Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supporting Information

Reverse the systemic biotoxicity of nanomaterials by downregulating ROS-related signaling pathways in multi-organs of zebrafish embryos

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Experimental section:

Reagents and Materials

Chloroauric acid ($HAuCl_4$), sodium citrate, Thulium(III) chloride hexahydrate ($TmCl_3 \cdot 6H_2O$), Ytterbium (III) chloride hexahydrate ($YbCl_3 \cdot 6H_2O$, 99.99%), Yttrium(III) chloride hexahydrate ($YCl_3 \cdot 6H_2O$, 99.99 %), Octadecene (90%, technical grade), Ammonium fluoride (NH_4F, 99.99%), Oleic acid (90%, technical grade), NaOH, glucose, $Zn(CH_3COO)_2 \cdot 2H_2O$,PEG-200, Tetraethyl orthosilicate (TEOS), Igepal CO520, Cyclohexane (AR), Methanol (AR), selenium powder, $NaBH_4$, $CaCl_2 \cdot 2.5H_2O$ thioglycolic acid ($C_2H_4O_2S$, AR), $ZnSO_4 \cdot 7H_2O$, $Na_2S \cdot 9H_2O$. The above reagents were purchased from Aladdin. ROS detection kit from Biyuntian. ANNEXIN V-FITC/PI Apoptosis Detection Kit from Solarbio.

Preparation of AuNPs

Prepare $HAuCl_4$ into a 0.01% aqueous solution, take 100mL and heat to boiling. Add 2mL of 1% sodium citrate with stirring, continue to heat and boil for 15 minutes.

Preparation of UCNPs

Reference.[1]

Preparation of C QDs

Add 1g of glucose as a carbon source, disperse glucose in 30mL of water, add 50mL (1mol/L) NaOH solution, sonicate for 4h, and obtain C QDs after separation and purification.

Preparation of ZnO QDs

0.84g of NaOH was dissolved in 50mL of absolute ethanol, 2.2g of $Zn(CH_3COO)_2.2H_2O$ was dissolved in 100mL of ethanol, stirred for 40min, added 6g of PEG-200, and continued to stir for 30min. Add NaOH solution, stir for 2h, add 1.5mL oleic acid, precipitate for 1h, centrifuge to collect the precipitate, add ethanol to dissolve.

Preparation of CdSe QDs

After 0.077g CdO (6×10^{-4} mol) and 0.68g (24×10^{-4} mol) oleic acid are mixed in a 50mL three-necked flask, argon is introduced, the temperature is raised to 100℃, kept for 30min, and then heated to 220℃, get cadmium oleate, when the mixture turns from reddish to clear solution, remove the heating mantle, stop heating, cool to room temperature, the obtained cadmium solution is cooled to room temperature, put it into the vacuum drying oven, the temperature is set to 150℃, remove the water for 30 minutes, take out the cadmium solution and immediately put it into a desiccator to cool to room temperature. Under anaerobic conditions, 1.4g Se was dissolved in 3.84g TBP and further diluted with 12.33g ODE to obtain a selenium solution. Add 1.5g (5.57 mmol) ODA, 0.5g (1.29 mmol) TOPO, and 2g (7.92 mmol) ODE to the above cadmium solution cooled to room temperature. In argon, heat the cadmium solution, and when the temperature reaches 280°C, quickly inject 3g of selenium solution (containing 3 mmol of selenium). After the injection, the temperature of the reaction mixture dropped, keeping the temperature at 260°C, at which the quantum dots were grown.

Preparation of CdSe@SiO_2

0.1g of CO520 was dissolved in 2mL of cyclohexane, ultrasonically dissolved, added 0.004g of NaOH, stirred to dissolve, added 0.5mL of TEOS and 0.1g of CdSe QDs, stirred for 12h, added a small amount of methanol to make the particles settle.

Preparation of CdSe/ZnS

Reference.[2]

Preparation of CdSe@PEG

Add 0.01g EDC to 1mL of CdSe QDs, stir at room temperature for 24 hours, continue to add 0.01g PEG-200, and stir at room temperature for 24 hours.

ROS detection

Add 100 μL of DCFH-DA (1:2000 dilution) to 96-well plates, place 1 zebrafish in each well, and observe the fluorescence of zebrafish under a fluorescence microscope after staining for 3 hours.

Take 20 zebrafish and grind uniformly, add DCFH-DA (1:2000 dilution), and detect the fluorescence intensity excited by 488nm under the microplate reader.

Take 20 zebrafish and add 1 mL of trypsin, incubate at 37°C for 15min, take the supernatant, add DCFH-DA (1:2000 dilution), incubate at 37°C for 30min, and detect cells containing ROS with a flow cytometer.

Apoptosis detection

Take 20 zebrafish and add 1 mL of trypsin, incubate at 37°C for 15min, take the supernatant, utilizing the ANNEXIN V-FITC/PI Apoptosis Detection Kit, incubate at 37°C for 30min, and detect cells containing ROS with a flow cytometer.

qRT-PCR detection

According to Takara's instructions.

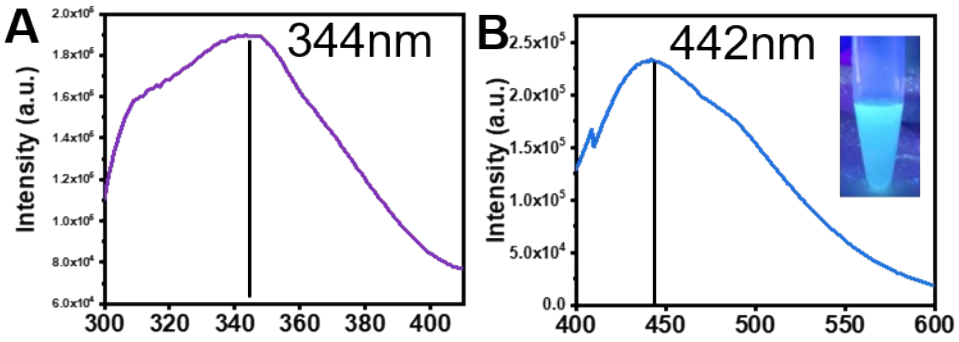

Fig. S1. A) Excitation spectrum of C QDs. B) Emission spectrum of C QDs.

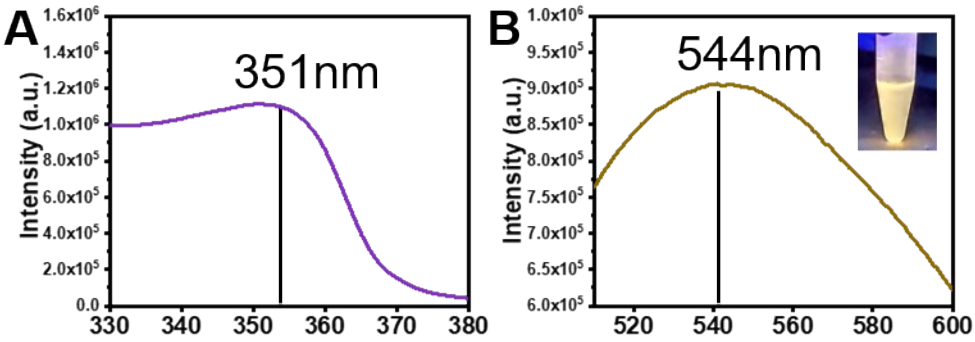

Fig. S2. A) Excitation spectrum of ZnO QDs. B) Emission spectrum of ZnO QDs.

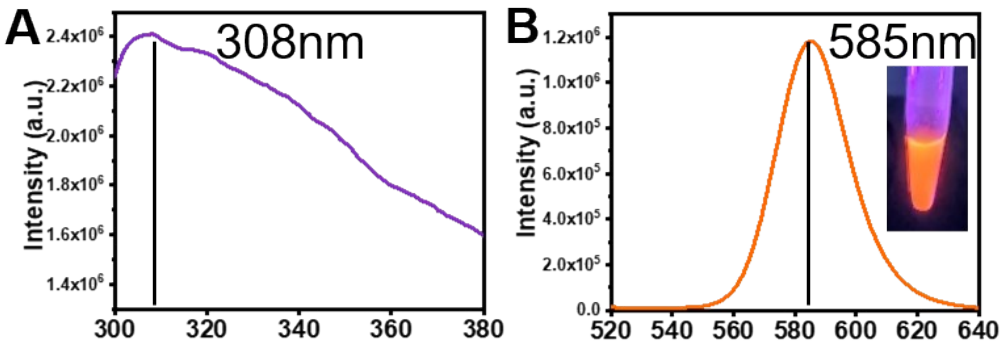

Fig. S3. A) Excitation spectrum of CdSe QDs. B) Emission spectrum of CdSe QDs.

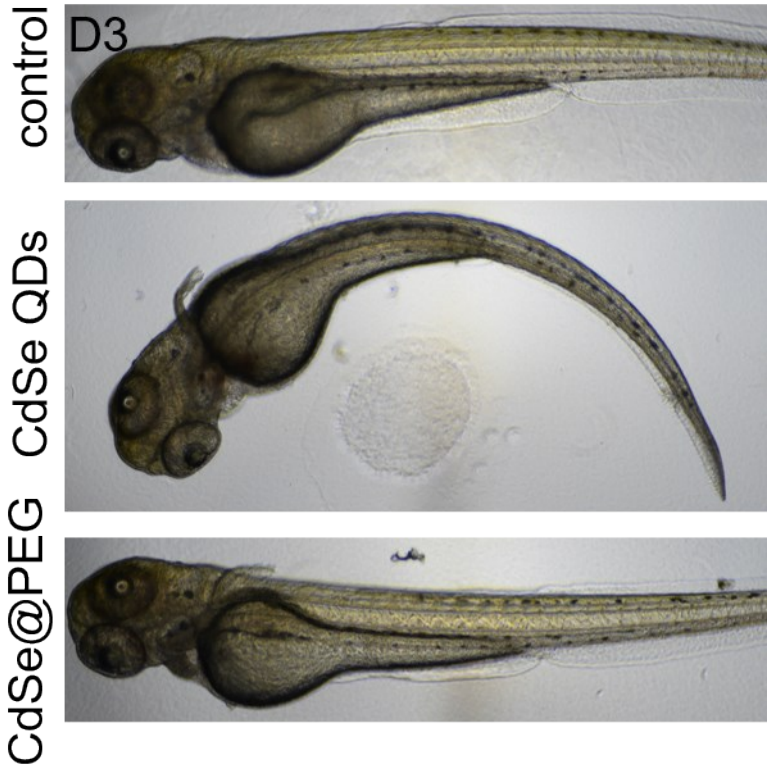

Fig. S4. PEG-modified significantly decreased the CdSe QDs induced malformation in zebrafish. The phenotype of zebrafish in the control, CdSe QDs and CdSe@PEG groups.

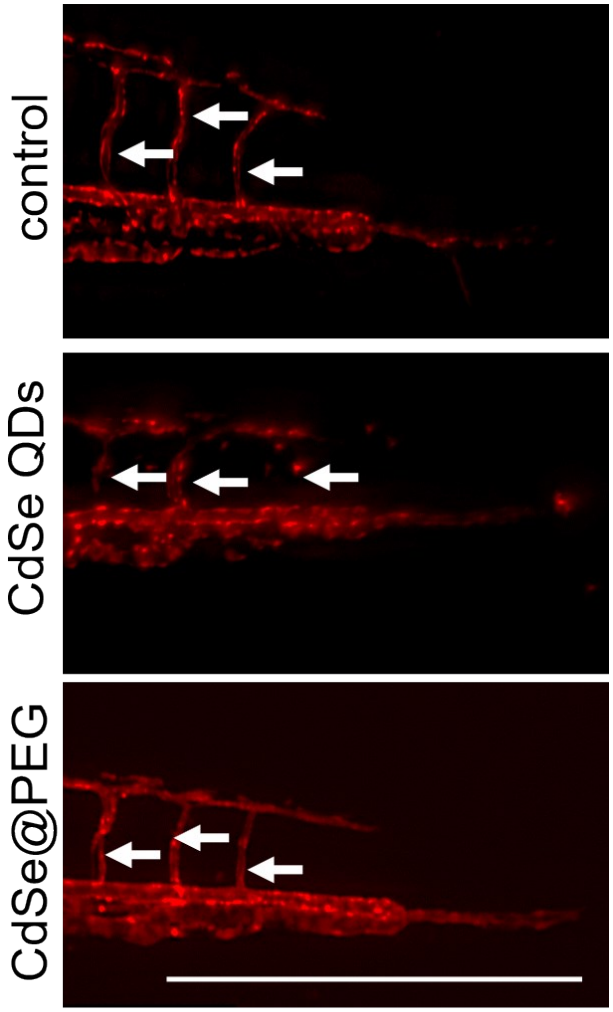

Fig. S5. PEG-modified significantly decreased the CdSe QDs induced vascular toxicity in zebrafish. The expression of zebrafish tail vascular endothelial cells in the control, CdSe QDs and CdSe@PEG groups. Scale bars: 100μm.

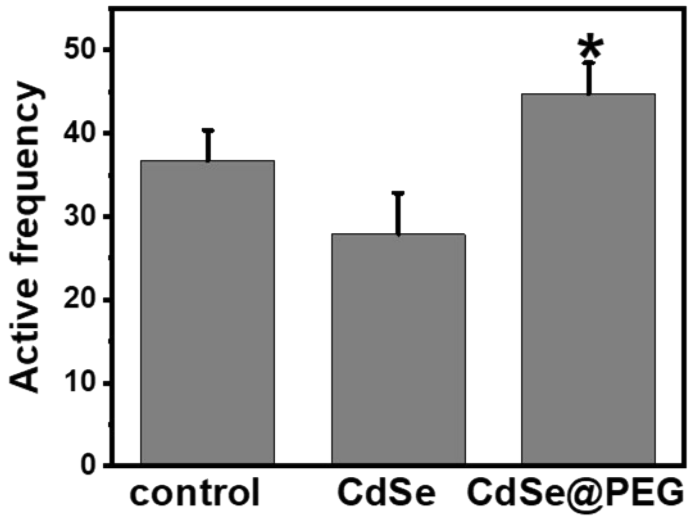

Fig. S6. The effect of CdSe QDs and CdSe@PEG on zebrafish behavior. Zebrafish active frequency in the control, CdSe QDs and CdSe@PEG groups.

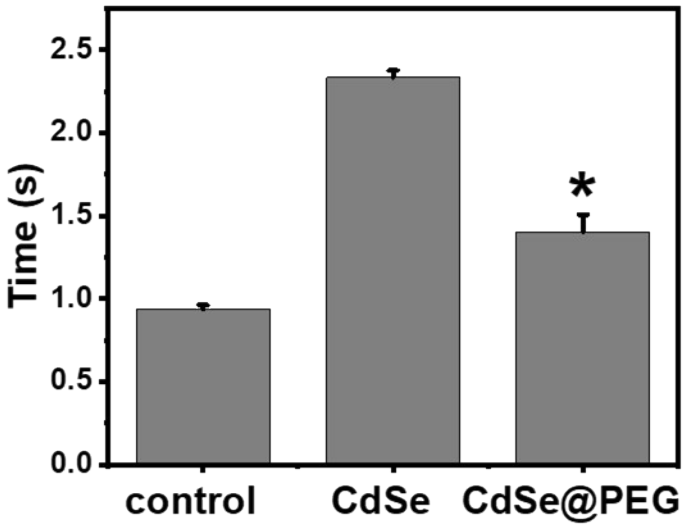

Fig. S7. The effect of CdSe QDs and CdSe@PEG on zebrafish behavior. Zebrafish resting time in the control, CdSe QDs and CdSe@PEG groups.

nox4-F	TCATTTCCCTCAGACGTGGC
nox4-R	GTGGGATATCGCCTCTGGTG
sod1-F	GTGACCGGCACCGTCTATTT
sod1-R	TGGCATCAGCGGTCACATTA
gpx1-F	TTTACGACCTGTCCGCGAAA
gpx1-R	TCTTGCAGTTCTCCTGGTGC
p53-F	CGCGGATTTGCTTTGTGGAT
p53-R	CCTGGGGGCTGAATACTTATCAA
caspase-3-F	CCATGCAGGTTGATGCCAAG
caspase-3-R	TCGTCATGGGCAACTGTTGTT

Fig. S8. The primer sequences for *nox4*, *sod1*, *gpx1*, *p53*, *caspase-3*, and *gapdh.*

References

[1] B. Zheng, H. Wang, H. Pan, C. Liang, W. Ji, L. Zhao, H. Chen, X. Gong, X. Wu, J. Chang, Near-Infrared Light Triggered Upconversion Optogenetic Nanosystem for Cancer Therapy, ACS Nano 11 (2017) 11898-11907.

[2] H.D. Duong, S. Yang, Y.W. Seo, J.I. Rhee, Effects of CdSe and CdSe/ZnS Core/Shell Quantum Dots on Singlet Oxygen Production and Cell Toxicity, J. Nanosci. Nanotechnol. 18 (2018) 1568-1576.