Preparation, Characterization and Toxicological Impacts of Monodisperse Quantum Dot Nanocolloids in Aqueous Solution

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Recently, mass production and extensive use of novel engineered nanomaterials (*e.g.*, carbon nanotubes, fullerene, nano-TiO_2, nano-Au and quantum dots) raise increasing concerns about their potential harmful effects on our environment and human health. Therefore, for better understanding of their environmental and biological fate upon accidental release during their manufacturing and transport processes, increasing number of studies on the toxicities of engineered nanomaterials were conducted during last few years. However, regarding the toxicity of engineered nanomaterials, contradictory results were frequently found in the current literature, probably due to the widely varying physicochemical properties of engineered nanomaterials and inconsistency of the dosage/exposure conditions. Among various engineered nanoparticles, semiconductor quantum dots (QDs) have received significant attention as a new type of fluorophore for the biological and medical imaging.[1-4] However, they also induced increasing concern as a potential pollutant, due to its unknown toxicity as a nanometer-scale material as well as the toxic components (*i.e.*, Cd, Zn, Se and etc). But, their colloidal stability, photochemical property, dissolution behavior and overall cytotoxic impact of these QD colloids have not been systematically studied and completely understood yet.

In this study, using various analytical tools, we have prepared and characterized water-soluble monodisperse QD nanocolloids with well-defined core and hydrodynamic size, functional groups at the interface, impurities, nanoparticle concentration and impurities in aqueous solution. Then, toxicological impacts of QD nanocolloids collected at different stage of preparation (*i.e.*, different degree of impurity) were tested on gram negative bacterial cells (*i.e.*, *Escherichia coli*).

Preparation, surface modification and characterization of QD nanoparticles. From XRD analysis of QD nanoparticles, we confirmed that the QDs synthesized using SiPOP mothod[3] predominantly have the wurtzite CdSe crystal structure (see supporting information, Figure S1). Additionally, absences of CdO or ZnO peaks in the XRD results indicated that negligible degree of oxidations occurred during the preparation of this QD nanoparticle. Furthermore, application of schrrer equation to the width of CdSe (110) peak resulted in the estimated value of CdSe core size, which is 3.25 nm (see supporting information, Figure S2). while estimation of CdSe core size from the position (559 nm) of the first excitonic absorption peak resulted in very similar value (3.24 nm).[6] To make this QD soluble in aqueous solution, ligand exchange reaction of TOPO capped QD (*i.e.*, ^{TOPO}QD) was performed using MAA (mercapto-acetic acid) and aqueous colloid of carboxylic acid group terminated ^{MAA}QD was prepared. Then, several cycles of wash-out procedure were conducted to remove residual MAA from aqueous solution. To assure the monodispersity of this QDs in aqueous solution, hydrodynamic sizes of QDs were also measured by dynamic light scattering methods (^{MAA}QD = 4.56 nm, see Fig. 1) and confirmed that the individual QD particles are well dispersed in solution with least degree of aggregation. Concentrations of QDs in aqueous solutions were determined from the intensities of the first excitonic absorption peak and extinction coefficients based on the method reported by Yu *et al.*[6] and presented in the units of moles of QD per liter (*i.e.*, [QD]).

Fourier Transform Infrared (FT-IR) spectroscopy was used to monitor changes in surface modifying groups of QDs as well as impurities included during ligand exchange process. During surface modification process, the replacement of TOPO and changes in adsorbed MAA molecules were monitored by FT-IR and corresponding spectra were presented in Fig. S3 (In supporting information). The IR spectrum shown in Fig. S3(e) shows spectrum collected from QD solutions after several cycles of wash-out process and showed quite distinct spectral features with that of Fig. S3(d). First of all, absence of thiol peaks around 2570 cm^{-1} indicate that there is no free MAA with thiol groups and most of the MAA are already lost their hydrogen and

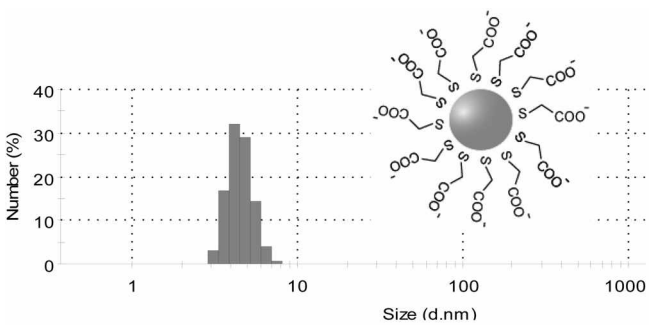

Figure 1. Hydrodynamic size distribution of ^{MAA}QD in aqueous solution prepared and used in this study.

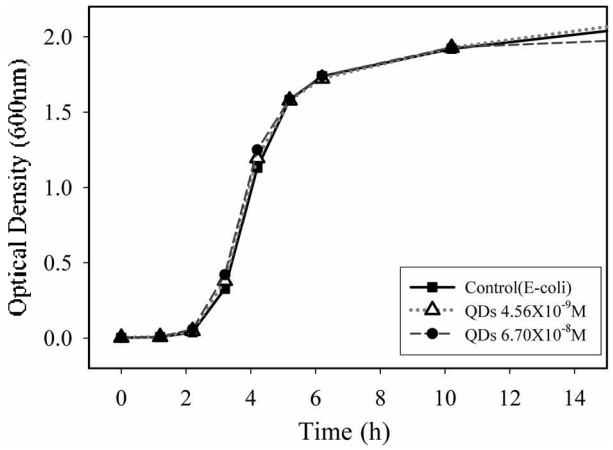

Figure 2. *E. coli* growth curves in the absence and presences of purified ^{MAA}QD.

adsorbed onto the QD surface. In addition, disappearance of peaks at 1713 and 1296 cm^{-1} and new peaks observed at 1589 and 1390 cm^{-1} confirm deprotonation of carboxyl groups of the QD surface and formation of negatively charged QD particles with carboxylate group on their interface with aqueous solution. In addition, further changes in IR peak widths and positions of this carboxylate functional group were observed. As shown in Fig. S3(c) and (e), there are significant increases in the peak widths (37 $cm^{-1} \rightarrow 72$ cm^{-1} and 26 $cm^{-1} \rightarrow 54$ cm^{-1} for asymmetric and symmetric stretching peaks of carboxylate group, respectively) as well as the shifts in peak positions (1570 $cm^{-1} \rightarrow$ 1589 cm^{-1} and 1387 $cm^{-1} \rightarrow 1390$ cm^{-1} for asymmetric and symmetric stretching peaks of carboxylate group, respectively). These increases in peak widths and positions are typically observed for the adsorbed species on the surface of particles and assure the adsorption of mercaptoacetate on the surface of QDs.

Cytotoxic impact of monodisperse QD colloids on *E. coli* cell growth: By monitoring changes in the growth curves of *E. coli* bacterial cultures, cytotoxic impacts of ^{MAA}QD solubilized in water has been tested. Among many components of ^{MAA}QD solution, we have tested the impacts of Cd^{2-} and Zn^{2-} ions on the bacterial culture. For each samples of *E. coli* cultured with ^{MAA}QD, the [Cd^{2+}] and [Zn^{2-}] were measured by AAS method after centrifugation of the cultures. Although slight increases in both metal concentration were observed as the amount of added ^{MAA}QD stock solution increase, but more or less they stayed in constant level during the time ranges of our growth curve experiments (48 hours) (maximum of ~1 ppm for [Cd^{2+}] and ~3.6 ppm for [Zn^{2+}]). Therefore, to test if these levels of released toxic ions can cause the observed cytotoxic impacts, we performed additional growth curve experiments in the presence of excess amounts of [Cd^{2+}] and [Zn^{2-}] (10 ppm for each). However, even in the excess amount of [Cd^{2-}] and [Zn^{2-}], no significant cytotoxic impacts were observed for both cations. Similar experiments were also performed for MAA added *E. coli* bacterial cultures. In contrast to the negligible cytotoxic impacts of [Cd^{2-}] and [Zn^{2-}], [MAA] used for the ligand exchange process have shown significant cytotoxic impacts on the growth of *E. coli* cells. Therefore, growth curve measurements were conducted using ^{MAA}QD stock solution prepared with a process involving several wash-out process to completely remove residual free MAA. Interestingly, as illustrated in Fig. 2, under the given growth conditions, there was no significant difference between the growth curves with and without QD-MAA nanoparticles, implying that the ^{MAA}QD nanocolloids by itself does not inhibit *E. coli* cells from growing. Therefore, we can infer that ^{MAA}QD nanoparticles does not have significant cytotoxic impact under the experimental conditions of this work ([QD] = 6.7×10^{-8} M, exposure time 48 hrs). Microscopic analyses were also conducted using fluorescence microscopy and TEM. From fluorescence image, orange colors originated from QDs were observed in the clusters of bacterial cells. TEM micrographs of *E. coli* cells embedded in ultrathin cross sections show that there is an association of the bacterial cells with clusters of ^{MAA}QD-like nanoparticles, while the bacterial cells cultured in the absence of ^{MAA}QD nanoparticles show no nanoparticles associated with the cell membranes. (see supporting information, Figure S4)

As a summary, overall result of this study indicated that, under experimental conditions tested here ([QD] = 6.7×10^{-8} M, exposure time 48 hrs), no significant cytototoxic impact of QDs were observed on *E. coli* cell growth, despite of the association of QD nanoparticles with microbial cells observed by TEM micrographs, while some misleading inhibitory effect (*e.g.*, extended periods of lag phase) was observed for certain sample mainly due to residual impurities included during QD preparations, such as free MAA. These results emphasize the importance of reference nanocolloids for impartial toxicity assessments of these novel materials, which are well prepared in appropriate aqueous media and characterized by various analytical techniques for their physicochemical properties.

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