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Supplementary Material

Effect of Micelle Encapsulation on Toxicity of CdSe/ZnS and Mn-doped ZnSe Quantum Dots

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1. Toxicity of Materials Used in Micelle Encapsulation

To rule out effects unrelated to quantum dots (QDs), we evaluated toxicity of the constituent materials involved in micelle encapsulation, including chloroform solvent and polyvinyl alcohol (PVA) surfactant, on HEPG2 cell viability. Solvent impurity guidance from the Food and Drug Administration suggests a chloroform concentration limit of 60 ppm in pharmaceutical products [1]. A quantitative colorimetric assay [2] indicated that concentrations below this limit were achieved at the tested QD concentrations. Briefly, chloroform was extracted from samples (or calibration standards) using 50% NaOH (200 μL) and pyridine (250 μL) added to each sample or calibration unit (300 μL). To promote extraction and phase separation, this mixture was vortexed (2000 rpm, 1 min), incubated at 90 °C in a water bath (2 min), and rapidly quenched in a cold-water bath. Phase separation was promoted by a brief centrifugation and 50 μL of the pyridine fraction was transferred for analysis. During this process, the block copolymer employed, polystyrene-poly(ethylene oxide) PS-PEO, is extracted to the pyridine along with the chloroform [3]. Samples for analysis were further diluted with 150 μL of pyridine and centrifuged (20,000 rcf, 1 min). At this point the supernatant displayed a pink to red color, which was assessed using UV-Visible absorbance at 540 nm to assay chloroform concentration. In the region investigated, chloroform displays a linear relationship between absorbance and concentration, which was determined against a standard curve [4]. Measured chloroform content did not exceed < 12.2 ppm, below the recommended limit of 60 ppm. Although liquid chromatography-mass spectrometry (LC-MS) should be used to confirm this result, these data suggest that chloroform is unlikely to pose a toxicity hazard to cells in the current study.

To evaluate potential toxicity of the PVA surfactant, which in interfacial instability synthesis is present at ~ 99% by weight, MTT analysis was performed on cells exposed to varying PVA concentrations (Figure S1). No significant changes were observed at concentrations up to 500 μg/mL, which is well above PVA concentrations anticipated in this study (e.g., < 12 μg/mL following purification). Similarly, toxicity of empty micelles (i.e., no QDs) was assessed at concentrations identical to those used in this study. No reduction in cell viability was observed (Figure S2).

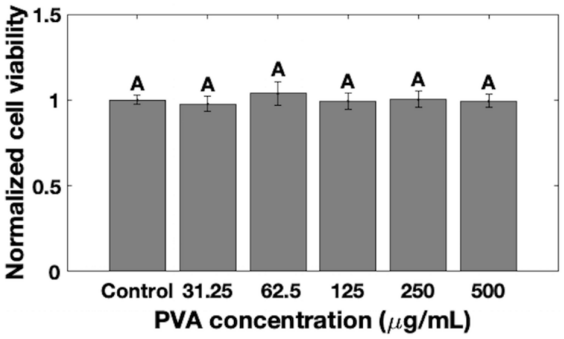

Figure S1. MTT cell viability assay of PVA treated cells as a function of concentration. Bars connected by the same letter are not statistically significant.

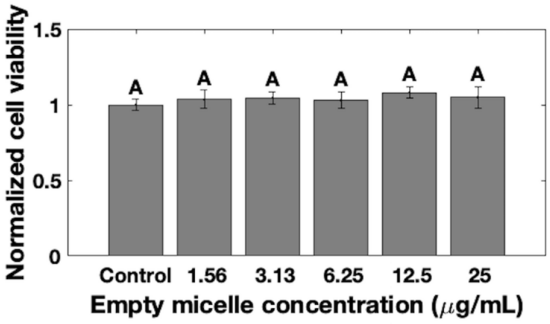

Figure S2. MTT cell viability assay of empty micelle treated cells assayed at the same concentrations employed in our study. Bars connected by the same letter are not statistically significant

2. Effect of QD Micelles at Different Concentrations on Cell Viability

Initially, we examined QD toxicity in the MTT assay using stock solutions at a different concentration than reported in our primary study. In these experiments, we observed that toxicity could be observed for the same dose if different stock solutions were employed to generate samples (Figure S3). MTT assay results for CdSe/ZnS micelle solutions generated from stock solutions of 250 μg/mL versus 35.0 μg/ml showed substantial differences in viability (~ 50% vs. ~ 100% at 25.0 μg/mL, respectively). Toxicity observed for samples diluted from the more concentrated stock solutions most likely resulted from loss of colloidal stability and subsequent aggregation. Aggregation may induce structural rearrangement of the surface coating, exposing QDs to oxidative attack. Alternatively, aggregation may cause local increases in the QD concentration on the cell surface as a result of reduced solubility, effectively increasing cellular uptake, which has been shown to increase toxicity response [5–7]. Therefore, all micelle solutions were prepared from 35.0 μg/mL stock solutions for the primary study.

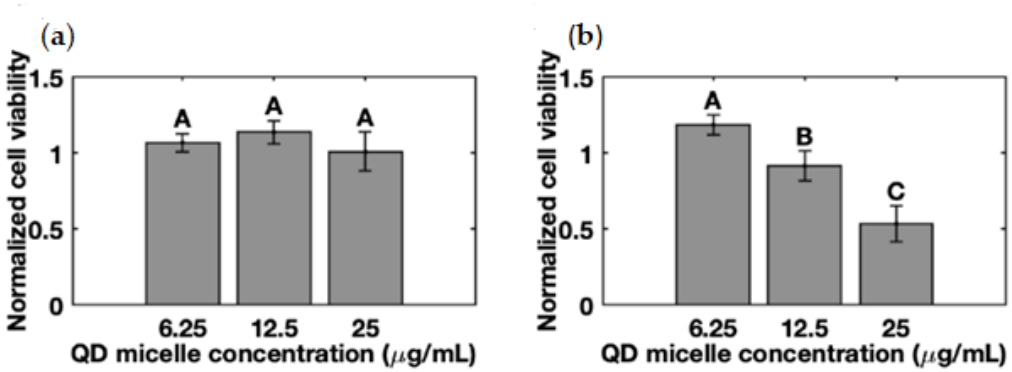

Figure S3. MTT cell viability assay results for cells treated with QD micelles prepared from different concentration stock solutions: (**a**) 35.0 μg/mL versus (**b**) 250 μg/mL. Samples were diluted to concentrations of 6.25, 12.5, or 25 μg/mL from the concentrated stock solutions. Bars connected by different letters display statistically significant differences. Thus, bars connected by B and C are statistically different from A.

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