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BRIEF COMMUNICATION

Labeling efficiency and toxicity evaluation of CdSe/ZnS quantum dots on *Escherichia coli*

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Abstract In comparison with conventional organic dyes, quantum dots (QDs) have unique optical and electronic properties, which provide QDs with a wide scope of prospective application in biology and biomedicine. However, the toxicity of QDs and the fluorescence intensity of labeled bacteria must precede their application in bacterial imaging and tracing in vivo. Here, we show that treatment with $CaCl_2$ significantly improved bacterial labeling efficiency of CdSe/ZnS QDs with the CdSe core size of ~3.1 nm (relative fluorescence unit (RFU) value and ratio of fluorescent *E. coli*) with rising CdSe/ZnS QDs concentration in a concentration-dependent manner. At 12.5 nmol/L CdSe/ZnS QDs concentration, labeled *Escherichia coli* (*E. coli*) DH5α appeared as short rod-shaped and luminescent with normal size, and the survival rate and ultrastructure did not change in comparison to the control. But the ratio of fluorescent bacteria and RFU were very low. However, the survival rate of transformed *E. coli* was significantly inhibited by high CdSe/ZnS QDs concentrations (≥25 nmol/L). Moreover, internalization of CdSe/ZnS QDs resulted in ultrastructure damage of transformed *E. coli* in a concentration-dependent manner (≥25 nmol/L). Therefore, CdSe/ZnS QDs may not suitable for tracing of bacteria in vivo. Moreover, our study also revealed that colony-forming capability assay and transmission electron microscopy could be used to comprehensively evaluate the toxicity of QDs on labeled bacteria. Our findings do provide a new direction toward the improvement and modification of QDs for use in imaging and tracing studies in vivo.

Keywords Quantum dots · CdSe/ZnS · Labeling efficiency · Toxicity · *Escherichia coli*

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Introduction

Quantum dots (QDs) are a novel class of fluorescent semi-conducting nanocrystals made up of groups II–VI or III–V elements in the Periodic Table, which have

a diameter range of 2–10 nm. In comparison with conventional organic dyes, QDs have unique optical and electronic properties, such as broad excitation spectra and narrow emission spectra controlled by varying the size and composition (Jamieson et al. 2007). In addition, their photostability is 1,000-fold better than conventional organic dyes. Owing to their high molar extinction coefficient, QDs possess very high levels of brightness (Gao et al. 2005). These properties, coupled with further advances in synthesis and optimization, provide QDs with a wide scope of prospective application in biology and biomedicine (Chan et al. 2002; Alivisatos et al. 2005; Lei et al. 2011).

With the remarkable advances in nanotechnology in recent years, the effect of this new material and technology on biosafety cannot be overemphasized. Majority of scholars believe that QDs for use in animals or humans should have optimum biocompatibility. It is believed that the toxicity of cadmium-based QDs is associated with the release of Cd^{2+} (Mahendra et al. 2008; Chen et al. 2012), but this leakage (of Cd^{2+}) can be prevented to a large extent by encapsulating the particles with an appropriate shell such as ZnS (Derfus et al. 2004; Kirchner et al. 2005).

As the largest group of microorganisms, the distribution of bacteria is ubiquitous. A study by Nogueira et al. (2012) showed that CdSe/ZnS QDs were less toxic to the structure of soil microbiota. Also, the toxicity of MAA-CdSe/ZnS QDs on the growth of both *Escherichia coli* and *S. aureus* was much less than that of MAA-CdSe QDs (Xiao et al. 2013). Hence, application of CdSe/ZnS QDs in bacteriological research has attracted a lot of attention recently (Fu et al. 2009; Carrillo-Carrión et al. 2011; Sanvicens et al. 2011).

There are two methods of labeling bacteria with QDs. In the first method, QDs can be covalently coupled to the bacteria surface through a coupling reagent or antibody (Fu et al. 2009; Sanvicens et al. 2011), while in the other QDs are internalized by bacteria (Hirschey et al. 2006). Due to the complexity of the in vivo environment, QDs coupled to the bacteria surface are generally unstable and likely fall off or degrade gradually. Bacterial labeling by internalization of QDs is more suitable for labeling and further tracing bacteria in vivo. The toxicity of QDs and the fluorescence intensity of labeled bacteria must precede their application in bacterial tracing in vivo. However, the ratio of fluorescent bacteria and fluorescence intensity of labeled bacteria are rarely mentioned if ever (Hirschey et al. 2006; Dumas et al. 2009; Schneider et al. 2009). Consequently, this area still needs to be more thoroughly and comprehensively investigated.

In this study, *E. coli* DH5α was treated with $CaCl_2$ to induce competence. $CaCl_2$-treated and $CaCl_2$-untreated *E. coli* DH5α groups were incubated with different concentrations of CdSe/ZnS QDs. Both groups were also incubated without CdSe/ZnS QDs followed by measurement of the fluorescence intensity and determination of the ratio of fluorescent *E. coli* DH5α. The survival rate of the *E. coli* DH5α was evaluated by colony-forming capability assay and ultrastructural change of the *E. coli* DH5α was observed under transmission electron microscope (TEM). Our study sought to highlight the optimum CdSe/ZnS QDs concentration with maximum fluorescent intensity and least QDs toxicity of the labeled bacteria in order to provide potential experimental basis for in vivo bacterial imaging and tracing for future research.

Materials and methods

Quantum dots

3-Mercaptopropionic acid (MPA)-functionalized water-soluble core/shell CdSe/ZnS QDs were purchased from Beijing Zhong Ke Wu Yuan Biotechnology Co., Ltd. The size of the CdSe core is ~3.1 nm, and the emission band of the QDs locates at ~570 nm.

Bacterial cultures

Escherichia coli DH5α was provided by the Laboratory of Medical Microbiology of Harbin Medical University. *Escherichia coli* DH5α was inoculated into Luria–Bertani (LB) broth and incubated at 37 °C with shaking overnight. The cultures were then seeded in fresh LB broth and incubated at 37 °C with shaking to achieve 0.3–0.4 turbidity at OD_{600}.

Incubation of bacteria with CdSe/ZnS QDs

CdSe/ZnS QDs were added to bacterial cultures to final concentrations of up to 12.5, 25, 50, 100, and 200 nmol/L in that order. The mixture was incubated for 45 min at

37 °C with shaking. The control *E. coli* DH5α group was treated similarly but without the addition and incubation with CdSe/ZnS QDs.

Competence preparation and transformation

Aliquots of bacterial cultures were placed into ice-cold centrifuge tube and centrifuged at 4,000 rpm for 10 min at 4 °C. The supernatant was discarded and the cell pellet was resuspended in 500 μL of ice-cold 0.1 mol/L $CaCl_2$ and incubated on ice for 30 min. The culture was centrifuged at 4,000 rpm for 10 min at 4 °C and the supernatant discarded again. The cell pellet was resuspended in 100 μL of ice-cold 0.1 mol/L $CaCl_2$. CdSe/ZnS QDs were added to the suspension until the final CdSe/ZnS QDs concentrations of up to 12.5, 25, 50, 100, and 200 nmol/L. The mixture was then incubated on ice for 30 min. Heat shock was performed by placing the tubes in a water bath at 42 °C for 90 s and then immediately returning them on the ice for 2 min. 800 μL of LB broth was added into the tubes followed by incubation at 37 °C for 45 min with shaking. The control *E. coli* DH5α group was treated in a similar manner but without the addition and incubation with CdSe/ZnS QDs.

Fluorescence microscopy

The above final untransformed and transformed bacterial culture mixtures were centrifuged at 4,000 rpm for 10 min at 4 °C and washed three times with 0.9 % NaCl. Under the DSY 5000X inverted fluorescence microscope, the bacterial samples were observed and imaged. The *E. coli* DH5α cells were counted in four different and randomly selected horizons under 100× objective first using bright field followed by fluorescence field. The ratio of fluorescent *E. coli* DH5α = (number of fluorescent *E. coli* DH5α under fluorescence field/number of *E. coli* DH5α under bright field) × 100 %.

Determination of relative fluorescence unit

Relative fluorescence unit (RFU) values of final untransformed and transformed bacterial culture mixtures were measured by NanoDrop 3300 fluorospectrometer. RFU values of $CaCl_2$-treated and $CaCl_2$-untreated *E. coli* DH5α but without incubation with CdSe/ZnS QDs were used to zero-calibrate the NanoDrop 3300 fluorospectrometer.

Determination of bacteria survival rate

The final transformed and untransformed bacterial culture mixtures were diluted to 1×10^4 CFU/mL, and 100 μL of this suspension was spread onto LB agar plates. The colonies were counted after an overnight incubation at 37 °C. $CaCl_2$-treated and $CaCl_2$-untreated *E. coli* without CdSe/ZnS QDs incubation was used as control. Survival (%) = (colony numbers of *E. coli* DH5α incubated with CdSe/ZnS QDs/colony numbers of control *E. coli* DH5α) × 100 %. The survival rate was used to evaluate the toxicity of CdSe/ZnS QDs.

Transmission electron microscopy

The final transformed bacterial culture mixtures were centrifuged and the supernatants were discarded. The bacteria pellet was fixed in 3 % glutaraldehyde in 0.1 mol/L PB (pH 7.4) at 4 °C. After washing with 0.1 mol/L PB (pH 7.4), the pellet was post-fixed in 1 % osmium tetroxide solution in 0.1 mol/L PB for 1 h. After washing with distilled water, the pellet was dehydrated first through a series of graded alcohols then with propylene oxide and was finally embedded by Epon812. Ultrathin sections were double-stained with uranyl acetate and lead citrate and observed under GEM-1220 TEM. Control group was $CaCl_2$-treated *E. coli* DH5α but without incubation with CdSe/ZnS QDs.

Statistical analysis

Data were expressed as mean ± SD ($\bar{x} \pm s$) and were statistically analyzed by IBM SPSS 19.0 software. Differences between different groups were tested for significance using Student's *t* test. A P value ≤ 0.05 was considered statistically significant. All experiments were repeated independently at least three times.

Results

Treatment with $CaCl_2$ significantly improved bacterial labeling efficiency of CdSe/ZnS QDs

Under fluorescence microscope, $CaCl_2$-untreated *E. coli* DH5α cells that were incubated with CdSe/ZnS QDs at concentrations <50 nmol/L and control

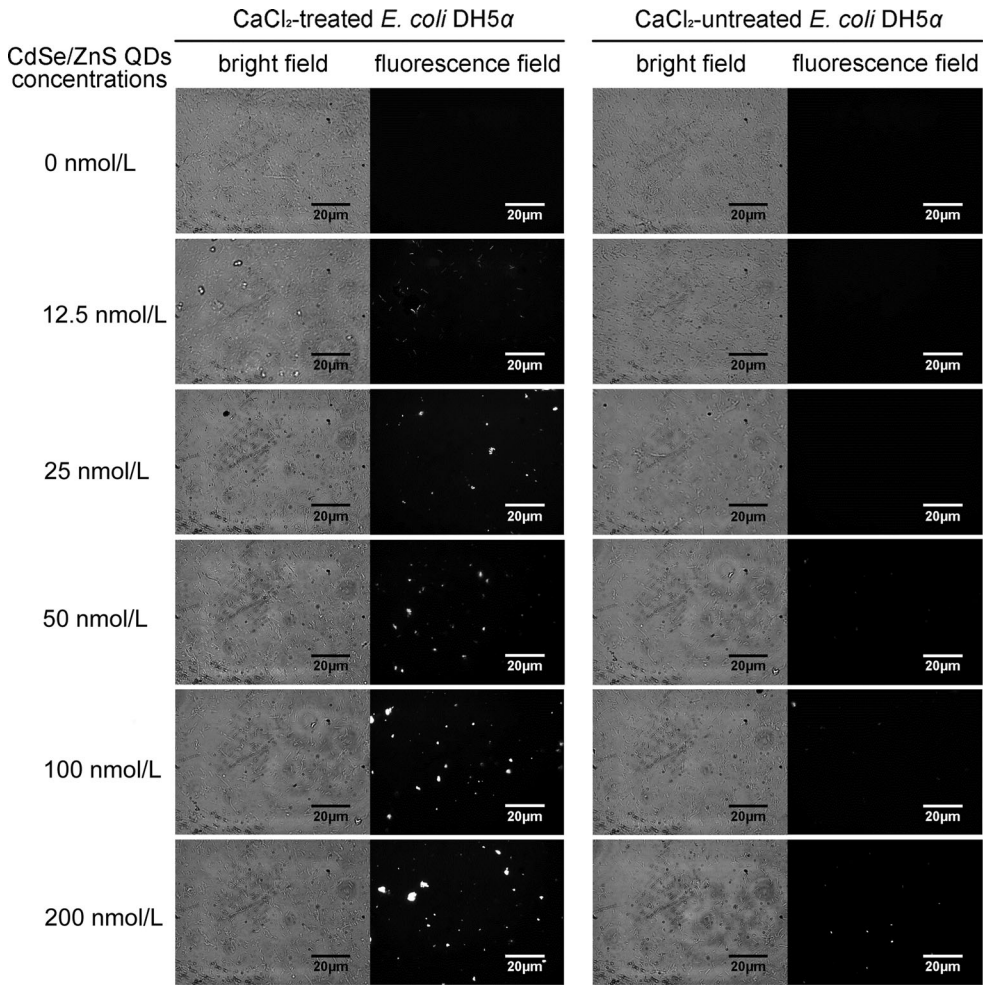

Fig. 1 Fluorescence and bright-field microscopy results of $CaCl_2$-treated and $CaCl_2$-untreated *E. coli* DH5α incubated with CdSe/ZnS QDs at different concentrations. Under the fluorescence microscopy, only a few luminescent dots were observed in the $CaCl_2$-untreated *E. coli* DH5α group incubated with CdSe/ZnS QDs at the concentrations ≥50 nmol/L, whereas numerous fluorescent *E. coli* DH5α were observed in the $CaCl_2$-treated *E. coli* DH5α group incubated with CdSe/ZnS QDs at different concentrations: short rod-shaped with normal size (12.5 nmol/L) and point-like or sheet-like (>12.5 nmol/L)

(incubated with no CdSe/ZnS QDs) did not show any fluorescence besides zero RFU values. Only a few luminescent dots were seen at ≥50 nmol/L CdSe/ZnS QDs concentrations (Fig. 1) and RFU values were all below 0.6 (Fig. 2a) with the ratios of fluorescent *E. coli* DH5α increasing to just below 6 % (Fig. 2b). However, numerous fluorescent *E. coli* DH5α were observed in all the $CaCl_2$-treated *E. coli* DH5α incubated with CdSe/ZnS QDs at the different concentrations (Fig. 1). At 12.5 nmol/L CdSe/ZnS QDs concentration, the fluorescent *E. coli* DH5α were short rod-shaped with normal size. As the CdSe/ZnS QDs concentration increased, the fluorescent cells were point-like or sheet-like exhibiting abnormal shapes and becoming stubby and aggregated in the corresponding bright field. At the same time, both RFU value and ratio of fluorescent *E. coli* DH5α gradually increased with rising CdSe/ZnS QDs concentration in a concentration-dependent manner ($P < 0.01$, $P < 0.05$, respectively) (Fig. 2a, b). At 12.5 nmol/L concentration of CdSe/ZnS QDs, RFU value and ratio of fluorescent *E. coli* DH5α were 0.6 and 24 %, respectively, while at 200 nmol/L concentration they were 12 and 40 %, respectively, and significantly higher than those of $CaCl_2$-untreated *E. coli* DH5α incubated with the same CdSe/ZnS QDs concentration ($P < 0.01$).

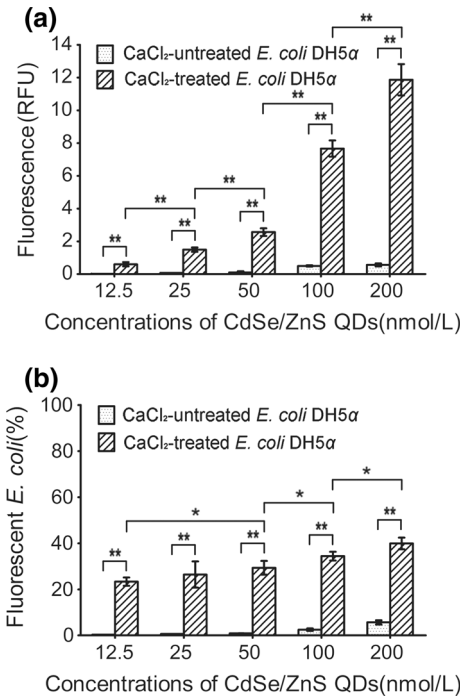

Fig. 2 **a** At higher CdSe/ZnS QDs concentrations, RFU values of the $CaCl_2$-treated *E. coli* DH5α group increased gradually in a concentration-dependent manner and were significantly higher than that of $CaCl_2$-untreated *E. coli* DH5α group incubated with the same concentration of CdSe/ZnS QDs. **b** The ratio of fluorescent *E. coli* DH5α of the $CaCl_2$-treated *E. coli* DH5α group also increased gradually in a concentration-dependent manner and was significantly higher than that of $CaCl_2$-untreated *E. coli* DH5α incubated with the same concentration of CdSe/ZnS QDs (*$P < 0.05$, **$P < 0.01$)

The survival rate of transformed *E. coli* DH5α was significantly inhibited by high CdSe/ZnS QDs concentration

With increase in the concentration of CdSe/ZnS QDs, both the number of colonies of transformed *E. coli* DH5α and the survival rate decreased gradually (Fig. 3a, b). At 12.5, 25, and 200 nmol/L CdSe/ZnS QDs concentrations, the survival rates of $CaCl_2$-treated *E. coli* DH5α were 86.26 % ($P > 0.05$), 60 % ($P < 0.05$), and 25 % ($P < 0.01$), respectively, indicating enhanced toxicity at concentrations ≥25 nmol/L. However, the survival rate of $CaCl_2$-untreated *E. coli* DH5α incubated with similar CdSe/ZnS QDs concentrations did not change significantly ($P > 0.05$) (Fig. 3c).

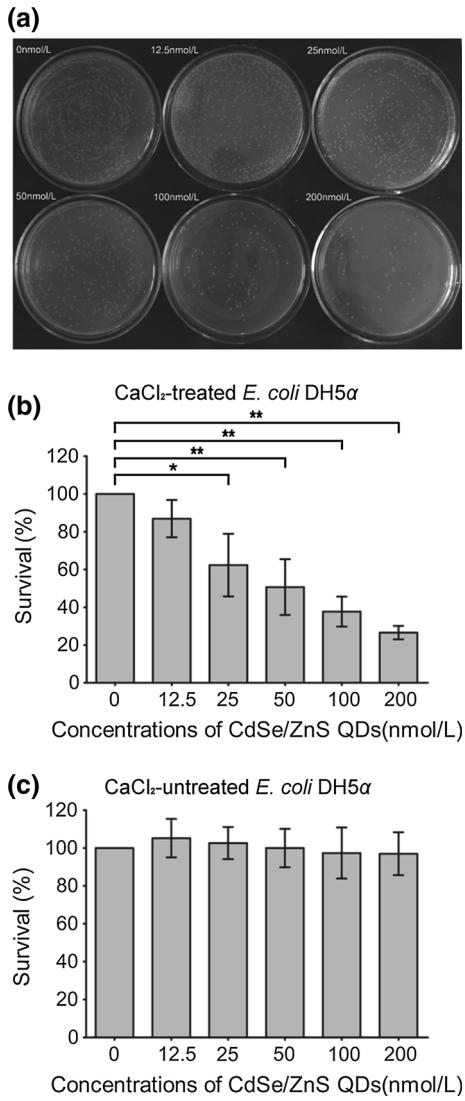

Fig. 3 The survival rate of $CaCl_2$-treated and $CaCl_2$-untreated *E. coli* DH5α incubated with CdSe/ZnS QDs at different concentrations. **a** The number of colonies of $CaCl_2$-treated *E. coli* DH5α decreased with the increase in CdSe/ZnS QDs concentration. **b** The survival rate of $CaCl_2$-treated *E. coli* DH5α gradually reduced with the increase in CdSe/ZnS QDs concentration. **c** The survival rate of $CaCl_2$-untreated *E. coli* DH5α did not change significantly (*$P < 0.05$, **$P < 0.01$)

Internalization of CdSe/ZnS QDs resulted in ultrastructure damage of $CaCl_2$-treated *E. coli* DH5α in a concentration-dependent manner

The TEM image of $CaCl_2$-untreated *E. coli* DH5α incubated without CdSe/ZnS QDs showed normal bacterial ultrastructure as follows: The *E. coli* DH5α

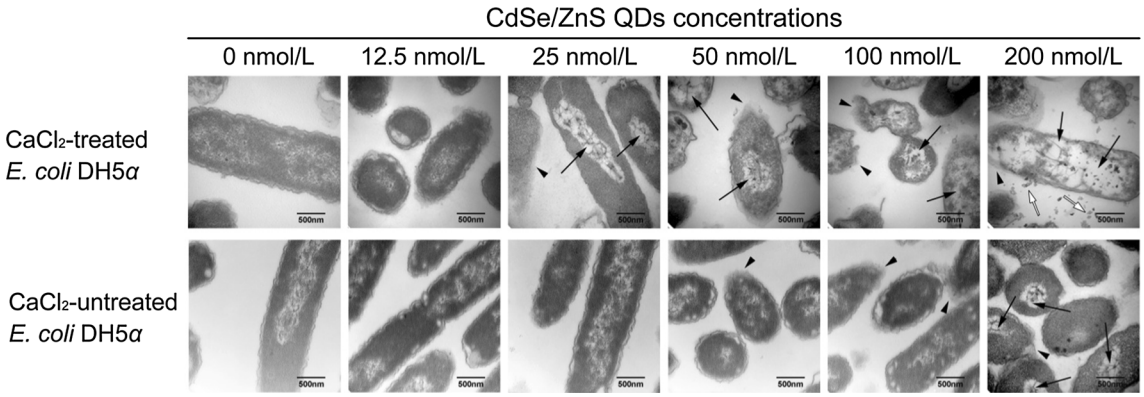

Fig. 4 Change in the ultrastructure of $CaCl_2$-treated and $CaCl_2$-untreated *E. coli* DH5α. The cell wall of $CaCl_2$-untreated *E. coli* DH5α incubated without CdSe/ZnS QDs was smooth, intact, and clear. Periplasmic space was also clear. The electron density of cytoplasm was high and uniform, while the electron density of nucleoid was relatively low. At ≥50 nmol/L CdSe/ZnS QDs concentrations, some slight and occasional damages could be observed. Ultrastructure of $CaCl_2$-treated *E. coli* DH5α without CdSe/ZnS QDs was normal. At 12.5 nmol/L CdSe/ZnS QDs concentration, *E. coli* DH5α showed no obvious change. With increased CdSe/ZnS QDs concentrations (≥25 nmol/L), the cell wall and cell membrane became blurred and periplasmic space disappeared (*arrowhead*). The electron density of cytoplasm and nucleoid reduced (*black arrow*) gradually and the bacteria exhibited abnormal shape as some were ruptured and unable to maintain original shape. Cytoplasm and nucleoid leaked out and many fragments (*white arrow*) were observed outside the bacterium

cell wall was smooth, intact, and clear. Periplasmic space was also clear. The electron density of cytoplasm was high and uniform, while the electron density of nucleoid was relatively low. $CaCl_2$-untreated *E. coli* DH5α incubated with CdSe/ZnS QDs at concentrations <50 nmol/L showed similar ultrastructure as above. At 50 and 100 nmol/L CdSe/ZnS QDs concentration, *E. coli* DH5α were not seriously damaged except that the bacterial cell wall and cell membrane became blurred occasionally and the periplasmic space partly disappeared. At 200 nmol/L CdSe/ZnS QDs concentration, the electron density of nucleoid was reduced. Meanwhile, as a control group, the TEM images of $CaCl_2$-treated *E. coli* DH5α incubated without CdSe/ZnS QDs also showed normal shape. At 12.5 nmol/L CdSe/ZnS QDs concentration, the ultrastructure of transformed *E. coli* DH5α was similar to the control: no obvious change. However, at 25 nmol/L CdSe/ZnS QDs concentration, not only the bacterial cell wall and cell membrane became blurred and the periplasmic space partly disappeared, but the electron density of nucleoid was reduced. At ≥50 nmol/L CdSe/ZnS QDs concentration, *E. coli* DH5α exhibited abnormal shape, surface roughness and further reduction in the electron density of nucleoid and cytoplasm along with serious cavitation. Moreover, some *E. coli* DH5α were ruptured and unable to maintain the original shape. Cytoplasm and nucleoid leaked out and many fragments were observed outside the cell. Evidently, under similar CdSe/ZnS QDs concentrations (except for 0 and 12.5 nmol/L CdSe/ZnS QDs), the extent of *E. coli* DH5α damage was much more marked in the $CaCl_2$-treated than $CaCl_2$-untreated cells (Fig. 4).

Discussion

The use of QDs as fluorescent probes has recorded a lot of meaningful advances not only on eukaryotic cells, but also on bacteria. Eukaryotes acquire extracellular materials by endocytosis (Chan et al. 2002; Kitakura et al. 2011). In contrast, the bacterial cell wall allows only water and very minute water-soluble molecules (<1 nm in diameter) to freely traverse into the cytoplasm, while the commonly used QDs are 2–10 nm in diameter. Presently, internalization of QDs by bacteria can be achieved by modifying QDs (Kloepfer et al. 2003, 2005; Hirschey et al. 2006) or inducing the bacteria to become competent to acquire extracellular materials. It has been reported that treatment of *E. coli* with $CaCl_2$ at the 0.1 mol/L concentration increases the permeability of bacterial cell wall, and could induce competency (Mandel and

Higa 1970). Induced competency is a method commonly used to introduce recombinant DNA into bacteria. Induced competency method is relatively simpler to carry out than the modification of QDs. Li et al. (2004) confirmed that QDs could be internalized by bacteria through $CaCl_2$-induced transformation. In our study, only a few luminescent dots could only be observed among the $CaCl_2$-untreated *E. coli* DH5α incubated with QDs at ≥50 nmol/L concentrations. At these concentrations, the ratios of fluorescent *E. coli* DH5α were low (<6 %) and the RFU values were <0.6, suggesting that CdSe/ZnS QDs, which were nonspecifically adsorbed to the bacterial surface, fell off due to the repeated washing process. This coincides with the finding by Lai and his colleagues who have also observed that with increase in the concentration of CdSe/ZnS QDs, only a few QDs remained bound to the $CaCl_2$-untreated bacterial surface (Lai et al. 2013). Contrastingly, numerous fluorescent *E. coli* DH5α were observed among the *E. coli* DH5α transformed at the different CdSe/ZnS QDs concentrations. At 12.5 nmol/L CdSe/ZnS QDs concentration, the fluorescent *E. coli* DH5α were short rod-shaped with normal size. Under bright field, as CdSe/ZnS QDs concentration increased, *E. coli* DH5α exhibited abnormal shapes, turned to be stubby and aggregated, while in the corresponding fluorescent field they were point-like or sheet-like. We believe this was due to bacterial damage and even death. Meanwhile, RFU value gradually increased in a concentration-dependent manner and was significantly higher than $CaCl_2$-untreated *E. coli* DH5α incubated with similar CdSe/ZnS QDs concentration. We suggested the enhancement of bacterial fluorescence intensity might have resulted from the increase in CdSe/ZnS QDs internalized by *E. coli* DH5α. In addition, when the concentration of CdSe/ZnS QDs reached 50 nmol/L and above, the ratio of fluorescent *E. coli* DH5α also increased gradually in a concentration-dependent manner confirming that bacterial labeling efficiency of CdSe/ZnS QDs could be significantly improved by $CaCl_2$-treatment: the higher the concentration of CdSe/ZnS QDs, the higher the bacterial labeling efficiency.

The toxicity of QDs on labeled bacteria was rarely mentioned in previous studies (Li et al. 2004; Hirschey et al. 2006). In our experiments, we found the survival rate of $CaCl_2$-untreated *E. coli* DH5α incubated with different concentrations of CdSe/ZnS QDs did not change significantly indicating CdSe/ZnS QDs do not cause damage to bacteria within short period. Moreover, compared to control, the survival rate of *E. coli* DH5α transformed at 12.5 nmol/L CdSe/ZnS QDs concentration did not drop significantly ($P > 0.05$), indicating the toxicity of CdSe/ZnS QDs at this concentration on *E. coli* DH5α is not obvious. However, the survival rate of transformed *E. coli* DH5α decreased gradually as the concentration of CdSe/ZnS QDs increased further ($P < 0.05$), pointing to the strengthened toxic effects of CdSe/ZnS QDs. To that effect, these findings suggest that at similar concentrations, the toxicity of CdSe/ZnS QDs on *E. coli* DH5α was much stronger inside than outside.

At present, major mechanisms of toxicity of cadmium-based QDs were attributed to the direct release of Cd^{2+} (Mahendra et al. 2008; Chen et al. 2012) or the photogeneration of reactive oxygen species (ROS) (Lu et al. 2008; Dumas et al. 2009). If QDs were internalized by cells, Cd^{2+} would be released directly inside cells (Kirchner et al. 2005) and could inhibit the synthesis of cellular DNA, RNA and proteins, and damage the DNA chains (Hossain and Huq 2002). Although QDs have a layer of ZnS shell which can limit the liberation of Cd^{2+} to a large extent (Derfus et al. 2004), when QDs are inside the bacterial cell, Cd^{2+} can still be released due to effects of change of pH and temperature, inorganic ions and organic substances in the surrounding environment (Mahendra et al. 2008; Li et al. 2012). In addition, QDs can transfer energy to nearby oxygen molecules to induce the formation of singlet molecular oxygen ($^1O_2$) and hydroxyl free radical (OH•) (Lu et al. 2008; Cooper et al. 2010). ROS are very active and can react with all biological molecules that they come into contacted with (Lu et al. 2008; Slaveykova et al. 2009). ROS can destroy the structure and function of the cell wall or cell membrane, damage bacterial internal components by oxidation, and eventually lead to bacterial cell death (Lu et al. 2008; Dumas et al. 2009; Senevirathna et al. 2009). In this study, experiments using TEM revealed that the ultrastructure of the transformed *E. coli* DH5α showed significant damages at increased CdSe/ZnS QDs concentrations (≥25 nmol/L). Some *E. coli* DH5α were ruptured and unable to maintain original shape. The cytoplasm and nucleoid leaked out and many fragments were observed outside the bacterium. Moreover, under similar CdSe/ZnS QDs concentrations (except for 0

and 12.5 nmol/L CdSe/ZnS QDs), the extent of *E. coli* DH5α damage was much more marked in the $CaCl_2$-treated than $CaCl_2$-untreated cells. These damages were consistent with the data from the evaluation of the survival rate. We believe that after being $CaCl_2$-treated, *E. coli* DH5α could internalize CdSe/ZnS QDs causing the Cd^{2+} and ROS to accumulate inside the *E. coli* DH5α thereby damaging the bacterial cell.

Both fluorescence intensity and ratio of fluorescent bacteria as well as the degree of damage to the labeled bacteria must be considered prior to experimental application of the transformed bacteria in imaging and tracing in vivo since bacterial damage will almost certainly affect their survival and pathogenesis in vivo. At 12.5 nmol/L CdSe/ZnS QDs concentration, labeled bacteria appeared as short rod-shaped and luminescent with normal size and the survival rate and the ultrastructure did not change significantly in comparison to the control. But, the ratio of fluorescent bacteria and RFU were very low. Evidently, the fluorescence intensity of bacteria and safety of CdSe/ZnS QDs on bacteria did not coincide. Our data did not achieve the ideal state in which there is maximum fluorescence intensity with little or no toxicity on labeled bacteria. This calls for further research on QDs to further improve them to be more suitable for application in imaging and tracing in vivo. For instance, the composition or the shell of QDs could be improved to increase the quantum yield; or modify the surface groups of the QDs to make it more compatible with the biological environment and lower the QD shell degradation (Winnik and Maysinger 2012).

In addition, while many workers have tended to evaluate the toxicity of QDs by OD_{600} measurement, it is important to mention that OD_{600} value may not accurately reflect the number of live bacteria since dead bacteria can still influence OD_{600} value. Moreover, OD_{600} value change within the short incubation period may not be very obvious. To that effect, we employed colony-forming capability assay, to determine the survival rate (live bacteria) and subsequently evaluate the toxicity of QDs despite short incubation period. Furthermore, we intuitively examined the bacterial ultrastructure under TEM to evaluate the any damage to bacteria at the sub-cellular level. These two methods together provide a new approach to comprehensively evaluate the biological safety of QDs at the cellular and sub-cellular levels. Our findings do provide a new direction toward the improvement and modification of QDs for use in imaging and tracing studies in vivo, and may form a basis for future studies to expand the application range of QDs in biological and biomedical research.

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