used in synthesis of orQDs, such as trioctylphosphine oxide and trioctylphosphine. Hydrophilic shell includes silica shell or polymer shell, which is generally used for improve hydrophibility of the orQDs (Reprinted with permission from [36], 2011 Biomaterials.).

at least 10-fold higher than that of CdTe/CdS/ZnS QDs at equal nanoparticle concentrations [39]. Therefore, the variant toxicity between CdTe and CdTe/CdS/ZnS QDs can be attributed to the different levels of released Cd^{2+} ions. We further revealed relationship between the cytotoxicity of aqQDs and free cadmium ions. Significantly, we found that the CdTe aqQDs were more cytotoxic than $CdCl_2$ solutions even when the intracellular Cd^{2+} concentrations were identical in the treated cells, implying that the cytotoxicity of aqQDs cannot be attributed solely to the toxic effect of free Cd^{2+} [39]. Very recently, we further studied short- and long-term *in vivo* biodistribution, pharmacokinetics, and toxicity of the aqQDs. We found that the aqQDs were initially accumulated in liver after short-time (0.5–4 h) post-injection, and then were increasingly absorbed by kidney during long-time (15–80 days) blood circulation. Moreover, biodistribution of aqQDs was obviously size-dependent. Furthermore, no overt toxicity of aqQDs in mice was observed even at long-time exposure time based on histological and biochemical analysis, and body weight measurement [35].

3. Inhibition of cell viability with CdTe QDs and $CdCl_2$ treatments

Most previous reports employed classic MTT assays that reflect the metabolic activity of cells to evaluate cytotoxicity of QDs. However, metabolic activity change does not necessarily correlate with other parameters of cellular status such as growth, proliferation and apoptosis. Therefore, in the present study, we investigated the effect of CdTe aqQDs on the proliferation of HEK293 cells by directly counting the number of viable cells. As shown in Fig. 2, cell proliferation curve for 3 days after treatment with CdTe QDs at various concentrations were determined. Fig. 2A shows that the growth curve of HEK293 cells treated with 37.5 nM CdTe aqQDs was not significantly altered as compared to that of the control, untreated cells, whereas high-dose (300 or 600 nM) treatment of CdTe aqQDs nearly completely inhibited cell growth even from the very beginning. At medium of concentrations of CdTe aqQDs (75 nM or 150 nM), although the number of viable cells was decreased, these cells could proliferate at a relatively normal rate for ~2 days after treatment.

Compared with our previous data from MTT assays [38] (Fig. 2C) , we find that CdTe aqQDs show less inhibition on cell proliferation than the reduction on cellular metabolic activity at mild concentrations (37.5 nM and 75 nM). These results reveal that the cytotoxicity of CdTe aqQDs is primarily associated with the inhibition of metabolic activity rather than QDs-induced direct cell death. The fact that aqQDs cause a relatively slow, long-term inhibition of cell proliferation suggests that reactive oxygen species (ROS) plays an important role in QD-induced cytotoxicity, an effect that is often associated with metabolic reduction. Indeed, two previous studies have proven that cadmium-based QDs can generate free radicals including ROS [31,40]. Elevated levels of intracellular ROS can cause oxidative stress and mitochondrial

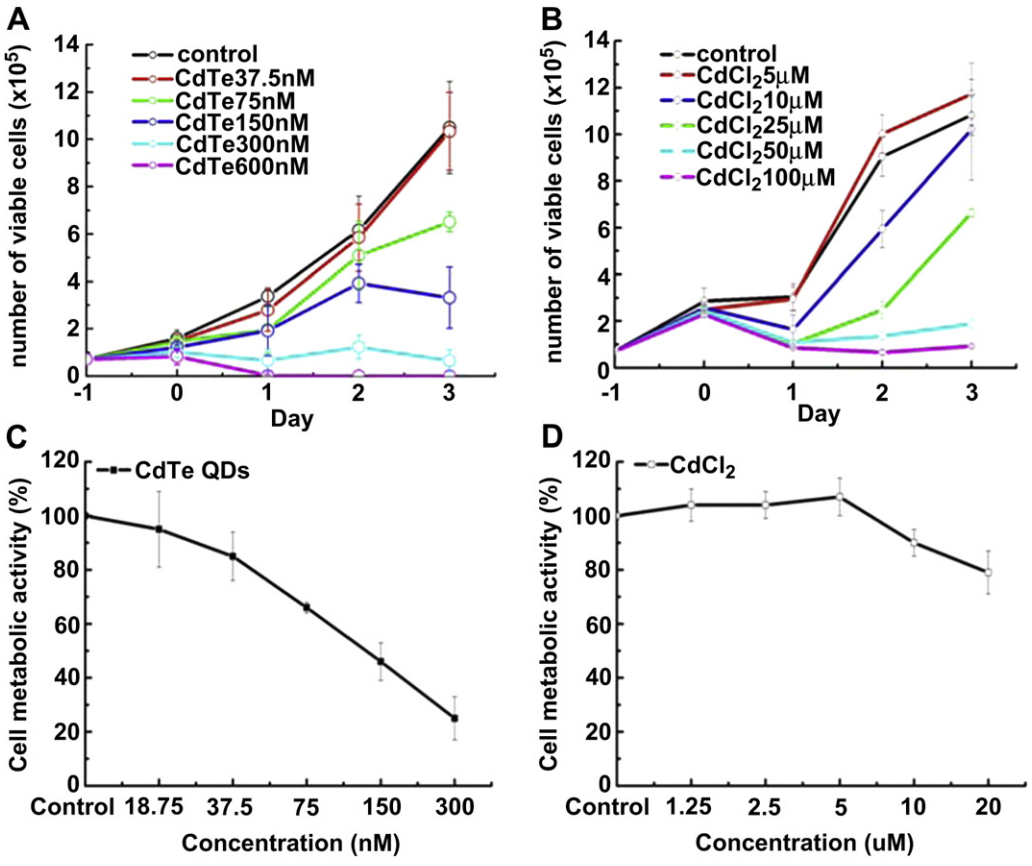

Fig. 2. Comparison of growth rate and metabolic activity change of CdTe QDs or $CdCl_2$ treated HEK293 cells. (A) and (B), cells in exponential growth phase (7×10^4) were plated and cultured for 24 h before serum starvation for 8 h. The cells were then treated with CdTe QDs or $CdCl_2$ at indicated concentration in complete medium for 10 h, washed with PBS and cultured in complete medium for 3 days. At every time point, cells were trypsinized, stained with trypan blue and the numbers of viable cells were determined using a hemocytometer. (C) and (D) metabolic activity of HEK293 cells after treatment with CdTe or CdCl2 in were measured using an MTT assay. The relative activity was calculated as a percentage from the viability of the control (untreated) cells (100%). The results are expressed as mean ± SD from three or four independent experiments ($n = 3$). (C and D are adapted with permission from [39], 2010 Biomaterials.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

damage, resulting in loss of metabolic functions [41]. Chan et al. found that when these oxidative damages are out of repair, cadmium-based QDs induced substantial apoptosis in the IMR-32 human neuroblastoma cell line. These apoptotic changes included JNK activation, loss of mitochondrial membrane potential, mitochondrial release of cytochrome C and activation of caspase-9 and caspase-3 [42].

Cadmium ions are well known to cause oxidative stresses inside the cell [43]. To further distinguish whether the inhibition of cellular growth is caused solely by released Cd^{2+} ions, we also investigated the effect of $CdCl_2$ treatment on the proliferation of HEK293 cells. Fig. 2B shows that the growth rates of HEK293 cells treated with 5 μM and 10 μM $CdCl_2$ were not significantly changed as compared to untreated cells. The proliferation was inhibited but not stopped by 25 μM of $CdCl_2$ whereas 50 μM or 100 μM of $CdCl_2$ completely inhibited cell proliferation. In our previous study [39], we utilized inductively coupled plasma-mass spectrometry (ICP-MS) to determine the concentrations of intracellular Cd^{2+} ions, which revealed that treatment with 10 μM of $CdCl_2$ resulted in significantly higher intracellular concentration of Cd^{2+} ion (>20 ng/10^5 cells) than that with 150 nM of CdTe QDs (<10 ng/10^5 cells). However, studies on both cellular metabolic activity and proliferation rate suggest that treatment of cells with 150 nM of CdTe QDs poses much stronger inhibition effects than that with 10 μM of $CdCl_2$ (Fig. 2B and D) . Therefore, we deduced that release of Cd^{2+} in cytoplasm does not correlate well with observed severe cytotoxicity induced by CdTe QDs and cannot be the only reason for proliferation inhibition of HEK293 cells.

4. Gene expression changes induced by aqQDs

To better illustrate the mechanism of cytotoxicity caused by CdTe QDs, a genome-wide gene expression profiling was performed to compare the effect of CdTe quantum dots and $CdCl_2$ treatment on HEK293 cells. Based on the results of MTT assays and proliferation assays, we chose cell samples treated with 37.5 nM of CdTe QDs and 10 μM of $CdCl_2$ as the "low toxic" conditions, and those with 300 nM of CdTe QDs and 60 μM of $CdCl_2$ as the "highly toxic" conditions. HEK293 cells were treated for 24 h with indicated conditions and total RNA were extracted for analysis. Differently expressed transcripts were identified using the Illumina GEX Bead Array platform. Table S1 listed differentially expressed genes found in all four types of treated cells. Compared with control sample, a total of 31 transcripts were significantly un-regulated and 3 transcripts were down-regulated by two-fold or greater. Out of the up-regulated ones, many genes are stress responsive and are involved in protective processes such as protein binding, metal ion binding, and intracellular oxidoreductive reactions (Table S1). Strikingly, 7 genes from metallothionein (MT) family, e.g. MT1A, MT1F, MT1G, MT1H, MT1X, MT2A, and MTE, were identified as up-regulated genes in both CdTe QDs and $CdCl_2$ treated cells (Table S1 and Fig. 3). The metallothioneins are small, cysteine-rich heavy metal-binding proteins which participate in an array of protective stress responses. MT proteins of higher eukaryotes evolved as a mechanism to regulate zinc levels and distribution within cells and organisms. These proteins can also protect against some toxic metals and oxidative stress-inducing agents. In mammals, there are four MT genes, MT-1 and MT-2, which display wide tissue distributions, and MT-3 and MT-4, which are more restricted in their expression [44]. A hallmark of the MT-1 and MT-2 genes is their rapid transcriptional induction by metals such as Zn^{2+} and Cd^{2+} [44,45]. In humans, the MT-1 and MT-2 proteins are encoded by a family of genes consisting of 8 functional (MT-1A, MT-1B, MT-1E, MT-1F, MT-1G, MT-1H, MT-1X, MT-2A) and 7 nonfunctional MT isoforms (MT-1C, MT-1D, MT-1I, MT-1J, MT-1K, MT-1L, MT-2B)

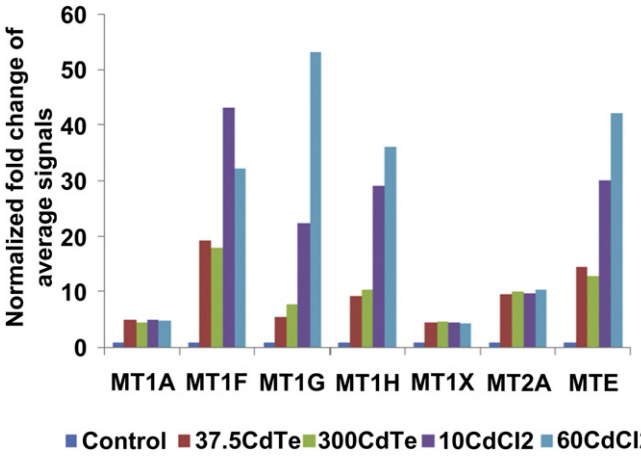

Fig. 3. Up-regulation of MT family genes by CdTe QDs and $CdCl_2$ treatment. HEK293 cells were treated at indicated conditions for 24 h and corresponding RNA samples were prepared, amplified and applied to Sentrix HumanWG-6_V3 expression BeadChip. Results were processed with Illumina GenomStudio software. Average signal intensity of untreated control cells were normalized to 1. All indicated genes has a Pval < 0.05 and fold change > 2.

[46,47]. To confirm the up-regulation of MT family genes identified by microarray analysis, we performed semi-quantitative reverse transcript (RT) PCR analysis of several MT genes. As shown in Fig. 4, we confirmed the up-regulation of MT-1A, MT-1B, MT-1E, MT-1F, MT-1G, MT-1H genes by RT-PCR analysis. However, quantitative studies revealed that the up-regulation of MT Proteins was not dosage dependent. In our studies, treatment with 5 different concentrations of CdTe QDs (from 18.75 nM to 300 nM) all caused similar levels of induction of the mRNA transcripts. This is consistent with our previous ICP-MS results that the intracellular concentrations of Cd^{2+} ions are not determined by the levels of CdTe QDs [39]. It again suggests that the protective transcriptional response caused by Cd^{2+} alone cannot explain the observed cytotoxicity of CdTe QDs.

In the case of $CdCl_2$ treatment, Cd^{2+} ions should be the only reason of toxicity. Indeed, we observed the up-regulation of 7 MT family genes by $CdCl_2$ treatment and the fold change of MT1F, MT-1G, MT-1H and MTE were significantly higher as compared to

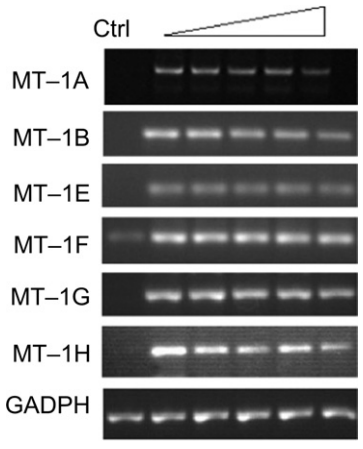

Fig. 4. The expression of MT genes of HEK293 cells after treatment with different concentrations of CdTe QDs. Cells were treated the same way as samples for microarray assays. Equal amount of RNA were used for each lane. From left to right: first lane is untreated control HEK293 cells. Lane2-lane6 were treated with gradient CdTe QDs concentrations (left to right) 18.75 nM, 37.5 nM, 75 nM, 150 nM, 300 nM. GAPDH levels are used as internal control.

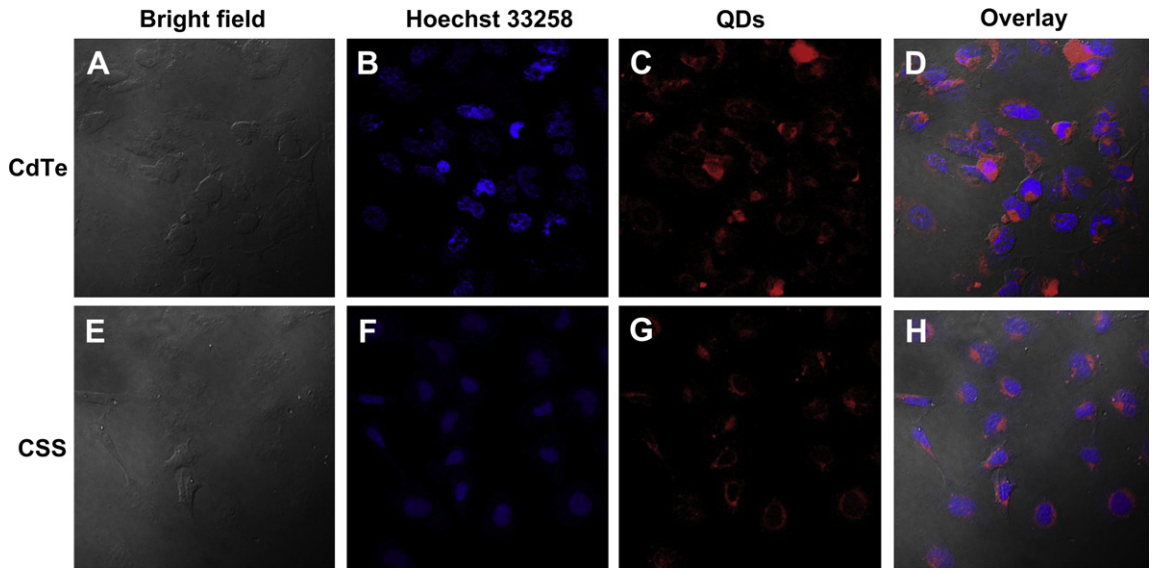

Fig. 5. Confocal micrographs of HEK293 cells treated with CdTe (150 nm, A–D) and CSS-QDs (150 nm, E–H): (A and E) visualization of cells in bright-field; (B and F) visualization of nuclei stained with Hoechst 33258; (C and G) visualization of CdTe and CSS-QDs in the intracellular compartment; (D and H) overlay of the three micrographs. (Reprinted with permission from [39], 2010 Biomaterials.).

CdTe QDs (Fig. 3). ICP-MS analysis also revealed that the Cd^{2+} ions concentrations were higher in those $CdCl_2$-treated cells [39]. Surprisingly, we did not observe significant difference in the gene expression pattern change caused by treatment with 37.5 nM of CdTe QDs and 10 μM of $CdCl_2$. Hence, the microarray analysis reveals that the genomic expression change caused by CdTe QDs comes primarily from released Cd^{2+} ions. Nevertheless, cadmium-based QDs possess additional toxicity that does not directly cause mRNA transcriptional level change, while instead more likely function via intracellular interaction and functional change of certain proteins or organelles.

5. Subcellular distribution of aqQDs

Intracellular localization of QDs is critically important for its toxicity to cells [48]. We previously identified the localization of cadmium-based QDs in HEK 293 cells via a fluorescence imaging study. The confocal fluorescence images indicated that both the CdTe and CSS-QDs were located predominantly in the cytoplasmic/perinuclear area [39] (Fig. 5). Optical imaging typically has a resolution limit of 200–300 nm that prevents studies on fine subcellular structures. Recently developed synchrotron-based scanning transmission X-ray microscope (STXM) provides a powerful technique to map the distribution of certain chemical elements at high spatial resolution due to the extremely short wavelength of X-rays. it is possible to image whole, hydrated cells up to several micrometer thick with nanometer-scale resolution [49]. In this study, we explore the subcellular localization of CSS-QDs with this element-sensitive, nanometer-scale resolution imaging approach to further illustrate the detailed subcellular distribution pattern of cadmium-based QDs.

STXM is an ideal approach to scan cellular structures without any tedious contrast enhancement methods such as entrapment, section, and fluorescent staining [50]. This approach has been used to inspect the subcellular distribution of proteins that were gold-labeled and silver-enhanced. Both cytoplasmic and nuclear

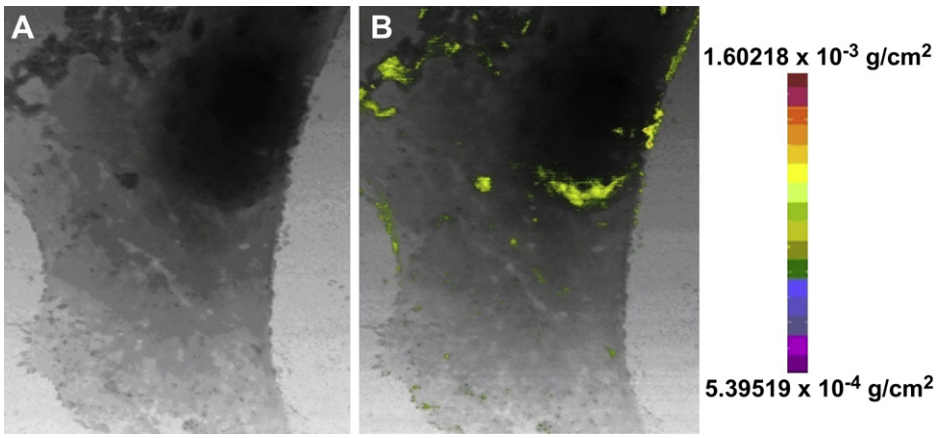

Fig. 6. STMX imaging of core-shell-shell QDs in HeLa cells. (A) image of a HeLa cell at 573eV. The image size, step and number of pixels are 18 mm × 18 mm, 50 nm and 360 × 360, respectively. (B) The Te distribution calculated using the subtraction and ratio methods. The image size, step and number of pixels are 18 mm × 18 mm, 50 nm and 360 × 360, respectively, after shifting and clipping. The yellow frame areas of the image denote the Te distribution. The range of ratio contrast (colorful regions) noted by the color bar which shows the subtraction contrast from 5.39519×10^{-4} g/cm^2 to 1.60218×10^{-3} g/cm^2, where 5.39519×10^{-4} g/cm^2 is the threshold and 1.60218×10^{-3} is the maximum of subtraction contrast. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

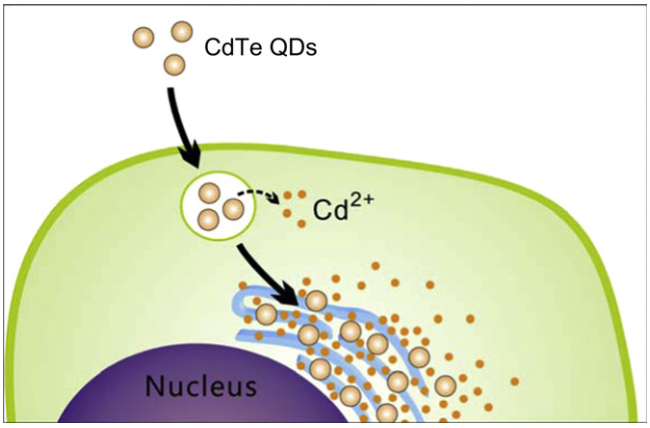

Fig. 7. Schematic illustration of the cytotoxicity induced by cadmium-based QDs. When CdTe QDs are transported across the cell membrane, free cadmium ions are released into the cytoplasm. Free Cd^{2+} induced a serious of protective responses including the up-regulation of MT family proteins. On the other hand, CdTe QDs are locally concentrated at perinuclear region and caused abnormally high local concentrations of Cd^{2+} ion that are responsible for the additional "nanoscale" cytotoxicity of QDs.

proteins in whole, hydrated mammary epithelial cells were observed at better than 50 nm resolution [51]. Recently, Tai et al. reported a new algorithm which can conveniently acquire the distribution of a number of elements quantitatively and precisely [52]. We herein studied the intracellular localization of CdTe/CdS/ZnS core-shell-shell structured QDs in HeLa cells by probing the subcellular distribution of Telluride element that can be readily imaged with STXM. As illustrated by the optical micrograph in Fig. 6A, sample process with graded dehydration has preserved the overall morphology of the cells relatively well. Images of HeLa cells incubated with CSS-QDs are shown in Fig. 6B, raster scanning of this cell were performed at two different energy (573 eV and 582 eV), which are the absorptive peak and valley of the Te element, respectively. Margin analysis provided topographical maps with subcellular distribution of the Telluride with a high resolution of 50 nm. Our result demonstrated that the CSS-QDs were distributed predominantly in the cytoplasm. The distribution was not uniform, rather it showed dotted pattern with differential intensity. Particularly, high-intensity dots were concentrated in the perinuclear area and marginal area of the cell. This result is in excellent accordance with our previous observation with fluorescence imaging studies [39]. We reason that such uneven distribution of aqQDs nanoparticles might cause abnormally high local concentrations of Cd^{2+} around the nuclei or certain cellular organelles such as mitochondria and enhanced damage to these organelles. This local concentration effect is responsible for the observed higher cytotoxicity of CdTe QDs than $CdCl_2$, even at lower average concentration of intracellular Cd^{2+}. Alternatively, CdTe QDs might have entered subcellular organelles including mitochondria or lysosomes and directly caused functional loss of these organelles. To prove the latter possibility, further colocalization studies with cellular organelles will be needed in future.

6. Conclusions and perspectives

It is critical to understand the toxicity and the related mechanism of quantum dots for the development of its practical biological and biomedical applications in imaging, diagnostics and therapy. However, it is difficult to compare and draw an unambiguous conclusion from so far reported studies because of the use of a variety of quantum dots, cell lines and analytic methods [53–55]. The synthetic methods and surface modifications of quantum dots will greatly affect its physicochemical characteristic, its interaction with the cellular membrane and subsequent uptake into the cells [56–58]. On the other hand, different cell types have varying threshold for the quantum dots-induced toxicity. Furthermore, cytotoxicity has been interpreted with different parameters such as cell number, cell growth, apoptosis, cellular morphology or metabolic activity change. All these variations in parameters complicate comparisons and make it difficult to reach consistent conclusions. In our studies, we employed a series of aqQDs with different coatings to provide a systematic evaluation of their cytotoxicity. Importantly, the chemical composition and structure of cadmium-based quantum dots determine the amount of released Cd^{2+} inside the cell, which can cause a series of stress responses [43,59]. Parallel tests with CdTe and CdTe/CdS/ZnS quantum dots indicated that the Cd^{2+} is the major source of cytotoxicity. We analyzed the gene expression profile of both CdTe and $CdCl_2$ treated cells. The MT family proteins that are involved in metal ion-induced protective response were identified as up-regulated in both samples. The similarity in transcription pattern changes for both treatments suggests that free Cd^{2+} ions indeed is the basis of the cytotoxicity of cadmium-based QDs. However, equal intracellular concentration of Cd^{2+} ion in $CdCl_2$ treated cells has much less effect on both metabolic activity and proliferation. This might be explained by a "high local concentration" effect of released Cd^{2+} ion at certain area/organelles inside the cell, as evidenced by uneven intracellular distribution of QDs in STXM imaging (Fig. 7). These studies provide relatively consistent data on the cytotoxicity of aqQDs and reveal possible underlying mechanisms. In future studies, we suggest performing whole animal toxicity studies that will help to better estimate the overall toxic effect and avoid conflict results from different cell types or tissues.

Acknowledgments

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Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.biomaterials.2011.10.070

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