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REVIEW ARTICLE

Toxicity of quantum dots on respiratory system

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Abstract

Quantum dots (QDs), as advanced nanotechnology products, are widely used in the biomedical field for diagnostic and therapeutic purposes due to their unique properties. Therefore, it becomes important for researchers to elucidate the adverse effects of QDs on human beings. This essay provides an overview of the toxic effects of QDs on respiratory system, which are summarized into two main parts: *in vitro* toxicity, including reduction of cell viability, genetic material damage and disordered immune cell reactions; as well as *in vivo* toxicity, involving accumulation of QDs, lung injury and inflammation, and potential long-term adverse effects. As the toxic severity of a QD type depends on its composition, dose, size, surface chemistry and structure, it is a big challenge to determine a benchmark of QDs. Thus, we have to remember that each QD type is a unique nanocrystal, which needs to be assessed individually. However, there are still some feasible recommendations for minimizing the toxicity provided in this review. Overall, more and more large-scale well-organized toxicity studies of different QD types on different species need to be conducted in order to provide guidelines of QDs' safety application.

Keywords

Nanotoxicology, quantum dot, respiratory system

History

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Introduction and varieties of quantum dots

Currently, nanoparticles, a kind of nanotechnology products, are produced and sold globally. Nanoparticles have become widely used in areas ranging from physics to biology due to their unique properties. In the field of nanotechnology, nanoparticles are usually classified into five categories which are carbon nanotubes (CNTs) and fullerenes, metals, ceramics, polymeric and semiconductors [known as quantum dots (QDs)] (Buzea et al., 2007). The reasons of QDs becoming an important class of nanoparticles are their photophysical and electrical properties that have huge potential values in energy and medical application.

Quantum dots are nanometer-scale crystalline semiconductors consisting of chemical elements from Groups III–IV or Groups II–VI of the periodic table. Sizes of QDs diameter are close to or less than Exciton Bohr radius, and typical ones range from roughly 1–10 nm (Leutwyler et al., 1996). A typical QD is composed of one kind of semiconductor material as core packaged with another kind of semiconductor material as shell. Sometimes, QDs need to be solubilized or biological interfacing for special biomedical applications. Thus, a large number of surface attachment materials, mostly biomolecules, need to be conjugated to QDs, such as peptides, antibodies and oligonucleotides (DNA or RNA) (Michalet et al., 2005). Figure 1 illustrates the basic structure of a typical QD demonstrated as Core/Shell-Conjugate (Pelley et al., 2009). A CdSe/ZnS-DNA QD, for instance, has a cadmium–selenium core and a zinc sulphide shell conjugated with DNA.

There are a variety of QDs reported to be used in commercial and research domains. Each type of them has its own strengths and weaknesses depending on its material, size, shape, structure and composition. So far, cadmium-containing QDs are used most frequently, and are the most suitable emitting material in biological imaging area, such as CdSe/ZnS, CdTe/ZnS Core/Shell QDs (Yong et al., 2013). Due to the wide range of emission wavelength of cadmium-containing QDs, they can be used in multiple *in vitro* and *in vivo* bio-imaging applications. However, the bare core cadmium-containing QDs can release toxic cadmium ions into surrounding biological fluids after long-term contact. A common useful method to overcome this challenge is to cap the cadmium-containing core with a ZnS shell in order to reduce toxicity from the heavy metal core (Hines & Guyot-Sionnest, 1996). The ZnS could enhance QDs' fluorescence efficiency and minimize oxidative and photo-bleaching action to increase chemical stability (Juzenas et al., 2008; Smith et al., 2008).

On the other hand, QDs containing non-cadmium chemical materials represent different features. For instance, comparing to cadmium-containing QDs made up of chemical elements from Group II to VI, InP QDs made of Group III to V elements have better structural robustness, which can improve their optical stability and reduce the toxicity via minimizing degradation in biological fluids (Xu et al., 2008). PbS QDs

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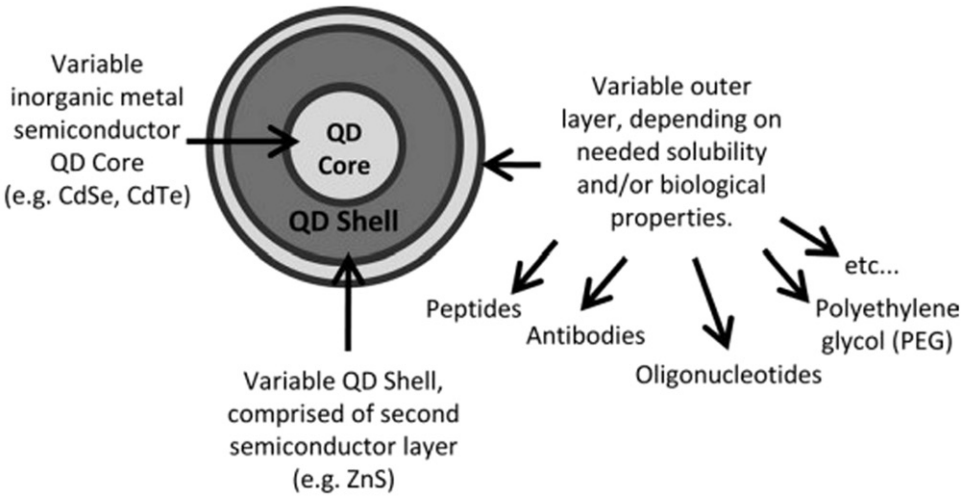

Figure 1. Basic structure of a typical QD (Pelley et al., 2009).

that are water dispersible could be used for near-infrared imaging of cancer cells when functionalized with mercapto ligands (Hyun et al., 2007). Silicon QDs as Group IV semiconductor nanocrystals are supposed to be less toxic than mental-containing QDs (Erogbogbo et al., 2008). Otherwise, they could be water dispersible and organic solvents dispersible when their surfaces are linked with octadecene, styrene or ethyl undecylenate (Li & Ruckenstein, 2004). These properly encapsulated silicon QDs have wide applications associated with cancer-related imaging, targeting or mapping.

There have been many other types of QDs synthesized and reported recently for multiple biomedical applications. For example, CdS/ZnS QDs, CdHgTe/CdS QDs, $CuInS_2$/ZnS, $CdTe_{0.25}Se_{0.75}$/CdS and Ag_2S QDs have been reported to be used as probes for *in vivo* labeling or imaging of blood vessels, tumors and cancer cells (Pelley et al., 2009; Qian et al., 2007; Santra et al., 2005; Yong, 2009; Yong et al., 2010; Zhang et al., 2013). These studied or applicable QDs could be a valuable foundation for further research on innovative QDs creation.

Applications of quantum dots

About three decades ago, QDs were first discovered and were only considered to be used in the electronics field because of their unique semiconductor and luminescent properties (Prasad, 2004). Since the development of nanotechnology, the optical properties of QDs made them suitable to be used as advanced fluorescent probes for biomedical and clinical applications (Michalet et al., 2005). With comparison to conventional organic fluorescent dyes, QDs have demonstrated more favorable optical properties including broad absorption and narrow emission spectra, better biocompatibility after modification, strong fluorescent intensity, brighter, photostability, more resistant to photobleaching and uncovered by fluorescence from marked tissues (Hoshino et al., 2011; Kairdolf et al., 2013; Mattoussi et al., 2012; Pelley et al., 2009; Shao et al., 2011).

Due to the fluorescent property of QDs, researchers have attempted to use QDs in fluorescence resonance energy transfer (FRET) analysis as efficient donors, such as QDs conjugated with biological molecules (e.g. antibodies) used in immunoassays (Wang et al., 2002), or proper QDs used in monitor of protein interactions (Hohng & Ha, 2005). Otherwise, QDs could be used in genetic technology to detect genes relevant to specific cancers, such as ERBB2 or HER2 related to breast cancer (Jamieson et al., 2007). In contrast with QDs used to label cells externally for cell tracking, intercellular labeling with QDs would be relatively difficult, such as labeling proteins (e.g. F-actin fibers) without impacting enzyme activities (Månsson et al., 2004; Medintz et al., 2005). QDs could also be used to label and detect pathogen and toxin other than normal or tumor cells (Jamieson et al., 2007).

Furthermore, it is fantastic that QDs can be used for whole animal body imaging. The fluorescence emission peak of QDs could be shifted to a wavelength of option with a fairly wide range, providing a series of colors from red to blue based on their sizes, shapes, structures and compositions (Costa-Fernández et al., 2006). QDs with proper structures and sizes could provide near-infrared regions of the electromagnetic spectrum (700–2000 nm), which are considered as optical signals with the largest penetrated depth in tissues because of lower scattering and absorption for tissues than blue–green luminescence (Mattoussi et al., 2012; Yong et al., 2009). Therefore, it is perfect for *in vivo* bio-imaging in deeply normal or tumor issues, like vasculature or lymphatic (Jamieson et al., 2007; Mattoussi et al., 2012; Shao et al., 2011). Moreover, bio-imaging in whole body can reveal biological processes at the intracellular and molecule levels, which is beneficial for non-traumatic disease diagnosis and identifying more appropriate therapeutic strategies. Moreover, due to unique features of QDs, they are capable to be used for treatment applications, including targeted drug delivery, drug discovery and photodynamic therapy (Azzazy et al., 2007; Choi et al., 2007; Yong et al., 2012), especially for theranostics in oncology in current clinical status (Akhter et al., 2013).

Exposure routes of quantum dots

Actually, there is no available and factual information demonstrating clearly routes of QD exposure. However, it is plausible to extrapolate common exposure routes from

nanoparticles with similar size, physicochemical properties and applications. We could separate potential exposure routes of QDs into three categories: workplace, environmental and bio-medical (Hardman, 2006).

Workplace exposures are the primary route currently, resulting from aerosolization and accidental inhalation, dermal contact or oral ingestion. The major exposure populations are QDs manufacturers, engineers, researchers and medical workers. For environmental routes, exposures might be though environmental media because of QDs' metal core and coatings. Factors including QDs stability, lifetime in media and environmental transformation or degradation would influence the health outcome after QDs exposure.

Bio-medical application is considered as an important exposure route due to the significant social and economic influence. Methods of exposure include injection as well as all routes mentioned in the workplace route. Apart from being used as fluorescent dyes in bio-imaging or sensing, QDs as are considerable as nano-carries for treatment of pneumological diseases, like asthma and chronic obstructive pulmonary disease (COPD). They could provide a controlled and prolonged duration of effect of the encapsulated drugs and a regional and cell-specific drug targeting within the lung, via varying the size, component or structure of QDs (Gessler, 2009). However, this exposure route is theoretical at present, since QDs are not approved for diagnostic or therapeutic purpose among humans.

With the substantial growth in the production and applications of QDs, potential health hazards associated with medical, environmental and occupational exposure to QDs are getting increasing attention from researchers. Recently, several article reviews overviewed the potential toxicity of QDs for organisms. Since most previous toxicity research attempted to elucidate adverse effects of one QD type on one kind of cell or animal, this literature review endeavor to summarize the potential risks of different types of QD on respiratory system ranging from cellular level to living tissues of tested animals via a variety of exposure routes, and identify valuable and feasible approaches to minimize toxicity and adverse health effects.

Evidence for respiratory toxicity of quantum dots

In 2009, Nanotechnology Research Center of National Institute for Occupational Safety and Health (NIOSH) published an important report relevant to occupational safety and health potentially impacted by nanomaterial ''Strategic plan for NIOSH nanotechnology research and guidance: Filling the knowledge gaps'' (NIOSH, 2009) to regulate applications of nanomaterial and minimize incidence of nanomaterial-related adverse events, especially respiratory diseases. Thus, it becomes interesting and meaningful to assess the adverse health outcomes occurring on respiratory system after various ways of QD exposures.

At present, there are a large number of available studies assessing the *in vitro* and *in vivo* toxicity of QDs on respiratory system. These adverse effects are partially similar to those induced by CNTs, a vital category of nanomaterial. For example, CNTs as delivery systems have been approved to cause pulmonary fibrosis and exacerbate lung disease in rodents (Bonner, 2011). However, QD-induced toxic effects seem severer compared with CNTs because of its noxious heavy metal core, especially the primary component cadmium.

Cadmium is considered as a widespread environmental and occupational pollutant and poison, which has been classified as a carcinogen in Group 1 by the International Agency for Cancer Research (IARC) (IARC, 2013). As most widely used QDs contain cadmium, researchers tend to believe that QDs would be toxic to human beings and the toxicity of cadmium-containing QDs would be closely related to the dissolution of QDs structures and removal of shells, resulting in releasing cadmium ions (Brunetti et al., 2013). Due to relatively sufficient toxicological researches on cadmium-containing QDs, toxic effects of them on respiratory system would be a major focus in this review.

Cell viability

Assessment of cell viability is the most common measurement of *in vitro* toxicity of QDs in the field of toxicology. Various methods, such as water-soluble tetrazolium salt (WST-8) cytotoxicity assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay have been widely used to measure cell viability. Therefore, toxicological studies in pulmonary cells could indicate respiratory toxicity of QDs. The toxicity study conducted by Brunetti et al. (2013) presented that cadmium-containing QDs were observed to reduce viability of A549 cells (human lung adenocarcinoma cells) obviously. This cytotoxicity of cadmium-containing QDs could be explained by combination of released free cadmium and intracellular oxidative stress (King-Heiden et al., 2009). As we know, ionic cadmium is highly toxic and might damage cell membrane by impacting outward potassium channel in cells. Oxidative stress could produce reactive oxygen species (ROS), which are capable of damaging cellular proteins, lipids and genetic material.

Some articles would attribute oxidative stress to the adverse effect of ionic cadmium. However, there is evidence showing that QDs with capping coats, which prevented cadmium ions leakage, were able to generate ROS resulting in cell death. After a large scale of analysis on >20 000 cells and replicated 10 times, Zhang et al. indicated that silanized CdSe/ZnS QDs coated with polymers, poly (ethylene glycol) (PEG) induced apoptosis or necrosis of IMR-90 cells (human lung fibroblasts). They also observed that a slight increase in apoptotic or necrotic cells followed by increasing dose of PEG-coated silanized QDs (Zhang et al., 2006a). One important discovery of this study was that toxic effects associated with heavy metal exposure were not induced by this kind of QD. Apart from oxidative stress, another mechanism of QDs' cytotoxicity is localization of QDs in vesicles in the perinuclear region or the cytoplasm, as previous studies have illustrated (Jaiswal et al., 2002). Other than human pulmonary cells, even for Chinese Hamster lung cells (CHL), cell activity was severely damaged when QD exposure concentration was up to 20 μg/ml (Li et al., 2009).

Gene level responses

Significant increased cadmium-containing QD-treated cells death compared to control group could be explained at the

genetic level. Choi et al. assessed the genotoxic effect of cadmium-containing QDs by MTT, LDH assays and real-time PCR analysis to evaluate A549 cell death, apoptosis and differential mRNA levels of genes. The results indicated that QDs presented toxicity for genetic material, and higher dose produced, severer response (Choi et al., 2012). Similarly, Jacobsen et al. (2009) observed that cells in bronchotracho alveolar lavage fluid obtained significantly increased level of DNA damage after QD exposure. Even though this feature of QDs is useful for photodynamic cancer therapy to kill tumor cells, it might be a potential risk for normal cells.

Additionally, QDs could render gene material damage resulting in severe health consequences, like oxidative stress and inflammation. McConnachie et al. (2013) used real-time PCR and observed an increased mRNA expression level of genes involved in both oxidative stress signaling and inflammatory pathways in QD-treat mice. Moreover, Nagy et al. investigated the up-regulation and down-regulation of genes by real-time PCR analysis to explore the mechanisms of cadmium-containing QDs driving normal human bronchial epithelial (NHBE) cell death, mitochondrial function and inflammation. The study discovered gene CASP9 involved with apoptosis, as well as genes CYP1A2 and UCP1 involved in mitochondrial function were up-regulated in cells exposed to positive QDs. For negative QDs, up-regulations of several genes associated with inflammation were observed. The up-regulated pro-apoptotic genes BAD and CASP9 were related to the increase in DNA fragmentation (Nagy et al., 2012).

There are some *in vivo* toxicity studies to demonstrate the genotoxicity of QDs. Ho et al. (2013) observed that cadmium-containing QDs induced gene expression of cytokines, chemokines and metalloproteinase 12 in mice lung tissues. Gagne et al. observed alterations in hepatic gene expression and immunocompetence in rainbow trout exposed to cadmium-containing QDs. The study discovered that 25 genes were specific to QDs, mainly implicated in inflammation, oxidative stress, xenobiotic biotransformation and endocrine system and relevant immune endpoints. On the whole, QD-treated fish displayed significant reductions in leukocyte counts, viability, and both resting and active phagocytic activities (Gagne et al., 2010). Therefore, QDs are able to render gene material damage in living rodents and aquatic organisms, which might be a potential cause of adverse health outcomes.

Immune cell reactions

As immune defense system plays an important role in preventing organism from variety of diseases, immunotoxicity of QDs posesa huge risk of severe whole-body health problems including diseases occurring in respiratory system. So far, cadmium-containing QDs have been reported to interact with blood components associated with the immune system directly, such as phagocyte and lymphocyte, and then induce immune responses, depending on the size, structure or component of QDs (Dobrovolskaia & McNeil, 2007). An *in vitro* study investigating the deleterious effects of CdTe QDs on model macrophages (J774A.1) and colonic epithelial cells (HT29) revealed that cells exhibited changes in metabolism and morphology at QD concentrations between 10^{-2} and 10 μg/ml and QDs could be internalized and changed in cell–cell contact, shapes and internal organization (Nguyen et al., 2013a). Among immune cells from different species, Bruneau et al. (2013) observed that human innate immune cells were most sensitive towards CdS/Cd-Te QDs, which cautions us for the potential risk of immune disorders if QDs were used in humans.

Immune cell reactions were observed in QD-treated animals as well as in QD-treated cells. Hoshino et al. (2009) observed that QDs affected the proliferation of immune cells in tested mice, indicating potential harm for immune system. Bruneau et al. (2013) suggested that QD elicited lymphoblastic transformation for adaptive immunity among tested mice and this transformation got stronger for higher QD concentration. Sadaf et al. (2012) observed that the level of white blood cells (WBCs) increased significantly in mice treated with CdTe QDs. Ho et al. (2013) found that QDs were potent in suppressing immunocompetence on a mass concentration basis. Moreover, QD exposure would generate a stronger immune response in animals with certain kinds of deficiency. Jacobsen et al. (2009) detected a slight insignificant increase in neutrophils and relative decrease in macrophages among apolipoproein E knockout (ApoE−/−) mice exposed to broncho-alveolar lavage (BAL) fluid. McConnachie et al. (2013) showed that QDs elicited a stronger and statistically significant neutrophil influx in lungs of both mice with normal or slightly diminished capacity for glutathione (GSH) synthesis 8 h after bronchoalveolar lavage (BAL).

Quantum dots disposition in cells of respiratory system or lung tissues

Quantum dots are easy to be taken up into cells or high blood-flow tissues due to their tiny size as well as specific surface coatings. For example, bio-conjugated CdSe/ZnS QDs capped with antibodies can be routed to targeted or non-targeted intracellular compartments through an endocytotic pathway, a complicated natural cellular process (Gao et al., 2004). This feature of QDs suggests potential therapeutic benefits, and on the other hand, severe health consequences of long-term accumulation of QDs in targeted or non-targeted cells and tissues. As a result, one observed respiratory toxicity of QDs is abundant accumulation in QD-treated cells in respiratory system or lung tissues of QD-treated animals.

Roberts et al. suggested that the majority of cadmium-containing QDs persisted in the lungs among rats treated by intratracheal instillation with saline out to day 14, while some deposit in the airways. Alveolar macrophages (AM) were the main cells to uptake QDs (Roberts et al., 2013). What's worse, QDs' deposition in lungs is not easy to clear. Ma-Hock et al. (2012) indicated that cadmium levels in the lungs of water soluble CdS/$Cd(OH)_2$ QDs-treated Wistar rats did not decrease 3 weeks after inhalation exposure. However, clearance rates vary with QDs with different functional coatings or mice with different genotypes. Roberts et al. (2013) discovered that QD-COOH had slightly greater clearance rate than QD-NH_2. McConnachie et al. (2013) suggested that clearance rate of QDs from the lung was much more rapid in

the mice with severe GSH synthesis deficiency due to genetic flaws than mice with normal or slightly diminished capacity for GSH synthesis.

Apart from cadmium-containing QDs that could accumulate in cells and tissues of respiratory system, photoluminescent carboxylated graphene QDs (GQDs) were reported recently that they could deposit in A549 cancer cells *in vitro* cell culture model and in lung after intravenous injection in long-term *in vivo* mouse model (Nurunnabi et al., 2013). Researchers also found that silicon-based QDs could be accumulated in the lungs of QD-treated mice with noticeably elevating silicon levels compared to untreated mice (Liu et al., 2013a).

Lung injury and inflammation

There are several studies suggesting that QD deposition in lung tissues results in lung damage (Ho et al., 2013; Jacobsen et al., 2009; McConnachie et al., 2013). However, there are other plausible explanations in the literature to elucidate mechanisms of QD-induced lung injury. Roberts et al. indicated that cadmium-containing QDs caused lung injury via increasing lung injury parameters, lactate dehydrogenase (LDH) and albumin. The injury was severest at days 7 and 14 post-exposure, and the dose of QD had a positive association with the severity of lung damage (Roberts et al., 2013).

Pulmonary inflammation is another primary lung damage induced by QDs, which could be reflected by increases in inflammatory cells and chemokines. Roberts et al. observed that the number of measured inflammatory cells (alveolar macrophages (AM), polymorphonuclear cells (PMN) and lymphocytes), as well as inflammatory chemokines, MCP-1 and MIP-2 recovered from lungs significantly elevated after exposed to the highest dose of QDs and peaked at days 7 and 14 after bronchoalveolar lavage. Similarly, the lung inflammation has a positive association with the exposure dose (Roberts et al., 2013).

Additionally, there is evidence showing lung inflammation was relevant to oxidative stress. A recent study investigated the pulmonary toxicity of CdSe/ZnS QDs with a tri-*n*-octylphosphine oxide, poly(maleic anhydride-alt-1-tetradecene) (TOPO-PMAT) coating in mice with partial or severe glutathione (GSH) synthesis deficiency via nasal instillation. The results showed that TOPO-PMAT CdSe/ZnS QDs induced oxidative stress, which caused increases in expressions of antioxidant proteins. The levels of the pro-inflammatory cytokines KC and TNFα increased in bronchoalveolar lavage fluid (BALF) among mice with normal or slightly diminished capacity for GSH synthesis other than severely GSH synthesis deficiency. Moreover, analysis of lung concentration of cadmium ions showed that TOPO-PMAT CdSe/ZnS QDs were more difficult to be eliminated from the lungs of the first two genotypes of mice. The study concluded that TOPO-PMAT CdSe/ZnS QDs have *in vivo* pro-inflammatory property relevant to GHS synthesis status (McConnachie et al., 2013). The findings may be extrapolated to humans to imply that people with GSH synthesis deficiency might be more susceptible to the inflammatory effects of TOPO-PMAT QDs.

Other than persistent inflammation, cadmium-containing QDs were observed to induce interstitial lymphocyte infiltration and granulomatous reactions in mice lungs in relatively long term, both led to reduced pulmonary function (Ho et al., 2013). Furthermore, QD-treated mice obtained beginning hepatic necrosis following acute pulmonary inflammation with edema after intratracheal instillation (Jacobsen et al., 2009). It should be noted that inflammation was also thought to be involved in the risk of atherothrombosis.

Lately, some researchers start to assess the deleterious effects of QD-labeled stem cells rather than individual QD, because QD-labeled cells are more valuable for diagnostic and therapeutic purposes in oncology. Ramot et al. assessed the potential toxicity of QD-labeled human embryonic palatal mesenchymal (HEPM) cells in male NOD/SCID mice by exposure via a single intravenous injection. They reported that multifocal organizing thrombi were observed in the pulmonary arteries of all QD-HEPM-treated mice in a 1-week study. They also found that the perivascular inflammation at the injection sites was severer in mice from the 1-week group than those from the 1-month group (Ramot et al., 2010).

Long-term potential adverse effects

Unfortunately, there has not been sufficient evidence to show QDs' long-term adverse effects. However, according to QD-induced damage in cellular and tissular levels, we have undeniable reasons to believe that QDs are able to induce long-term adverse health consequences. Genetic material damage and immune cells reactions elicited by QDs implicate potential risks of carcinogenicity and respiratory bacterial or viral infections. QD-induced toxicity in L929 cells indicated potential pulmonary fibrogenic reactions (Liu et al., 2013b). Abundant accumulation of QDs and lung tissue inflammation might induce pulmonary granuloma, asthma and reduced lung function, resulting in systematic diseases. Therefore, it is urgent to conduct chronic toxicity tests to investigate QDs' long-term toxic effects on respiratory system via different cell models and species. As the ultimate purpose of toxicity studies of QDs is to explore the adequate safety precautions to be applied in human beings, occupational studies would be a good choice.

To further clarify the variety of QD types and their corresponding adverse effects, Table 1 summarizes basic characteristics of recently reported toxicity studies associated with respiratory system. This table is also useful to identify a particular QD type from various types when a benchmark is hard to determine.

Adverse effects of extrapulmonary systems

Quantum dots are able to impact organs remote from the lung, such as liver, spleen or kidney. After QDs are administrated to tested animals by injection, they are mainly accumulated in the organs of reticuloendothelial system (RES), including liver, spleen and lymph nodes (Zhang et al., 2013). Findings of Ma-Hock et al. (2012) indicated increased cadmium level in the livers of tested rats. Both of Chen et al. (2008) and Lin et al. (2008) observed QDs deposition in the livers of treated mice after intravenous injection. In addition, QDs presented cytotoxic for hepatic cells. Tang et al. (2013a) suggested

Table 1. Summary of QD toxicity studies associated with respiratory system.

QD types	Model	Method(s) used	Exposure conc. with/without admin.	Study duration	Respiratory toxicity	References
CdSe/ZnS; InP/ZnS	A 549 cells	WST-8 test; LDH leakage assay; *in vitro* gene expression level by real-time $_{Q}$PCR	1 pM to 5 nM	24 and 48 h	CdSe/ZnS QDs produced a significantly larger decrease in cell viability compared to InP/ZnS QDs. The toxic effect of CdSe/ZnS QDs was clearly observable after 24-h incubation at 1 nM concentration. CdSe/ZnS QDs induced significant membrane damage in cells, even at 1 pM after 24 h.Cells incubated with CdSe/ZnS QDs started to result in some detectable over-expression of genes correlated with oxidative stress from 1 pM after 24 h.	Brunetti et al. (2013)
Silicon QDs (SiQDs)	Mice; rhesus macaques	Histological analysis; tissue analysis	200 mg/kg; intracenous transfusion	14 weeks	Elevated levels of silicon were present in the liver, spleen and lung of mice 3-month post-treatment.Histopathology 3 months after treatment showed adverse effects of the nanoformulation in the liver and lung of mice, but showed no such effects in monkeys.	Liu et al. (2013a)
CdSe/ZnS-TOPO-PMAT	Gclm +/+, Gclm +/− and Gclm −/− mice	Total lung GSH determination; fluorogenic 5′ nuclease-based assay; quantitative RT-PCR; ELISA; cytokine assay; toxicokinetic modeling	6 µg Cd equivalents/kg body weight; bronchoalveolar lavage	8 h	QD treatment had no effect on lung GSH levels, regardless of genotype. QD treatment resulted in marked increases in stress response and inflammatory cytokine mRNA expression level in Gclm +/+ and Gclm +/− mice. QD treatment resulted in an increase in inflammatory cytokines KC and TNFα protein levels in Gclm +/+ mice. The rate of clearance of Cd from the lung was much more rapid for the Gclm −/− mice with a half-life of 4.6 h compared to the Gclm +/+ and +/− genotypes with half-lives of 46 h and over 400 h, respectively.	McConnachie et al. (2013)
Photoluminescent carboxylated graphene QDs (GQDs)	A549 cancer cells; SKH1 female nude mice; SD rats	MTT assay; LDH release assay; noninvasive optical imaging study; histological analysis	50, 100, 250, 500 µg/ml (*in vivo*); 2.5, 5, 10 mg/kg (*in vitro*); intravenous injection	24 and 48 h (*in vivo*); 2, 4, 6, 8, 10, 12 and 24 h, 1, 8, 22 days (*in vitro*)	GQDs caused increase in LDH release and reduce in cell viability in dose- and time-dependent manner. The quantitative analysis of biodistribution, showing GQD accumulation in liver, lung and spleen at 2-h post-injection. Moderate pathological changes were observed in the liver and lung at 21 days after the GQDs administration at a higher dose (10 mg/kg).	Nurunnabi et al. (2013)

(*continued*)

Table 1. Continued

QD types	Model	Method(s) used	Exposure conc. with/without admin.	Study duration	Respiratory toxicity	References
CdSe/ZnS-COOH; CdSe/ZnS-NH_2	Male Sprague–Dawley rats	LDH and albumin test; inflammatory chemokines assay; microscopy and histopathological analysis; QD lung deposition, clearance and distribution analysis	12.5, 5.0, 1.25 μg/rat; intratracheal instillation	0, 1, 3, 5, 7, 14 and 28 days	After treatment with the 5.0 μg dose, both QDs caused an increase in both lung injury parameters, primarily at days 5, 7 and 14 after treatment. Lung inflammation was found to be dose-dependent and peaked at days 7 and 14 post-exposure for both forms of QD. Cd persisted up to 28 days for both forms of QD, but clearance rate was slightly greater for QD-COOH over time. Accumulation of the QD were discovered in and around a lymphatic vessel.	Roberts et al. (2013)
CdSe/ZnS with positive, negative or PEG coating	BALB/c mouse	Organ index tests; histopathological analysis; long-term toxicity test	2.5, 4.096, 5.12, 6.4, 8, 10 nmol/kg; intravenous injection	14 days, 15 weeks	For positive QD-treated mice, no animal death occurred at dose <6.4 nmol/kg, while the mortality was 100% at 10 nmol/kg. And the death occurred within 24-h post-injection. For QDs with negative and PEG coating, no death was observed at 10 nmol/kg. Positive QDs was largely present in the lung at 10 nmol/kg, while QDs with negative and PEG coating preferred to accumulate in the liver. PEGylated QDs displayed the slightest chronic injuries in the long-term toxicity examination in comparison to positive or negative ones.	Tang et al. (2013b)
CdSe/ZnS	A 549 cells	MTT assay; ROS measurement; LDH assay; real-time PCR analysis	1.6, 4.1, 8.3, 16.6 μg/ml	24 h	CdSe/ZnS QD induced cell apoptosis in a dose-dependent manner. Intracellular ROS content increased only slightly in cells treated with CdSe/ZnS QDs alone. The LDH assay revealed significant and consistent increases in LDH release in proportion to increases in QD concentration, indicating increasing necrotic cell death. Significant DNA damage was evident in A549 cells treated with QDs (OTM: 5.56 ± 2.63, 11.05 ± 4.61 and 15.10 ± 5.77 for control, 4.1 and 8.3 μg/ml QDs, respectively).	Choi et al. (2012)
$CdS/Cd(OH)_2$	Male Wistar rats	Histological examination; Cd organ burden analysis; TEM image	6 h/day (0.52 mgCd/m^3); head–nose exposure	5 days	Results indicated that QD caused neutrophil inflammation in the lungs that partially regressed after 3-week recovery period.	Ma-Hock et al. (2012)
Positively charged CdSe; negatively charged CdSe	Normal human bronchial epithelial (NHBE) cells	WST-1 test; LDH assay; ROS measurements; gene expression analysis	20, 80, 60 μg/ml (negative QDs); 0.5, 5,	6 or 24 h	Positively charged QDs were found to be highly necrotic at much lower concentrations compared to negative QDs (3.5 μg/ml compared to 80 μg/ml, respectively).	Nagy et al. (2012)

			20 μg/ml (positive QDs)		Cells exposed to 3 or 5 nm QDs containing longer ligands were significantly less proliferative compared with QDs capped with the shorter ligands. Comparing gene expression changes between negative and positive 3 nm QDs at concentration of 20 μg/ml, the positive QDs up-regulated genes involved with mitochondrial function, apoptosis. Negative QDs induced expression of several genes associated with inflammation. Overall, positive QDs are significantly more cytotoxic compared to negative QDs.	
Positively charged CdTe (Cd-S-CH_2-CH_2-NH_3+Cl^-); negative CdTe (Cd-S-CH_2-CH_2-COO^-Na^+)	Apolipoprotein E knockout mice ($ApoE^{-/-}$) mice	Comet analysis on BAL cells; preparation of RNA and cDNA from lung tissue; real time RT-PCR	137.5 μg/mouse; instillation	3 and 24 h	The mice had developed acute pulmonary inflammation with edema and beginning hepatic necrosis at 24 h after QD-instillation. Instillation of both QDs caused a highly significant increase in the level of all three cytokine mRNAs at both time points. A slight insignificant increase in neutrophils and accordingly decrease in macrophages were detected at 3 h in BAL fluid. BAL cells contained significantly increased level of DNA damage at 3 h. Both QDs caused a highly significant increase in leakage of protein in BAL fluid following 24 h.	Jacobsen et al. (2009)
CdS	CHL cells	MTT assay; intracellular ROS measurement; GSH measurement	2.5, 5, 10, 20, 40, 60, 80 μg/ml	24 h	Cell activity was severely damaged when the QD concentration was up to 20 μg/ml. CdS QD treatment resulted in an increase (0.2–0.3 fold) of ROS generation over control levels. After 24 h treatment with CdS QD (20 μg/ml), CHL cells manifested a significant decrease (50%) of intracellular GSH level.	Li et al. (2009)
Silianized CdSe/ZnS-PEG	IMR-90 cells	Phenotypical measurements with high content image analyzer (HCA, Cellomics KineticScan HCS Reader); affymetrix high throughput analysis	8 or 80 nM	48 h	Cells exposed to high or low dosages of Silianized CdSe/ZnS-PEG increased the percentage of cells slightly in an apoptotic or necrotic state, but not significant compared to the control (1.8–2% versus 1.2–1.5%). There were far more down-regulated and up-regulated genes affected by low dose. A significant portion of the down-regulated genes are related to the M phase of the mitotic cell cycle, as well as up-regulated genes include those for carbohydrate binding proteins, intracellular organelle related proteins and stress–response genes.	Zhang et al. (2006a)

CdTe QDs were toxic in aquatic organism (i.e. zebrafish) liver cells by interfering with DNA repair and increasing reactive oxygen species (ROS). Nguyen et al. (2013b) found that cadmium-containing QDs elicited oxidative stress due to the effect of cadmium ions and generated ROS, resulting in extrinsic and intrinsic apoptosis in hepatocellular carcinoma HepG2 cells in a time- and dose-dependent manner.

Since spleen and lymphatic systems are two other important organs in the RES, QDs were taken up eventually by them after intravenous injection (Smith et al., 2008). Otherwise, QDs could be persistent in blood vessels, posing potential risk of cardiovascular diseases. Even though some studies suggested that QDs could be removed from the RES via blood circulation, they would persist for several months before removal (Fischer et al., 2006).

Since QDs could be cleared through kidneys into urine, kidney is another important targeted organ. Currently, nephrotoxicity of QDs has been approved. Luo et al. suggested that cadmium-containing QDs caused increase in level of intracellular reactive oxygen species (ROS) of mouse renal adenocarcinoma (RAG) cells and induced autophagy, even apoptosis. The autophagy induced by oxidative stress played an essential role in defense of cytotoxicity of QDs (Luo et al., 2013). Additionally, an *in vivo* study using male Sprague–Dawley mice showed that the concentration of cadmium in kidney, a primary target of cadmium-containing QDs, increased slightly in QD-treated rats by intraperitoneal injection compared to untreated ones (Roberts et al., 2013). Ma-Hock et al. (2012) also observed abundant accumulation of QDs in kidney of rats after inhalation exposure. Cadmium-containing QDs, even worse, was showed to redistribute with time from body mass to liver and kidney a few days post-dosing (Yang et al., 2007).

Factors determining toxicity of quantum dots

Dose and size

In addition to inherent toxicity of component materials, toxicity of QDs also depends on multiple other factors, including the concentration, size, charge and outer coating materials. Dose and size are considered as the main factors determining the toxic severity of an individual QD.

Paracelsus, a German-Swiss Renaissance physician, said ''*What is there that is not poison? All things are poison and nothing without poison. Solely the dose determine that a thing is not a poison.*'', which shows a close relationship between the dose and toxicity of a substance. Li et al. (2009) provided evidence that the cytotoxicity of cadmium-containing QDs was positively associated with concentrations. Roberts et al. (2013) indicated that higher dose of QDs elicited severer lung injury and inflammation and the highest dose of QDs induced the easiest accumulation in the respiratory system.

On the other hand, size plays another important role in affecting QDs' toxicity. It has been proved by several studies that cadmium-based QDs with a smaller particle size are easier to distribute to nucleus of cells or organs of RES (Lovrić et al., 2005; Xu et al., 2013; Zhang et al., 2013). Moreover, when the exposed concentration was the same, 3 nm QDs could induce severer intracellular ROS production in primary human bronchial epithelial cells than 10 nm QDs (Nagy et al., 2012).

Outer coating

In order to make QDs biologically active or compatible, synthesized QDs are capped with outer coatings, which could improve water solubility, durability and stability. Outer coatings are classified into capping shells and functionalized surface. There is sufficient evidence to conclude that nude core QDs have severe toxicity due to releasing free heavy metal ions and generating ROS (Rzigalinski & Strobl, 2009). As mentioned previously, a shell is able to reduce release of metal ions and generation of ROS to a great extent (Tsoi et al., 2012). In addition to ZnS, which is a common shell for cadmium-containing QDs, silica material has also been used as capping shells for minimizing toxicity as well as special biomedical applications (Sadaf et al., 2012). For example, the siloxane shell was capable of preventing ZnO QDs from releasing zinc ions (Aboulaich et al., 2012). However, the toxicity attributed to the degradation of the shell material was considered as a big challenge by some researchers (Rzigalinski & Strobl, 2009). Zhang et al. (2006b) observed that the ZnS shell deteriorated in living cells, suggesting the potential cytotoxicity of the shell chemistry.

For functionalized surface group of QDs, several studies have indicated that surface materials could influence effects of certain types of QD, thus QDs with different surface coatings would display different toxicity. For *in vitro* toxicity, Bradburne et al. (2013) showed that CdSe/ZnS QDs with surface capping ligand presented obvious cellular toxicity to tested THP-1 cells (monocytic), such as reducing cellular viability. Other researchers further observed that QDs with long ligands resulted in greater toxicity than those with short ones (Nagy et al., 2012). For *in vivo* toxicity, Fischer et al. discovered that the half-life of pharmacokinetics of QDs coated with mercaptoundecanoic acid (QD-LM) was significantly longer than that of QDs with bovine serum albumin (QD-BSA). Moreover, the accumulation of QD-BSA was located in significantly lower parts of lung than QD-LM. Thus, QD-LM presented high toxicity for respiratory system (Fischer et al., 2006). Roberts et al. (2013) found that CdSe/ZnS QDs functionalized with carboxyl (QD-COOH) and amine (QD-NH2) showed slightly different degrees of toxicity at different time points, which may be partly due to different lung clearance rates as QD-COOH displayed a mildly greater clearance rate over time.

Recent research from Tang et al. (2013b) suggested that even though QDs coated with polyethylene glycol (PEG) deposited dramatically in the liver and could result in adverse health outcomes, QDs with PEG presented relatively slight toxicity. Some previous evidence also indicated that PEG capped QDs presented good blood compatibility, and tended to attenuate the toxicity of uncoated QDs with different core/shell materials by inhibiting ROS generation (Tang et al., 2013b; Zhang et al., 2013). However, Ho et al. (2013) suggested that PEG coatings failed to prevent adverse responses caused by administration of QDs via the lung, where they were mainly trapped in the lung and needed a few time to be removed (Yang et al., 2013). Otherwise, PEG is

still useful for minimizing the toxicity of other nanoparticles (Ju et al., 2013).

Charge

Sometimes, outer coatings could make QDs positively or negatively charged. Each of the two kinds of QDs exhibits different toxicity. Nagy et al. observed that positively charged QDs using amino undecanethiol (AUT) or cysteamine (CYST) as outer coatings were more toxic for human bronchial epithelial cells than negatively charged ones using mercaptoundecanoic acid (MUA) or mercaptopropanoic acid (MPA) as outer coatings. Research suggests that positive QDs elicited alterations in genes associated with mitochondrial function, whilst lower toxic negative QDs induced increases in gene expression of pro-inflammatory cytokines related to DNA damage (Nagy et al., 2012). Zheng et al. (2013) drew similar conclusions that negative QDs were relatively less cytotoxic.

When positively charged QDs were coated with specific compounds, the toxicity may be changed. Tang et al. found that positive QDs coated with cationic polymer PDDA caused abundant accumulation in the lung rather than the liver of QD-treated BALB/c mice. These positive QDs also rendered the most severe acute respiratory toxicity caused by pulmonary embolism, which was mostly attributed to the surface material PDDA. Furthermore, PDDA presented greater toxicity when coated with QDs (Tang et al., 2013b).

To conclude, as there are so many factors affecting the toxicity of a QD, it is important to remember that each QD type is characterized with its unique property and its potential toxicity and needs to be assessed individually.

Minimizing the toxicity of quantum dots and outlook

Various methods seem plausible to minimize the toxicity of QDs. One basic method is to use materials with low intrinsic toxicity as cores of QDs, as the main toxicity of a QD is attributed to the heavy metal released from a QD's core. For instance, indium-containing QDs can be safer alternatives to cadmium-containing QDs because they have much lower intrinsic toxicity. Brunetti et al. (2013) showed that InP/ZnS QDs induced less damage in cells and genetic materials than the counterpart CdSe/ZnS. Aboulaich et al. (2012) suggested that ZnO QDs displayed weak *in vitro* toxicity, and the concentration of zinc ions leaked from QDs was too low to be responsible to the inhibitory effects against tested bacterial cells. Otherwise, in view of factors determining the toxicity of QDs mentioned in the previous part of this review, possible ways to minimize the toxicity include reducing doses, magnifying sizes and capping appropriate coatings.

Due to the increasing prevalence of QDs and the potential social and economic benefits, it is crucial to elucidate the mechanism as well as sources of QDs toxicity in order to avoid hazards by misapplications. As each type of QD is a unique nanocrystal with regard to its component, size, shape and surface chemistry, further large-scale carefully designed studies need to be conducted to assess both the acute and chronic toxicity of various QDs in animal models as well as *in vitro* cell cultures. We hope that properly designed QDs, as a good option for doctors, could be selected and approved to be used in the diagnostic and therapeutic applications of certain important diseases in human beings in the near future.

Declaration of interest

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