Biomaterials 33 (2012) 1238–1244

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Leading opinion

The cytotoxicity of cadmium-based quantum dots☆

Nan Chen [a,1], Yao He [b,1], Yuanyuan Su [a,b,1], Xiaoming Li [a,1], Qing Huang [a,*], Haifeng Wang [c], Xiangzhi Zhang [a], Renzhong Tai [a], Chunhai Fan [a]

[a] Laboratory of Physical Biology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China
[b] Institute of Functional Nano and Soft Materials (FUNSOM) and Jiangsu Key Laboratory for Carbon-based Functional Materials and Devices, Soochow University, Suzhou, Jiangsu 215123, China
[c] Chinese National Human Genome Center at Shanghai, 250 Bi Bo Road, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 14 October 2011
Accepted 24 October 2011
Available online 10 November 2011

Keywords:
Cadmium based quantum dots
Cytotoxicity
Metallothioneins
Nanomaterials

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

☆ *Editor's Note*: This paper is one of a newly instituted series of scientific articles that provide evidence-based scientific opinions on topical and important issues in biomaterials science. They have some features of an invited editorial but are based on scientific facts, and some features of a review paper, without attempting to be comprehensive. These papers have been commissioned by the Editor-in-Chief and reviewed for factual, scientific content by referees.

* Corresponding author. Tel./fax: +86 21 39194520.
E-mail address: huangqing@sinap.ac.cn (Q. Huang).

[1] These authors contributed equally to this work.

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.10.070

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

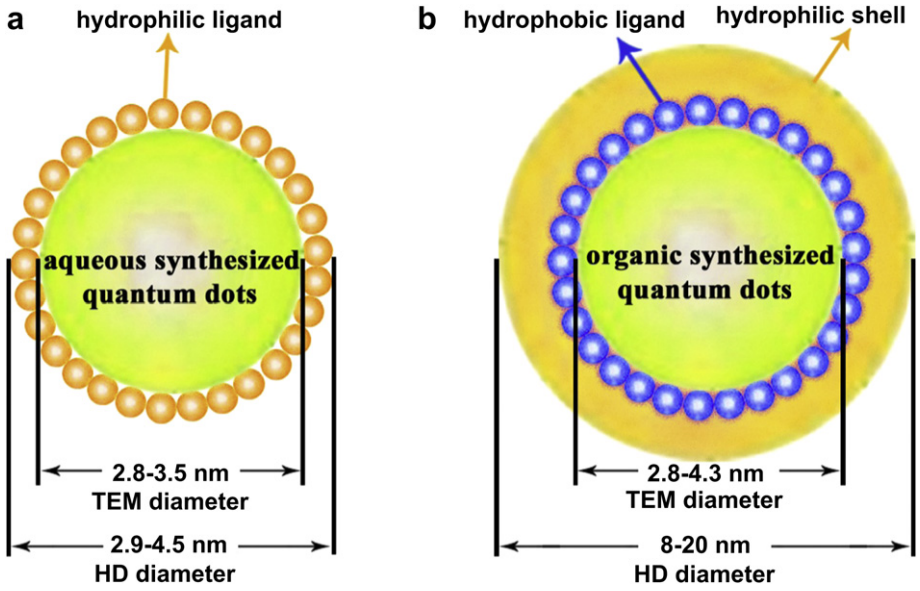

Fig. 1. Schematic structures of aqueous synthesized QDs (aqQDs) and organic synthesized QDs (orQDs). Hydrophilic ligand indicates thiol- and carboxyl-modified short chain organic molecules with hydrophilic property (e.g., 3-mercaptopropionic acid and thioglycolic acid). Hydrophobic ligand indicates long 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.).