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Cytotoxicity Tests of Water Soluble ZnS and CdS Quantum Dots

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Cytotoxicity tests of zinc sulfide (ZnS) and cadmium sulfide (CdS) quantum dots (QDs) synthesized via all-aqueous process with various surface conditions were carried out with human endothelial cells (EA hy926) using two independent viability assays, i.e., by cell counting following Trypan blue staining and by measuring Alamar Blue (AB) fluorescence. The ZnS QDs with all four distinct types of surface conditions were nontoxic at both 1 μM and 10 μM concentrations for at least 6 days. On the other hand, the CdS QDs were nontoxic only at 1 μM, and showed significant cytotoxicity at 10 μM after 3 days in the cell counting assay and after 4 days in the AB fluorescence assay. The CdS QDs with (3-mercaptopropyl)trimethoxysilane (MPS)-replacement plus silica capping were less cytotoxic than those with 3-mercaptopropionic acid (MPA) capping and those with MPS-replacement capping. Comparing the results of ZnS and CdS QDs with the same particle size, surface condition and concentration, it is indicated that the cytotoxicity of CdS QDs and the lack of it in ZnS QDs were probably due to the presence and absence of the toxic Cd element, respectively. The nontoxicity of the aqueous ZnS QDs makes them favorable for *in vivo* imaging applications.

Keywords:

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1. INTRODUCTION

Quantum dots (QDs) are semiconductor nanocrystals with distinctive photoluminescence properties. Compared to traditional organic dyes, QDs are much brighter with higher quantum yields and are resistant to photobleaching.[1] In addition, QDs have tunable emission wavelengths and can be excited over a broad excitation wavelength range.[2] Applying QDs in biological imaging is one of the most exciting new nanobiotechnology developments in the past decade.[3–6] QDs can be utilized as diagnostic and therapeutic tools for both scientific study and clinical applications to better understand, detect, and treat human diseases.[7–9]

The *in situ* imaging using QDs has been successfully demonstrated in animal models.[10–13] However, the use of QDs in humans has not yet been realized to date, due to concerns of the potential toxicity. As a nanomaterial, QDs may have adverse effects on the environment and human health, by releasing toxic elements, interrupting cell metabolism and proliferation, and resulting in environmental risk, gene mutation, chronic diseases, cell death or organelle damage.[14–19] The influence of nanoparticles on health is an important issue, which has become a newly emerging research topic and has not been fully understood. Elucidating the properties of QDs and their effects on the human body is crucial before any clinical use can occur.

Most current commercial QDs are comprised of diverse metallic elements known to be toxic even at very low concentrations, including cadmium, lead, and arsenic, etc. If these QDs are exposed to conditions promoting degradation, such as an oxidative environment, toxicity related to the release of free metal ions is expected. To prevent core degradation, an additional shell layer is added, making these QDs relatively biocompatible and nontoxic to some extent.[20,21] However, as several groups have found,[22,23] the QDs *in vivo* are prone to cellular internalization and retention rather than being excreted. This suggests possible toxicity could result from the accumulation and release of toxic elements thereafter.

To realize the potential of QDs for *in vivo* imaging, it is necessary to produce intrinsically nontoxic QDs. Recent studies have shown that water-soluble zinc sulfide (ZnS) QDs could be synthesized via an environmentally friendly, all-aqueous process. With 3-mercaptoprorionic acid (MPA) as the capping molecule, the ZnS QDs exhibited bright blue emission with a quantum yield (QY) of 31%.[24] With (3-mercaptopropyl)trimethoxysilane (MPS) as the capping molecule, the ZnS QDs exhibited good chemical stability

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for over 50 days under the ambient conditions (at room temperature and under the laboratory lighting condition).[25] With the MPA partially replaced by MPS, the resultant ZnS QDs exhibited higher QY of 75% and remained stable for over 60 days under the ambient conditions.[26] Since Zn is a biologically essential element and is usually considered nontoxic in moderate amounts, these highly luminescent and stable aqueous ZnS QDs offer a great potential for *in vivo* imaging applications.

However, in addition to the chemical toxicity, there are other factors that might affect the organism functionality, such as size, shape, charge, concentration, capping material, functional group, solubility and stability of QDs. Even for absolutely inert compositions, QDs with ultra small size, i.e., less than 10 nanometers, may stick to cell membrane or be ingested by cells, thereby disturbing metabolism or causing death.[27] Therefore, although ZnS QDs do not contain toxic elements, they still need to be examined to ensure the safety and biocompatibility before they can be used in the human body.

In this study, we carried out cytotoxicity tests of aqueous ZnS QDs prepared with four different surface conditions, using cultured human endothelial cells as a model system. Since the potential toxicity of nanoparticles in the human body is the focus of the study, human cells should be used to better predict the QDs toxicity. We chose to work with human endothelial cells, because they are amongst the first cells that would come in contact with the QDs if they are used *in vivo*. For comparison, the cytotoxicity of aqueous cadmium sulfide (CdS) QDs was also examined. In addition to different surface conditions, we studied the cytotoxicity at different QD concentrations, i.e., 1 μM and 10 μM, in the cell culture medium. These concentrations were chosen because they cover the range of applications for effective bioimaging.[28] After incubation of cells with the QDs for certain time, the cytotoxicity tests were conducted by cell counting after Trypan blue staining and by fluorescence assay with Alamar Blue (AB). Performing two independent tests is advantageous to ensure valid conclusions are drawn. In addition, the continuous AB fluorescence assay provides additional information on the potential long-term cytotoxicity. Our results indicated that the aqueous ZnS QDs were indeed nontoxic at both 1 μM and 10 μM for at least 6 days. In contrast, the CdS QDs were nontoxic only at 1 μM, and showed clear toxicity at 10 μM after 3 days in the cell counting assay and after 4 days in the AB fluorescence assay.

2. MATERIALS AND METHODS

2.1. Materials

All chemicals for QDs synthesis were purchased from Sigma-Aldrich (St. Louis, MO) and Alfa Aesar (Ward Hill, MA) and used as received. Human endothelial cells (EA hy926) were kindly provided by Dr. Cora-Jean Edgell, University of North Carolina (Chapel Hill, NC) and cultured as previously described.[29] All cell culture media and supplements were purchased from Cellgro (Herndon, VA) and Hyclone (Logan, UT). Disposable cell culture supplies were from VWR (West Chester, PA).

2.2. Aqueous QDs with Various Surface Conditions

The MPA-capped ZnS QDs with a ratio of MPA:Zn:S = 8:4:1 and the MPA-capped CdS QDs with a ratio of MPA:Cd:S = 2:3:1 were synthesized as previously described.[24, 30] The MPS-capped ZnS QDs with a ratio of MPS:Zn:S = 1/2:2:1 were also prepared.[25] The MPS-capped CdS QDs cannot be synthesized by mixing the precursor with MPS directly. Therefore, no MPS-capped CdS QDs were available. The MPS-replaced QDs were first synthesized with MPA and followed by capping molecule replacement with MPS. The obtained MPS-replaced ZnS QDs had a ratio of MPS:Zn:S = 1/2:4:1[26] and the MPS-replaced CdS QDs had a ratio of MPS:Cd:S = 1/2:3:1.[28] For QDs with MPS-replacement plus silica coating, 0.2 ml of $Na_2Si_3O_7$ solution with Si concentration of 63 mM was added to 8 ml of the MPS-replaced ZnS or CdS QDs suspension. The resultant ZnS QDs had a ratio of Si:(MPS:Zn:S) = 1:(1/2:4:1) and the CdS QDs had a ratio of Si:(MPS:Cd:S) = 1:(1/2:3:1).

A total of seven different types of aqueous QDs were applied in the cytotoxicity tests, i.e., MPA-capped ZnS, MPS-capped ZnS, MPS-replaced ZnS, and MPS-replaced plus silica-coated ZnS, as well as MPA-capped CdS, MPS-replaced CdS, and MPS-replaced plus silica-coated CdS. All the QDs suspensions were prepared in deionized (DI) water and had a nominal concentration of 1.6 mM based on the sulfur concentration. The precursor ratios were chosen for their respective optimal photoluminescence performance. All the QDs had a similar particle size of about 5 nm with reasonably narrow size distribution of 4–6 nm and nearly spherical shape, as characterized by transmission electron microscopy and dynamic light scattering.[24–26, 28, 30]

Immediately before addition to the cell cultures, all QDs suspensions were micro-centrifuged (MiniSpin plus, Eppendorf, Westbury, NY) with 10 kD filter (Millipore Co., Billerica, MA) and rinsed with DI water for three times to remove the excess capping molecules and free ions. After microcentrifugation, the QDs remained in the retentate suspension and no precipitation or aggregation was observed.

2.3. Cell Culture

Human endothelial cells (EA hy926) used for the cytotoxicity tests were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 4.5 g/l glucose, 50 units/ml penicillin and 50 mg/ml streptomycin, and 10% fetal bovine serum (complete DMEM, thereafter), and

cultured at 37 °C in a humidified atmosphere with 5% CO_2 and 95% air. All cytotoxicity tests were carried out on cells immediately after they reached confluence.

Cells cultured under identical conditions except for the absence of QDs were considered as controls. Each experiment was repeated 3 times. Where applicable, the data were expressed as mean ± standard deviation (SD). One-way ANOVA and two-tail t-test were used for statistical data analysis with $P < 0.05$ considered as being statistically significant.

2.4. Cell Counting with Trypan Blue Staining

Human endothelial cells (EA hy926) were seeded in T-25 culture flasks at a density of 150,000 cells/flask and allowed to attach for 3 h. The cells in each flask were then incubated with complete DMEM containing one specific type of QDs at 1 μM or 10 μM respectively. After 3 days, dead cells floating in the supernatant and live cells adhering on the flask surface were collected separately. Adhering cells were detached by trypsinization with 1 ml of trypsin for 2–3 min. The collected cells were then stained with Trypan blue and counted in a hemacytometer (Hausser scientific, Horsham, PA) under a PhotoZoom inverted microscope (Leica Microsystems Gmbh, Wetzlar, Germany).

2.5. Alamar Blue Test

Cytotoxicity of QDs was also examined using the Alamar Blue (AB) (Biosource, Alameda, CA) fluorescence assay, as previously described.[31, 32] EA hy926 cells were first seeded in 24-well culture plates at a density 10,000 cells/well and allowed to attach for 3 h. Then the medium in each well was replaced with complete DMEM containing one specific type of QDs at 1 μM or 10 μM respectively. After 2 days of incubation, the supernatant in each well was replaced with 1 ml fresh complete medium containing 5% (v/v) AB. After 3 h of incubation, two 100 μl aliquots of AB-containing medium were collected from each well for fluorescence measurement using a CytoFluor Multi-well Plate Reader (PerSeptive Biosystems Inc., Framingham, MA). Subsequently the supernatant in each well was replaced with fresh QD-containing medium and the cells were cultured continuously. The AB fluorescence assay was repeated two more times until day 6, when the cells in the control group reached confluence.

To translate the fluorescence readings into cell numbers, a calibration curve was established. We placed known numbers of cells (0–200,000) in 24-well plates, allowed to attach for 3 h, and then treated the cells with the medium containing 5% (v/v) AB for 3 h as above. By fluorescence measurement for the supernatant from each well, the relationship between the measured fluorescence intensity and the cell number was obtained and used as the calibration curve.

2.6. Microscopic Examination

For assessing the effect of QDs on EA hy926 cell morphology, the cells were cultured in 6-well plates with medium containing one type of QDs at 1 μM or 10 μM. After 3 days of incubation, the supernatant with floating cells was removed, and the adhering cells were fixed with 10% buffered formalin for 15 min at room temperature. Following a gentle wash with PBS, the cells were incubated for 15 min in PBS containing 0.2% Triton-X 100 (Sigma-Aldrich, St. Louis, MO) for cell membrane permeabilization, 2 μg/ml Hoechst 33258 (*bis*-benzimide, Sigma-Aldrich, St. Louis, MO) as a nuclear stain, and 1 μg/ml rhodamine-phalloidin (Phalloidin TRITC-labeled, Sigma-Aldrich, St. Louis, MO) as a specific stain for microfilaments. The cells were then washed and soaked in PBS for 15 min to eliminate non-specific staining. The cells were observed under a Leica DMRX microscope (Leica Microsystems Gmbh, Wetzlar, Germany) equipped with appropriate fluorescence filters. Digital images were acquired using a Leica 300F camera (Leica Camera AG, Solms, Germany).

3. RESULTS

3.1. Cell Counting with Trypan Blue Staining

As a diazo dye, Trypan blue is only permeable to cells with compromised membranes. Dead cells are stained blue while live cells remain colorless. Because cytotoxic agents will compromise the cell membrane integrity, cell counting with Trypan blue staining is routinely applied to determine the number of viable/dead cells, hence, the cytotoxicity of the introduced agents.[33]

In our study, seven different types of QDs were added to the cell culture at two different concentrations, 1 μM and 10 μM. After 3 days of incubation, cells floating in the supernatant and cells adhering to the flask surface were collected separately, stained with Trypan blue, and counted in a hemacytometer. It is known that live cells would attach to the flask surface and dead cells would float in the supernatant. After Trypan blue staining, the observation under a microscope confirmed that, more than 92% of the attaching cells appeared colorless, which were live cells. Meanwhile, all the floating cells appeared dark blue, which were dead cells. Note that the detaching process may damage some cells even though they were alive before being removed from the flask surface. This may explain why about 8% of the attaching cells were stained dark by the Trypan blue. As a result, we used the total number of attaching cells as live cells number, no matter whether they looked blue or colorless. And the number of floating cells was considered as dead cells number.

The total cell number is a sum of the live cell number and the dead cell number. Figure 1 shows the cell counting results of total cell number obtained in three independent experiments with statistical analysis. As can be seen in Figure 1(a), after incubation for 3 days, the samples with all four types of ZnS QDs at both 1 μM and 10 μM indicated similar total cell numbers to that of the control, which were more than 4 times of the initial cell number. On the other hand, in Figure 1(b) the samples with three types of CdS QDs at 1 μM had the total cell number more than 3 times of the initial cell number, which was statistically similar to their control. But the samples with CdS QDs at 10 μM showed significantly decreased total cell number, especially with the MPA-capped and the MPS-replaced CdS QDs. Clearly, the high concentration of CdS QDs impeded the increase of the total cell number during incubation, which may result from a lower proliferation rate and/or a higher death rate.

In Figure 2, we plot the fractions of live and dead cells with all types of ZnS and CdS QDs at both 1 μM and 10 μM. The fraction of live/dead cell is defined as the number of the live/dead cells divided by the total cell number. Apparently, the sum of the live cell fraction and the dead cell fraction is unit. As shown in Figure 2(a), the samples with ZnS QDs exhibited essentially the same fractions of live/dead cells as those of the control. This indicated that the ZnS QDs did not affect the cells proliferation, i.e., were nontoxic to the cells, and that the nontoxicity was independent of the surface conditions and the two concentrations used. By contrast, as seen in Figure 2(b), the samples with all three types of CdS QDs at 1 μM exhibited no discernable difference in their fractions of live/dead cells from those of the control, indicating that at this concentration, the CdS QDs could be considered nontoxic to the cells. However, with the CdS QDs at 10 μM, the fractions of live cells became significantly smaller than

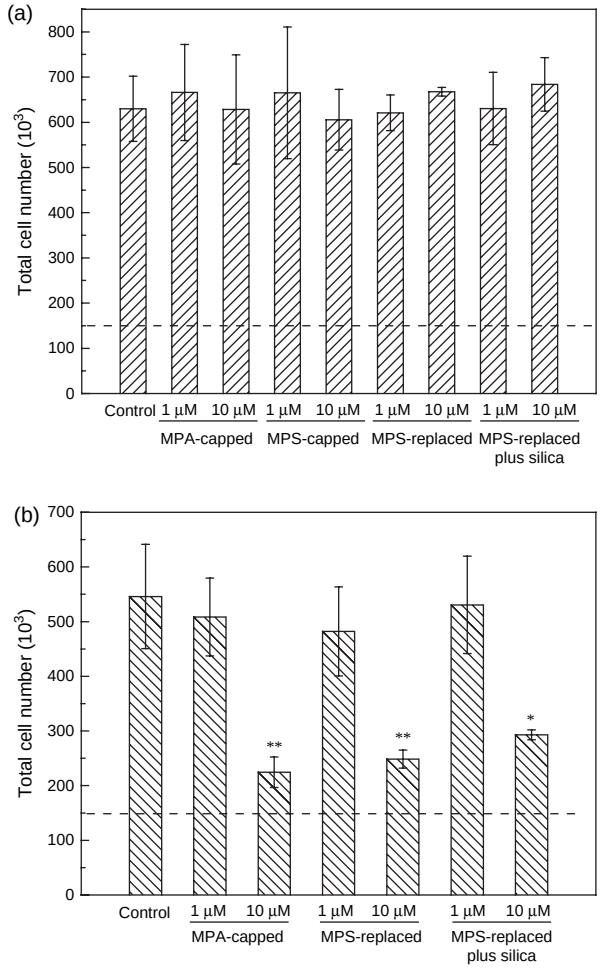

Fig. 1. Total cell number after incubation for 3 days with (a) ZnS QDs and (b) CdS QDs with different concentrations and surface conditions. *indicates $P < 0.05$ and **indicates $P < 0.01$, values are significantly different from the control. The dashed line indicates the initial cell number was 150,000 before incubation.

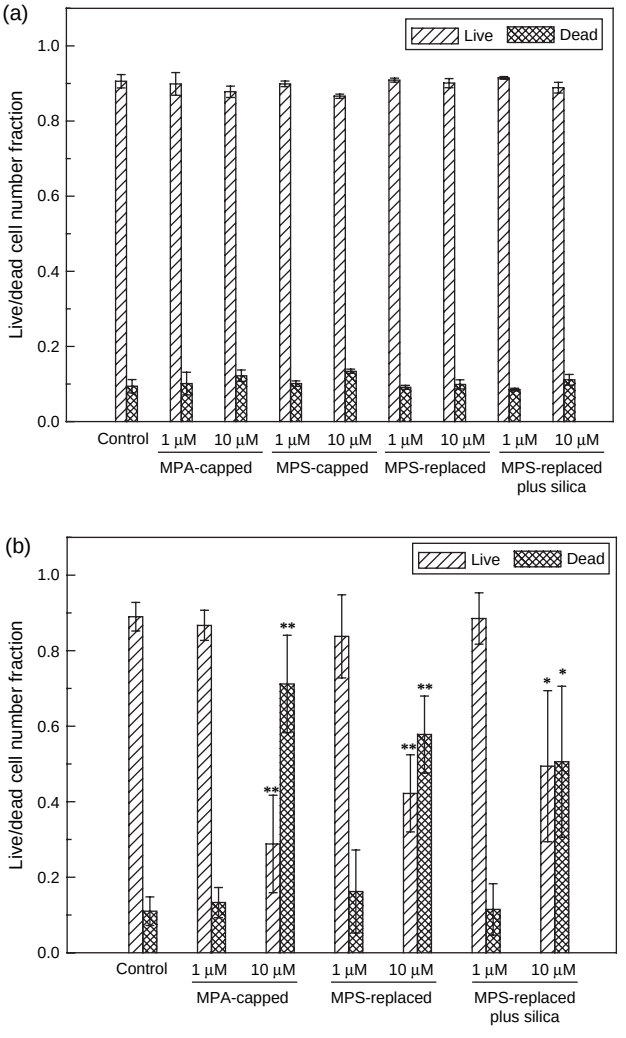

Fig. 2. Fractions of live and dead cells for all samples incubated for 3 days with (a) ZnS QDs and (b) CdS QDs with various surface conditions at 1 μM and 10 μM. *indicates $P < 0.05$ and **indicates $P < 0.01$, values are significantly different from the control.

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that of the control, and accordingly the fractions of dead cells were much larger. In other words, the CdS QDs at this high concentration killed many more cells during incubation and were evidently toxic to the cells. Among the CdS QDs with three different surface conditions, the CdS QDs with MPS-replaced plus silica coating showed relatively lower cytotoxicity than the MPA-capped and the MPS-replaced ones.

To confirm the above cell counting results, cells were examined by fluorescence microscopy after incubated with QDs for 3 days. Since the dead cells were not bound to the plate surface and were removed with the supernatant, only the live cells were fixed, stained for DNA and F-actin, and observed under microscope. As examples, Figure 3 shows micrographs of the control (a), the cells incubated with MPA-capped ZnS QDs at 1 μM (b) and 10 μM (c), the cells with MPA-capped CdS QDs at 1 μM (d) and 10 μM (e), respectively. As can be seen, the cells incubated with the ZnS QDs at both 1 μM and 10 μM looked spread-out, covered the surface densely, and reached confluence, similar to the control. In contrast, the cells with CdS QDs looked balled-up and smaller, and did not cover the surface as dense. This was particularly evident in the sample with CdS QDs at 10 μM where the surface was barely covered and the cells looked quite round. Therefore, the CdS QDs affected not only the cell density but also the cell morphology, further substantiating the cytotoxicity of CdS QDs at these concentrations. Clearly, all

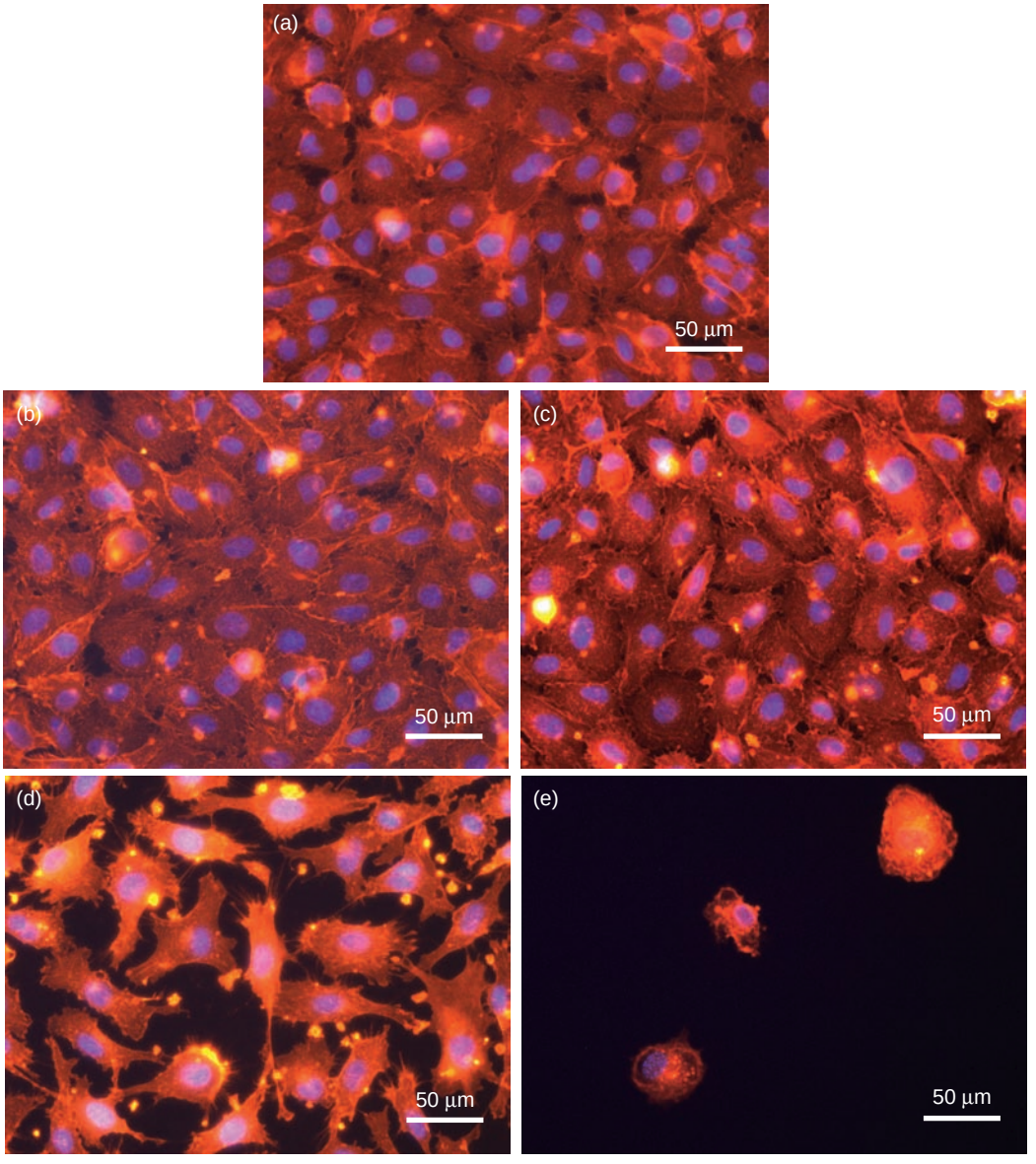

Fig. 3. Optical micrographs of (a) the control, (b) cells with MPA-capped ZnS QDs at 1 μM, (c) cells with MPA-capped ZnS QDs at 10 μM, (d) cells with MPA-capped CdS QDs at 1 μM, and (e) cells with MPA-capped CdS QDs at 10 μM, after incubated for 3 days. In the fluorescence images of endothelial cells, the red was microfilaments stained by the rhodamine-phalloidin, and the blue was nucleus stained by the Hoechst 33258.

the micrographs are qualitatively consistent with the data shown in Figures 1 and 2.

3.2. Alamar Blue Fluorescence Assay

Because the non-fluorescent Alamar Blue dye can be reduced to a pink fluorescent dye by cell metabolic activity, mainly by acting as an electron acceptor for enzymes such as NADP and FADH during oxygen consumption,[34] the cell metabolic activity can be quantified by measuring the fluorescence intensity of the reduced dye. Therefore, Alamar Blue fluorescence assay can also be used to continually measure the effect of QDs on the proliferation and metabolism of cells.

In our study, EA hy926 cells were incubated with QDs at either 1 μM or 10 μM for up to 6 days, during which cells were treated every other day with AB-containing medium for fluorescence measurements. The fluorescence intensity versus time of cells incubated with the various types of ZnS QDs and CdS QDs is plotted, respectively, in Figures 4(a) and (b). For the cells with all types of ZnS QDs at both 1 μM and 10 μM, as well as the cells with the three types of CdS QDs at 1 μM, the measured fluorescence intensity increased over time for 6 days and was statistically indistinguishable from that of the control. In contrast, the cells with all three types of CdS QDs at 10 μM showed no significant difference from the control on day 2, but started to show reduced fluorescence intensities at day 4 and day 6, indicating reduced cell viability over time with CdS QDs at this concentration. Figures 4(a and b) provide a temporal evolution of the AB intensity of all samples with ZnS QDs and with CdS QDs, allowing one to see how the AB intensities changed over time. This time dependence may be related to the mechanism of cytotoxicity, whether it is due to the dissolution of elements or other factors.

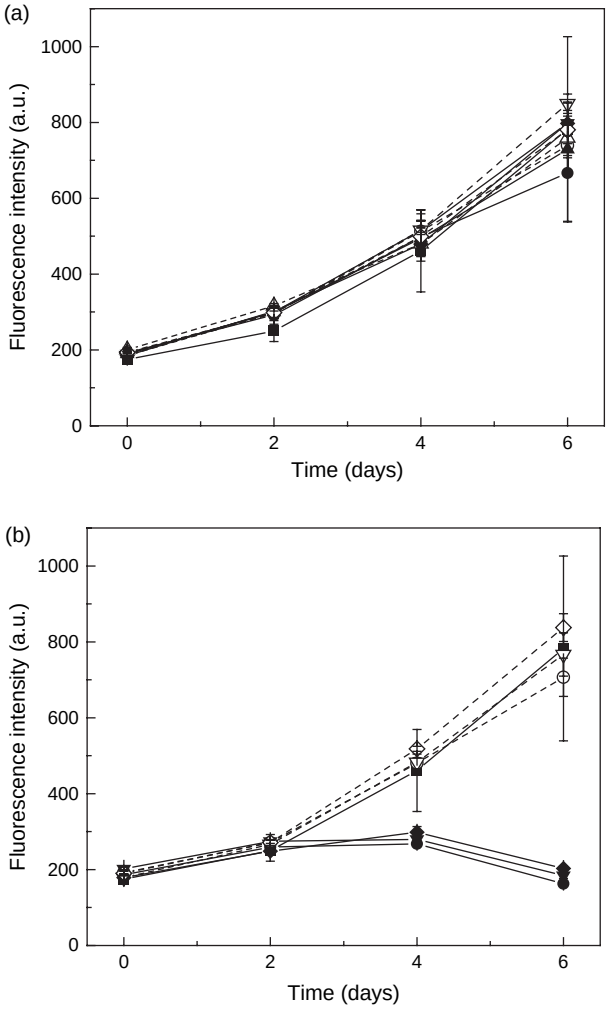

Fig. 4. Fluorescence intensity versus time of cells incubated with (a) ZnS QDs and (b) CdS QDs with various concentrations and surface conditions: —■— control, --○-- 1 μM MPA-capped, —●— 10 μM MPA-capped, --△-- 1 μM MPS-capped, —▲— 10 μM MPS-capped, --▽-- 1 μM MPS-replaced, —▼— 10 μM MPS-replaced, --◇-- 1 μM MPS-replaced plus silica coating, and —◆— 10 μM MPS-replaced plus silica coating.

In order to make the results shown in Figure 4 more explicit, we converted the results of Alamar Blue fluorescence intensity to an 'estimated cell number.' The estimated cell number is not the actual number of cells but the number of living cells that are metabolically active, which is proportional to the AB fluorescence intensity. To do this, we generated a calibration curve as shown in Figure 5, by measuring the AB intensity of known number of live cells and fitting the experimental data with a polynomial curve. The measured AB fluorescence intensities of cell samples incubated with QDs for various amounts of time, as shown in Figure 4, were then converted to the estimated cell numbers according to this calibration curve.

We defined the ratio of the estimated cell number after 6 days of incubation to the initial cell number as an 'estimated proliferation factor' (F), which provides a simple

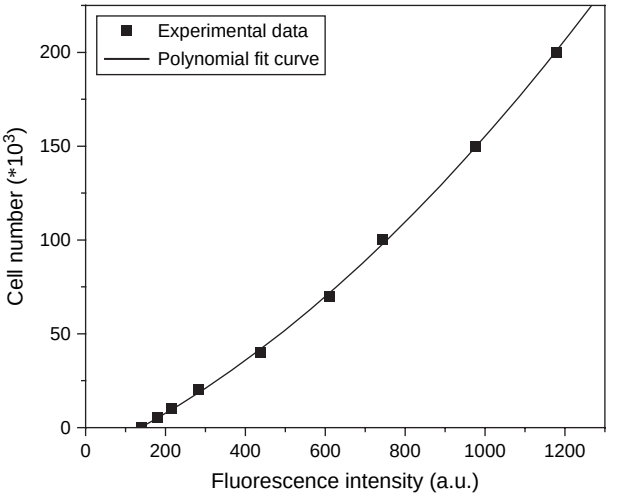

Fig. 5. Calibration curve of known cell numbers versus the measured AB fluorescence intensity. The solid curve is a polynomial fit of the experimental data (solid square dots).

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quantitative assessment of how many folds the number of live and active cells increased after 6 days of incubation. The estimated proliferation factor F is a dimensionless quantity. We plot the estimated proliferation factors of samples incubated with all types of ZnS and CdS QDs in Figures 6(a) and (b), respectively. As can be seen, cells with four types of ZnS QDs at 1 μM and 10 μM and cells with three types of CdS QDs at 1 μM showed the comparable proliferation factors with that of the control. In contrast, cells with CdS QDs at 10 μM showed significantly reduced proliferation factors. It is worth noting that, among the samples with CdS QDs at 10 μM, the sample with the MPS-replaced plus silica capping CdS QDs showed relatively higher proliferation factor than the samples with the MPA-capped and the MPS-replaced CdS QDs. These results are consistent with the previous cell counting data.

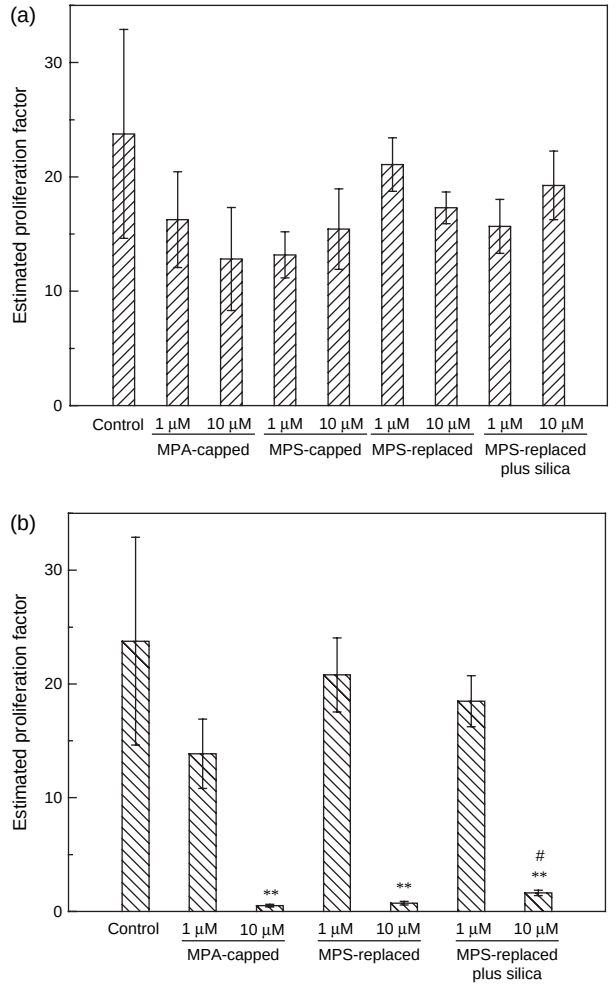

Fig. 6. Estimated proliferation factor of samples incubated with (a) ZnS QDs and (b) CdS QDs at 1 μM and 10 μM with various surface conditions. **indicates $P < 0.01$, values are significantly different from the control. # indicates $P < 0.05$, value of the sample with MPS-replaced plus silica coating CdS QDs at 10 μM is different from those of the samples with MPA-capped and with MPS-replaced CdS QDs at 10 μM.

4. DISCUSSION

Prior studies suggested that the cytotoxicity of QDs was dependent on specific surface modification.[35,36] Some hydrophilic surface coatings contribute to the cytotoxicity of QDs, such as MPA, polyethylenimine (PEI), etc. In addition, cytotoxicity was more pronounced with the smaller QDs than with the larger QDs at the same concentration.[37] Cytotoxicity of QDs was also found to be dose dependent.[38]

In our study, all the QDs tested had a similar particle size of about 5 nm with nearly spherical shape, as characterized by transmission electron microscopy and dynamic light scattering.[24–26,28,30] Also, we measured the zeta potential of the aqueous QDs which showed that QDs of all surface modification conditions examined in this study carried negative charges on the surface. The zeta potential of the ZnS and CdS QDs with various surface conditions differed very slightly. Therefore we did not think the size, shape, and surface charge were major factors to cause the difference in the cytotoxicity effect of the QDs.

With the same surface condition and concentration, the cytotoxicity of ZnS and CdS QDs were compared. The results suggest that the apparent lack of toxicity of ZnS QDs is probably due to the fact that they are devoid of toxic elements. In contrast, the toxicity of CdS QDs increased with increasing particle concentration and/or a less protective capping layer such as MPA, suggesting that the toxicity of the CdS QDs is likely due to the leaching of Cd from the QDs. In both the cell counting assay and the Alamar Blue fluorescence assay, the CdS QDs with MPS-replaced plus silica coating indicated relatively lower cytotoxicity than the MPA-capped and the MPS-replaced CdS QDs. This finding suggests that the CdS QDs were better covered by the MPS-replaced plus silica coating, probably due to the formation of the inorganic silica cell. Both the silica and MPS would have silanol groups through hydrolysis and can cross link by condensation reaction to form a siloxane network. This could act as an inorganic silica shell covering the QD surface, thereby protecting the QDs from exposing to the external environment and reducing the dissolution and release of the elements from QDs to the solution. On the other hand, the MPA capping was not stable, which could be attributed to the fact that MPA may easily detach from the surface due to the disulfide bond formation,[39] causing the toxic element Cd to be exposed and released.

In this preliminary study we did not measure the Cd release directly. To examine in depth the mechanism of cytotoxicity, in future work, we will study cells response to QDs as well as to a 'Cd control'—a solution with known Cd concentration—to further investigate the effect of QDs instability on their cytotoxicity. In addition, study on the dissolution rate of Cd ions from QDs will be carried out with measurement of Cd concentration in QD suspension

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as a function of time. The uptake of QDs is another possible reason that CdS QDs exhibited toxicity to the cells. The fluorescence of QDs from inside cells may be investigated as a quantitative indication of the uptake of QDs in cells. The effect of QD uptake may also be examined with the methoxyphenyl-tetrazolium salt (MTS) assay that quantifies mitochondrial activities of cells.

While Zn is an 'essential' trace metal and often found in active center of enzymes, Cd is detrimental even in trace amounts. Our cytotoxicity results of aqueous CdS QDs are consistent with the results reported by Lovric[40] and Derfus[20] on CdTe and CdSe QDs. Although a variety of syntheses, storage conditions, and coating strategies have been proposed to alleviate the cytotoxicity of Cd-core QDs, there is always a concentration threshold above which cytotoxicity occurs, such as a few μM found in Kirchner's study.[21] According to our results, the threshold concentration for CdS QDs may be a value between 1 μM and 10 μM, which can be determined with a more detailed dose response experiment. In contrast, the present study showed that the ZnS QDs were nontoxic at 10 μM regardless of the surface conditions, encouraging for their potential *in vivo* imaging applications. More experiments across a larger range of concentration (i.e., 1 μM to 100 μM) will be carried out in the future to determine the threshold of ZnS QDs concentration for safe *in vivo* applications.

In vitro cytotoxicity studies of QDs using different cell lines and colorimetric assays are increasingly being reported[41] with a wide range of QD concentrations, exposure times, and surfaces. Standardization in experimental set up, such as choice of model (cell line, animal species), exposure conditions (cell confluence, exposure duration, QD concentration ranges and dosing increments), as well as biochemical and bioassays (*in vivo* and *in vitro*), is necessary in order for comparisons between studies conducted by different groups to be useful. In our study, we used the EA hy926 endothelial cell line to demonstrate the different cytotoxicity of ZnS and CdS QDs. Although primary cell lines are more relevant for future *in vivo* applications, our focus here is the different effects of ZnS and CdS QDs on cells due to their different chemical constituents, capping molecules and concentrations. In the future, we will use primary human endothelial cell lines such as HUVEC and human macrophage cell lines such as J774A.1 to provide a more realistic prediction for the *in vivo* application of the quantum dots. Furthermore, other potentially exposed cell types, such as macrophages which are likely to accumulate around injected particles, will be applied for further cytotoxicity tests and the sub-lethal effects of the QDs on cell function will be examined.

5. CONCLUSIONS

The cytotoxicity of ZnS and CdS QDs synthesized via all-aqueous process with various surface conditions was investigated with cultured human endothelial cells using two independent cell viability tests. Both the cell counting results and the Alamar Blue fluorescence assay consistently showed that the ZnS QDs with all four types of surface conditions were nontoxic at both 1 μM and 10 μM concentrations for at least 6 days. In contrast, the CdS QDs were nontoxic only at 1 μM, but showed significant cytotoxicity at 10 μM after 3 days in the cell counting assay and after 4 days in the AB fluorescence assay. The CdS QDs with MPS-replaced plus silica capping exhibited less cytotoxicity than those with MPA-capping and MPS-replaced capping. Compared with the same surface condition, concentration and particle size, our results suggested that the cytotoxicity of CdS QDs and the lack of it for ZnS QDs were likely due to presence and absence of a specific toxic element, respectively. The nontoxicity of the aqueous ZnS QDs offers great potential for their biomedical applications including *in vivo* imaging. More work will be carried out to further study the mechanism of cytotoxicity of QDs, and to examine the toxicity and sub-lethal effect of ZnS QDs to other cell types.

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