Detection of Apoptotic Cells by Using Biofunctional Quantum Dots

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Apoptosis, which is widely observed in different cells of various organisms, is the unique morphological pattern of programmed cell death characterized by chromatin condensation, membrane blebbing and cell fragmentation

QDs have already been utilized for labeling different cellular organelles as well as tracking their functions

Clearly, as Cd-containing nanoparticles cannot be considered as 100% biocompatible, it is more important to correlate the study of apoptosis with the cytotoxicity of quantum dots categorically to emphasize on the suitable experimental conditions inhibiting cytotoxic events.

Cytotoxic effects of Quantum Dots

Three main reasons for the cytotoxic effects of QDs were identified in the literature

Release of Cd^{+} ions from CdSe QDs by UV exposure

QDs surface interaction with different cell types

QDs surface chemistry imparts stability towards aggregation

To our knowledge, only few studies exist where the cytotoxic effects of CdSe and CdSe/ZnS nanoparticles are investigated.

Derfus et al. (2004) have reported that the release of Cd^{2+} ions from CdSe particles is enhanced by oxidation, either through exposure to air or UV irradiation, but is repressed by encapsulating the particles with appropriate shells, as with ZnS and an additional organic shell.

Shiohara et al. (2004) claims that for different sized CdSe/ZnS particles, the main source of cytotoxicity is not their cadmium content but rather the particle surface interaction with different cell types.

Furthermore, Kirchner et al. (2005) have investigated that for different surface modifications of CdSe and CdSe/ZnS nanoparticles, their surface chemistry imparts stability towards aggregation, which plays an important role for cytotoxic effects.

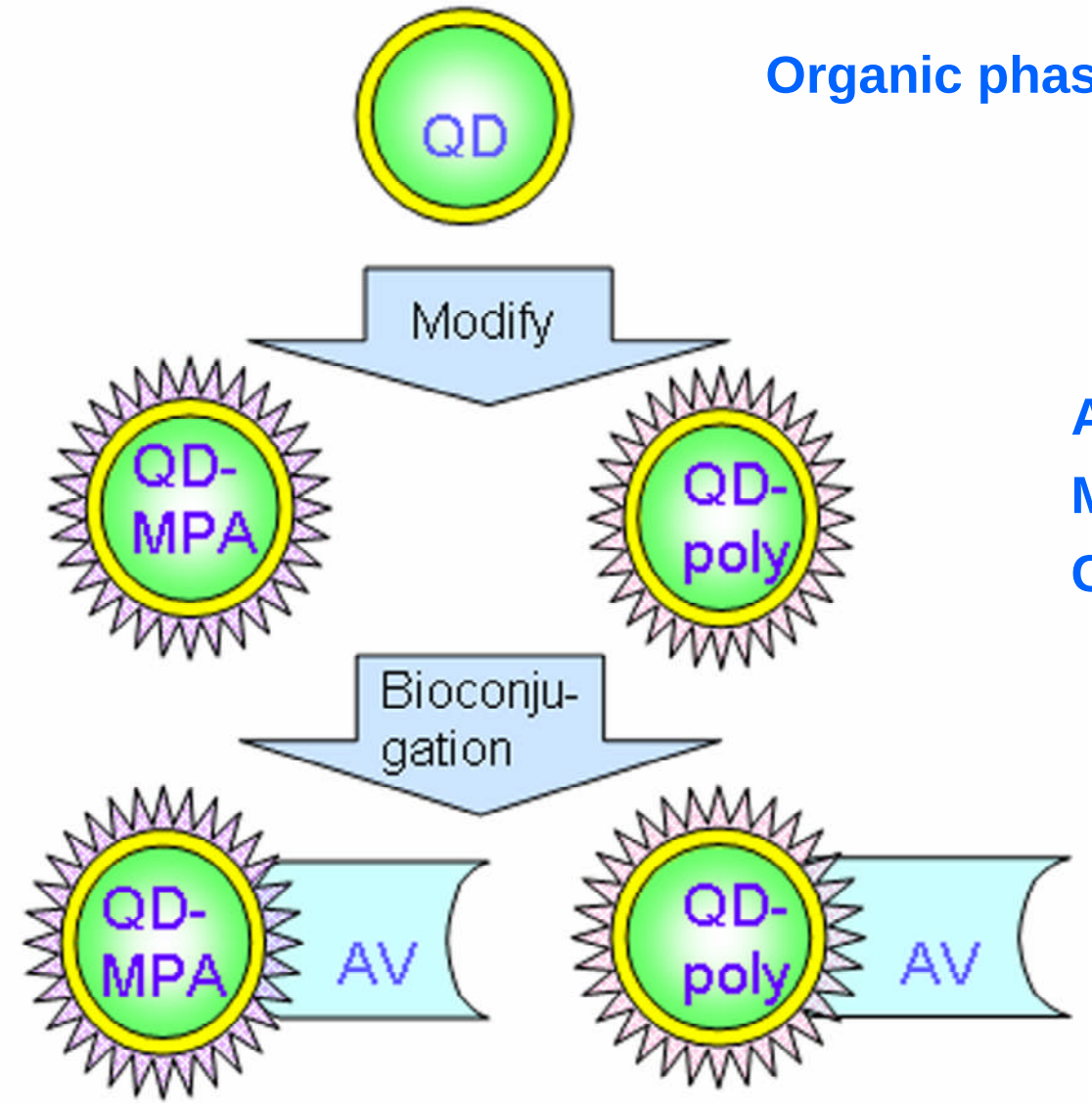
Protocols

Osteoblast cells (Human osteoblast cell line-hFOB1.19) were incubated with various μM concentrations of CdSe-core and CdSe/ZnS core-shell QDs for 24 hrs.

curcumin induced Apoptosis/Necrosis of osteoblast cells were detected using CdSe/ZnS core-shell QDs as fluorescent probes for confocal microscopy

And various Biochemical methods have been attempted to quantify the cytotoxicity as well as the apoptotic signaling pathway induced by these quantum dots.

Bioconjugation of QDs



Organic phase CdSe/ZnS core shell QDs

Aqueous phase transferred MPA- and polymer coated CdSe/ZnS core shell QDs

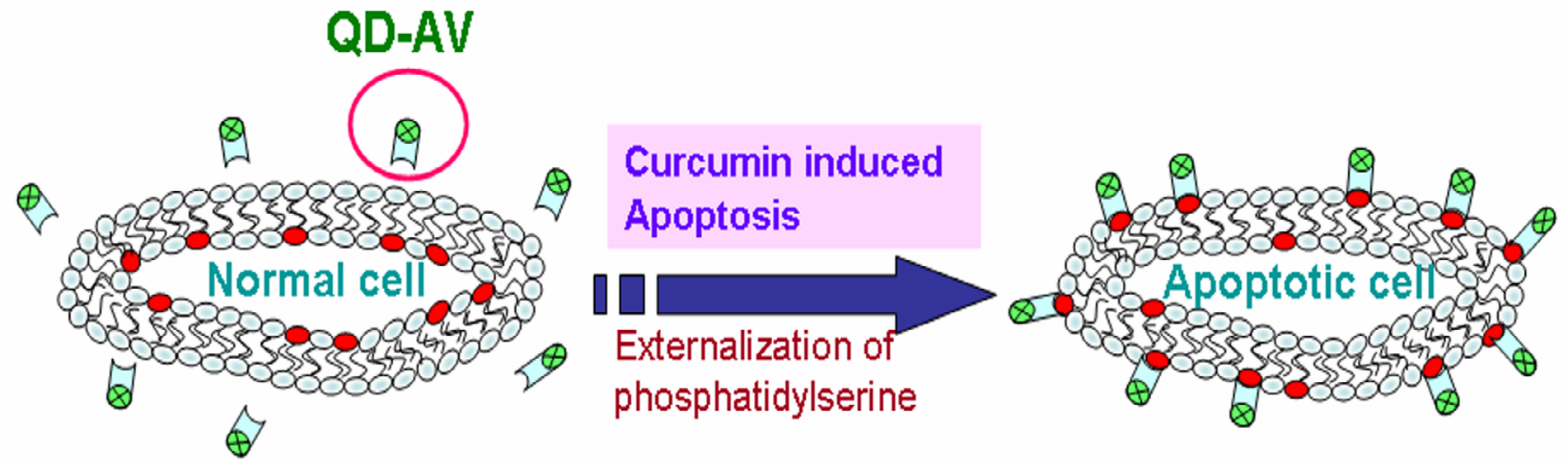
Annexin V (AV) protein conjugated CdSe/ZnS core shell QDs

Curcumin, a common herbal spice, stimulates apoptotic/necrotic signaling

Annexin V, a lipid binding protein, having strong affinity towards phosphatidylserine (PS), which is normally found in the inner surface of the plasma membrane facing the cytoplasm of living cells, but in turn flips and faces the extra-cellular fluid during apoptosis.

As shown in the table, QD-AV conjugates are better biolabeling probes than the usual PI organic stains

Apoptosis Detection using QDs

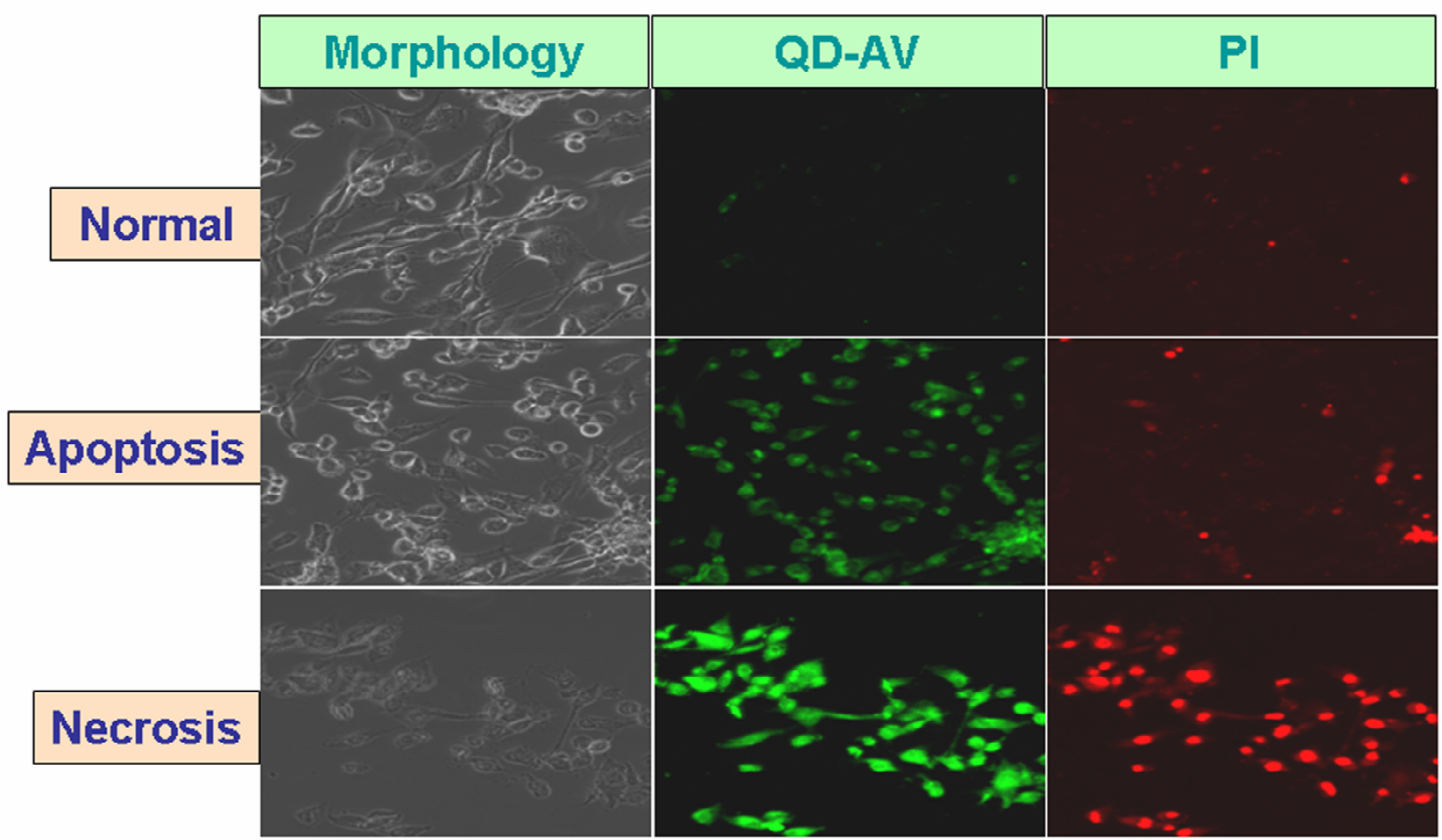


	QD-AV	PI
Normal cells	-	-
Apoptotic cells	+	-
Necrotic cells	+	+

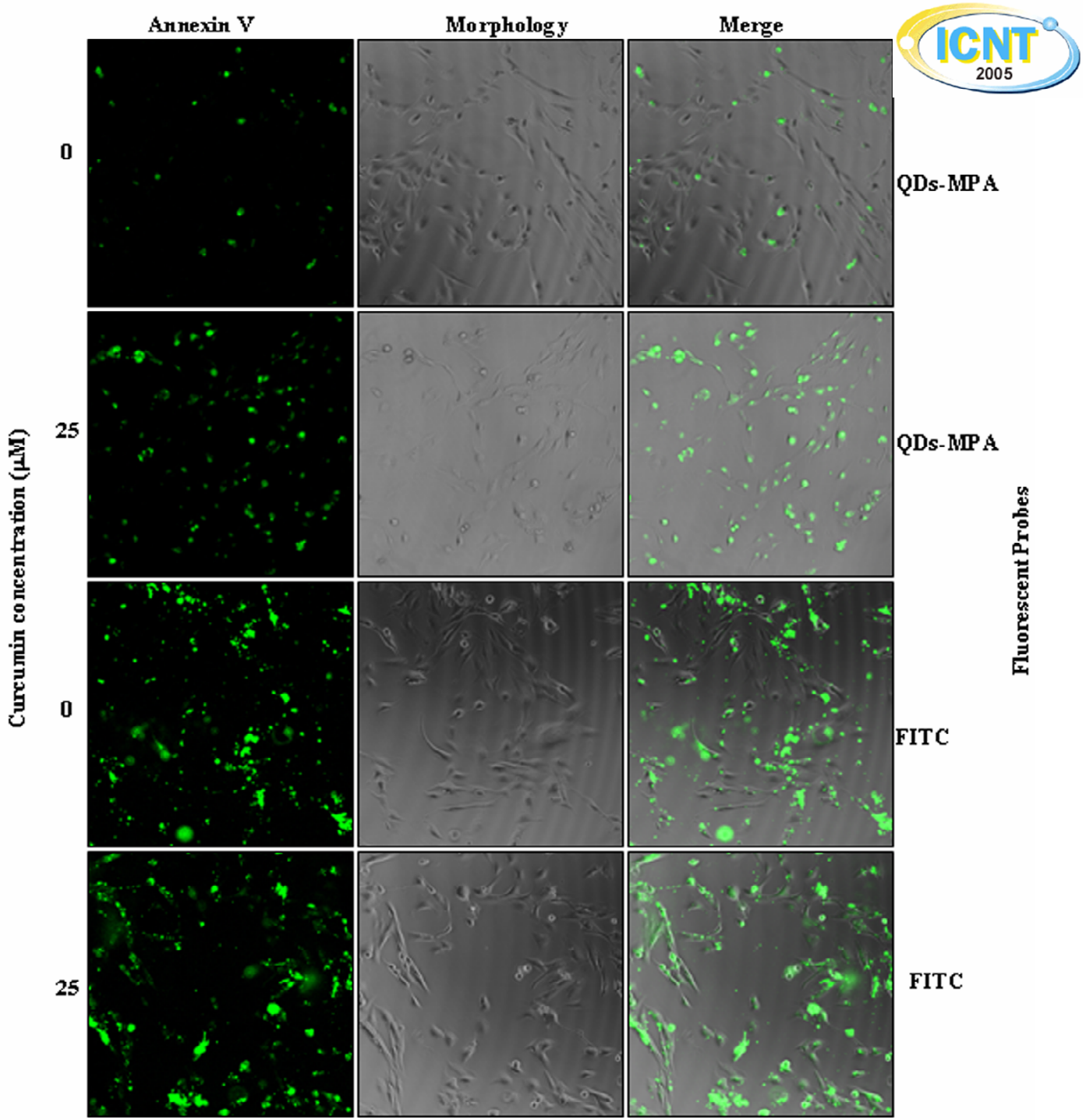
curcumin dose-dependant apoptotic character of human osteoblast cell line (hFOB1.19) is apoptosis at low concentration (up to 25 μM curcumin) and necrosis at high concentration (>200 μM curcumin).

the morphological changes in the apoptosis and necrosis stages have been portrayed in a regular fashion using the QDs than the usual standard PI stain, without affecting their integrity with the normal cells.

Apoptosis and Necrosis detection

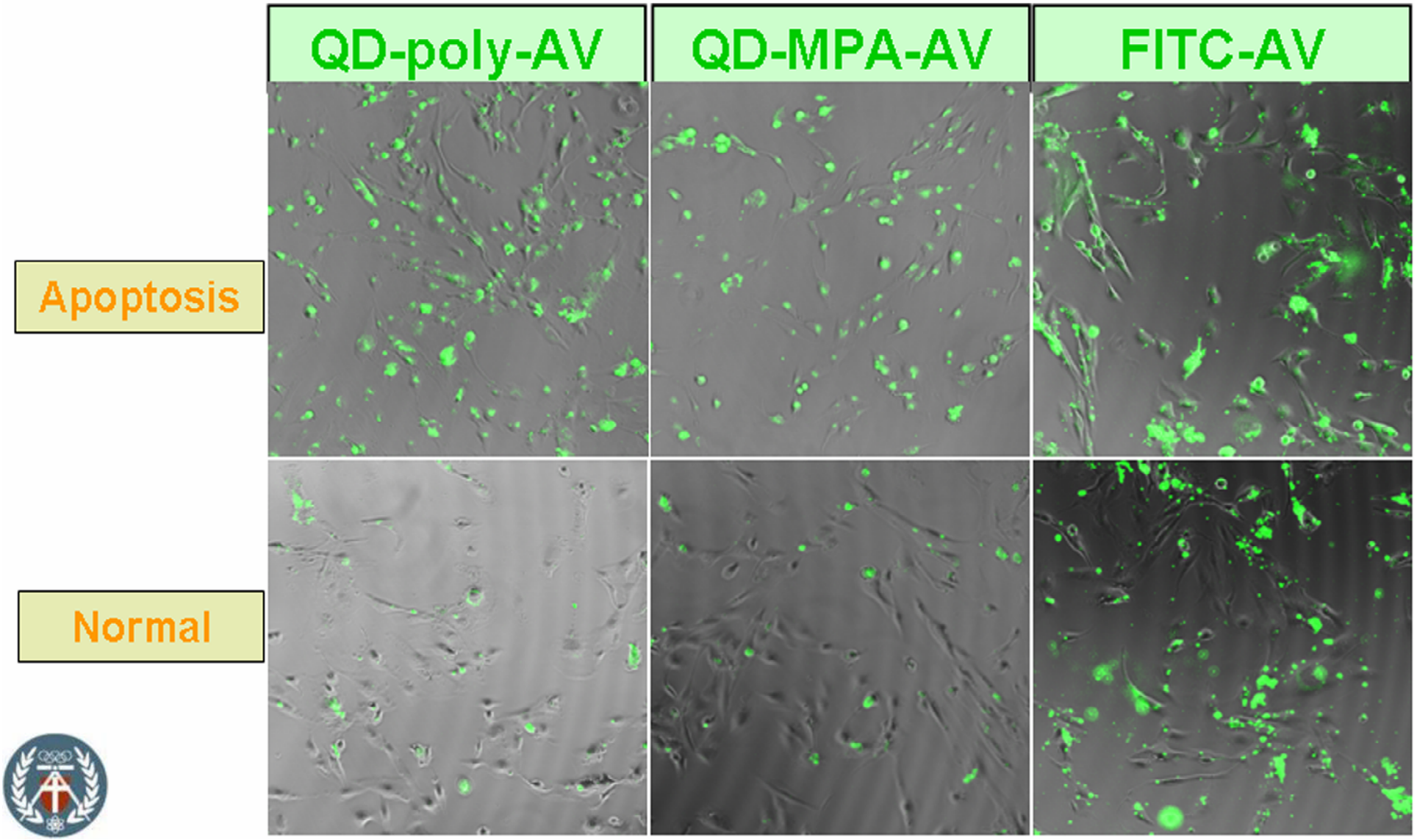


Curcumin-induced Apoptosis of Osteoblast cells illustrated using QD-AV and FITC-AV conjugates



In comparison, Annexin V-FITC conjugates does not differentiate between live and apoptotic cells, as FITC even binds with live cells, demonstrating the sensitivity of QDs in precisely biolabeling apoptosis.

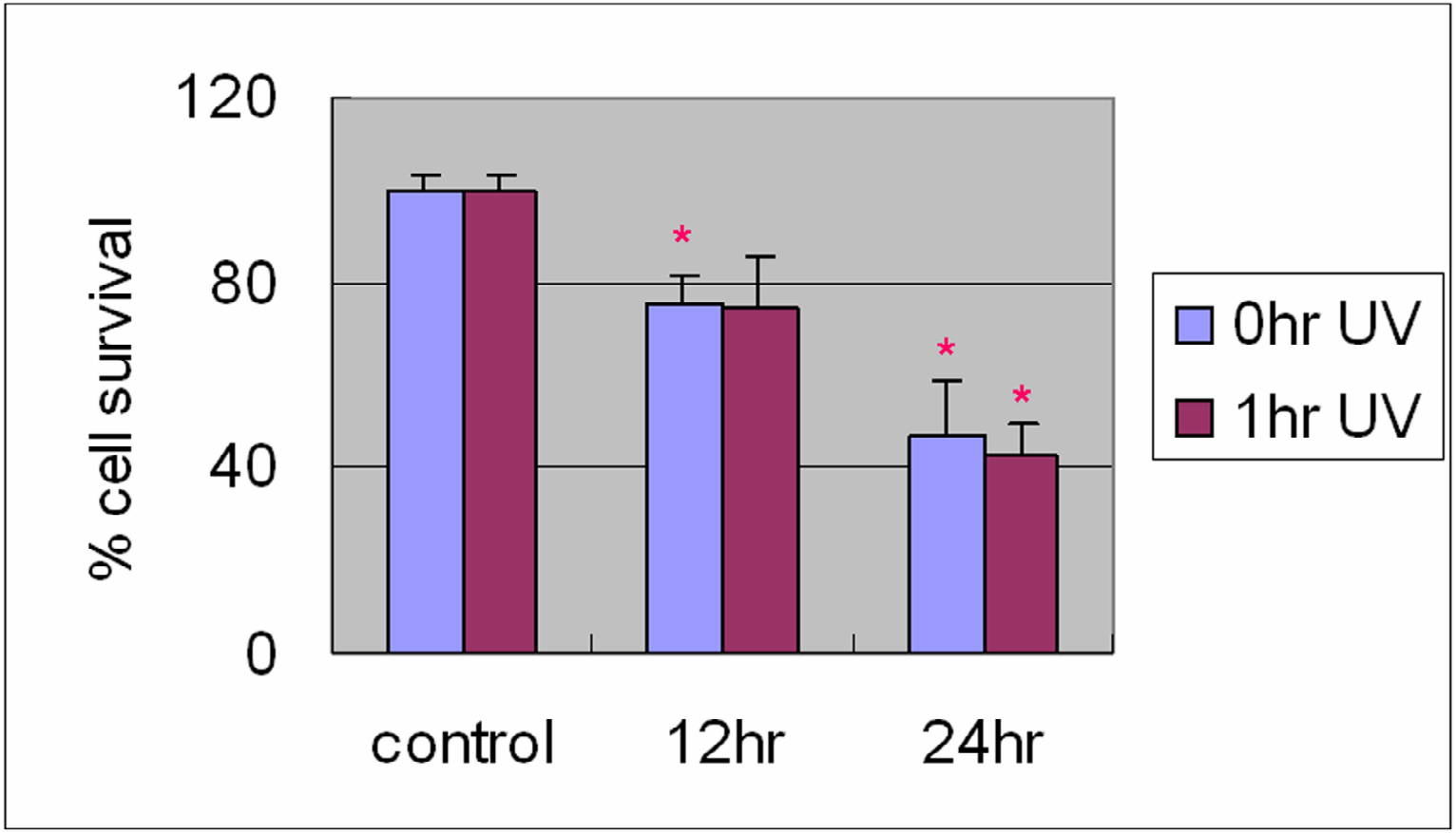
Curcumin-induced Apoptosis of Osteoblast cells using QD-AV and FITC-AV conjugates



In all our further measurements, the osteoblast cells were incubated with various μM concentrations of CdSe-core and CdSe/ZnS core-shell QDs for 24 hrs.

And various Biochemical methods have been attempted to quantify the cytotoxicity as well as the apoptotic signaling pathway induced by these quantum dots.

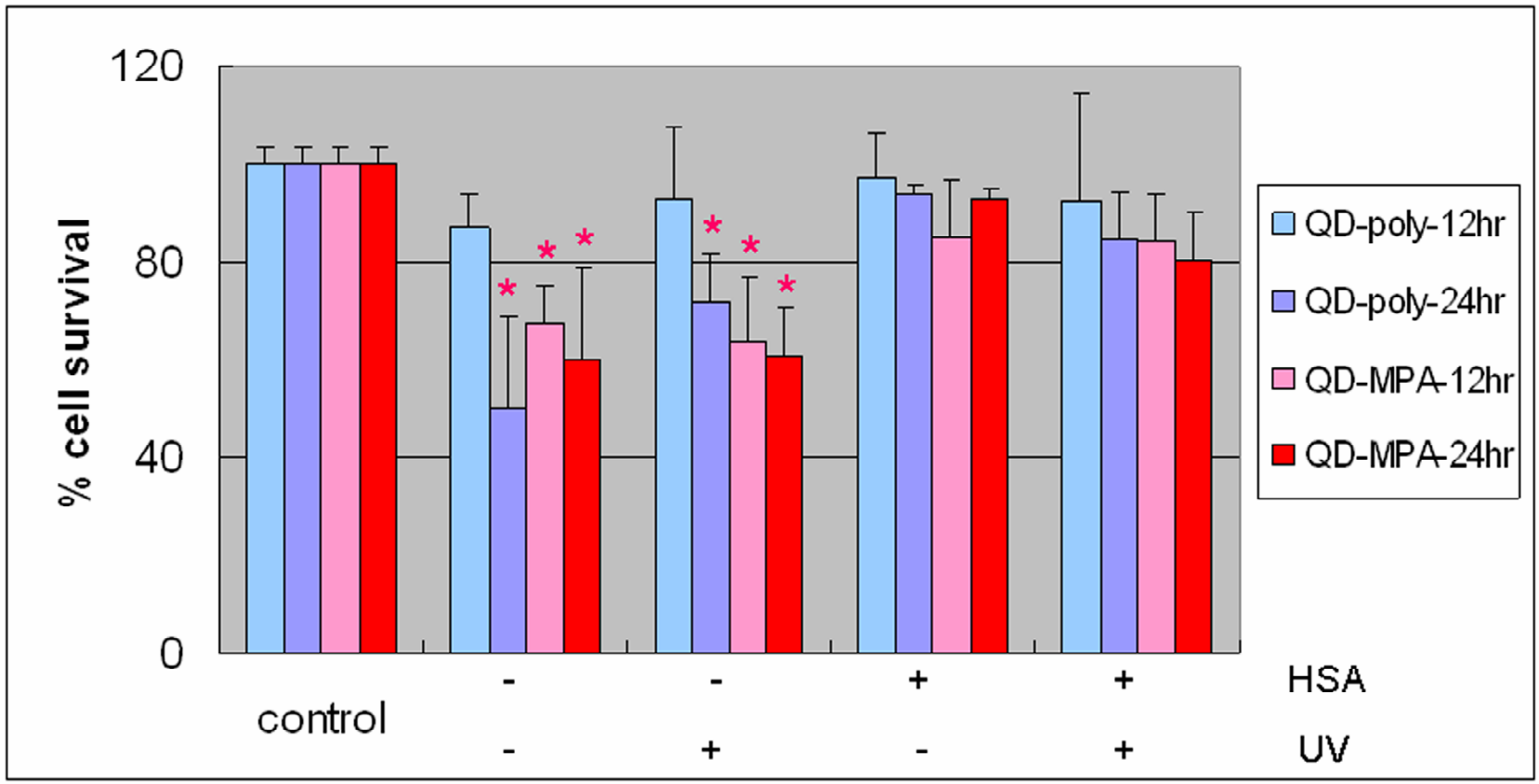
Cytotoxicity of CdSe-core QDs



*P<0.001

CdSe-core QDs are cytotoxic to cells under UV exposure

Cytotoxicity of CdSe/ZnS core-shell QDs



specific surface modified QDs imparts stabilization towards cytotoxic effects

Cell viability (MTT assay)

Approximately 50-60% of cells died by treating CdSe-core QDs with osteoblast cells, while the CdSe/ZnS core-shell QDs had no effect on the cell viability.

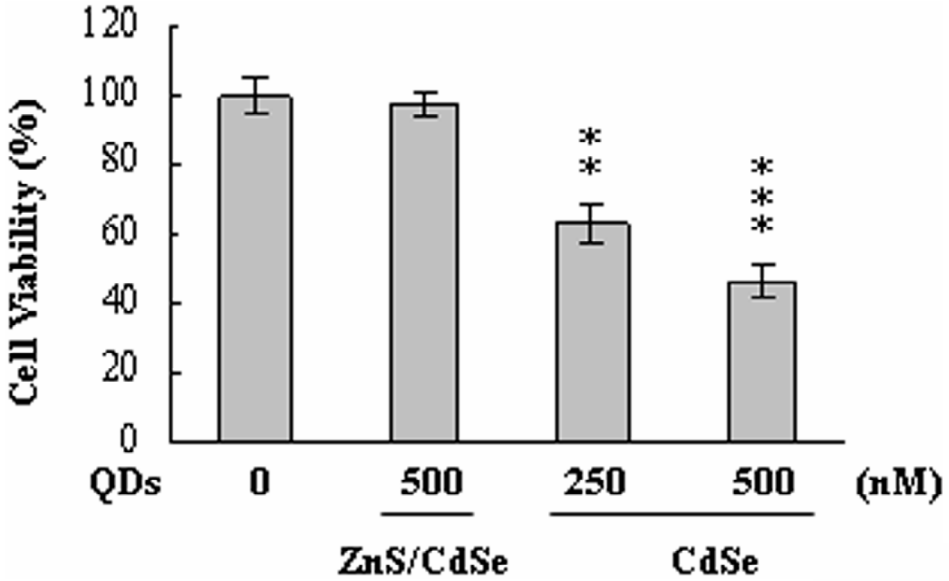
Apoptosis associated parameter (TUNEL apoptosis assay kit)

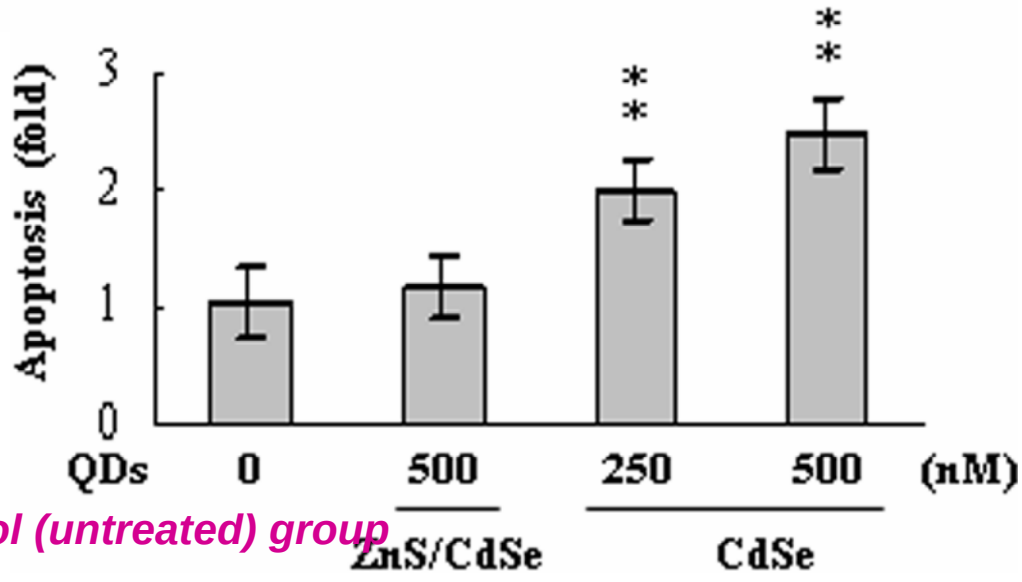
From the quantitative determination on the amount of histone-associated oligonucleosome DNA fragments using an ELISA kit, we found that CdSe-core QDs induced a 2.5-fold increase in this apoptosis-associated parameter.

Both quantifies the non-toxic nature of core-shell QDs as compared with highly toxic core QDs (the different μM concentrations used were given along the x-axis).

Values are presented as means ± SD. *P < 0.05 and **P < 0.01 versus control (untreated) group.

Osteoblast cell viability and Apoptosis after CdSe-core and CdSe/ZnS core-shell quantum dots exposure





*P < 0.05 and **P < 0.01 versus control (untreated) group

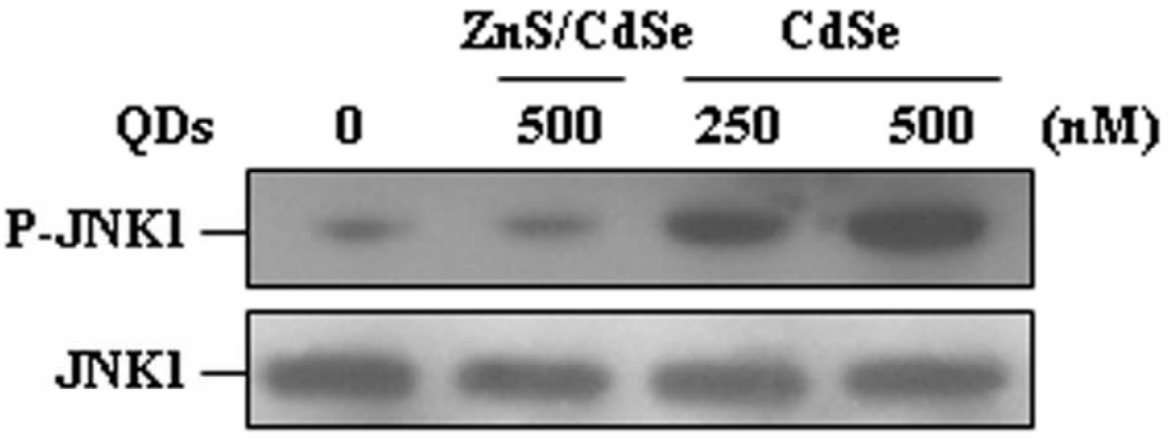
JNK activation in CdSe-core QDs-treated osteoblasts

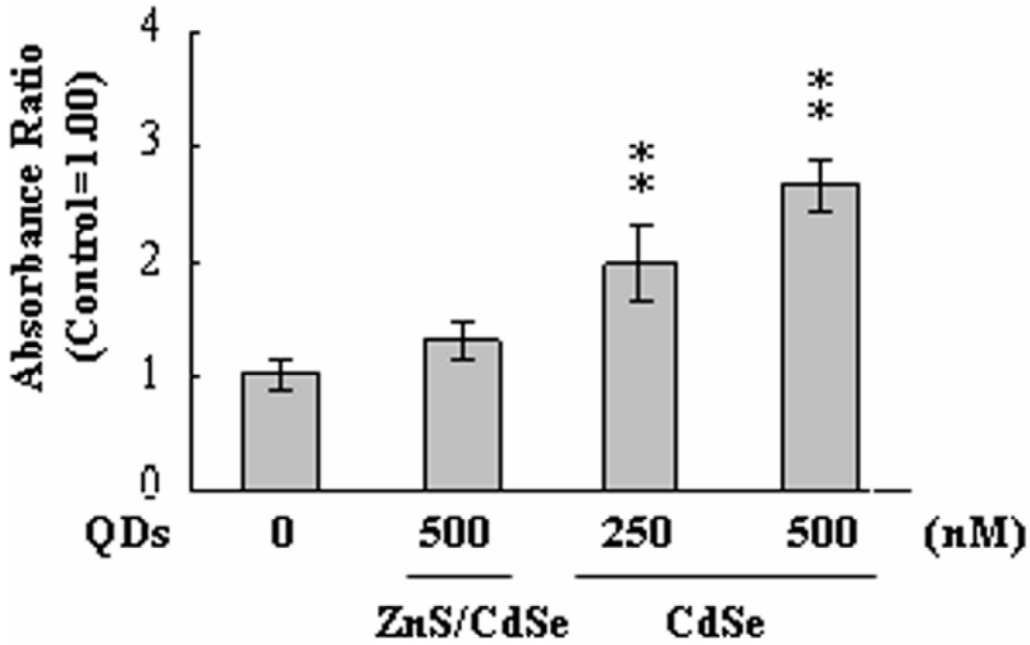
Cell extracts (60 mg) were immunoblotted with anti-p-JNK antibody.

The lower panel shows an immunoblot of JNK1 protein from 60 mg cell extract.

JNK/AP-1 activity was evaluated by ELISA detection of phosphorylated c-Jun.

JNK activation in quantum dots-treated osteoblast cells





*P<0.05 versus control (untreated) group

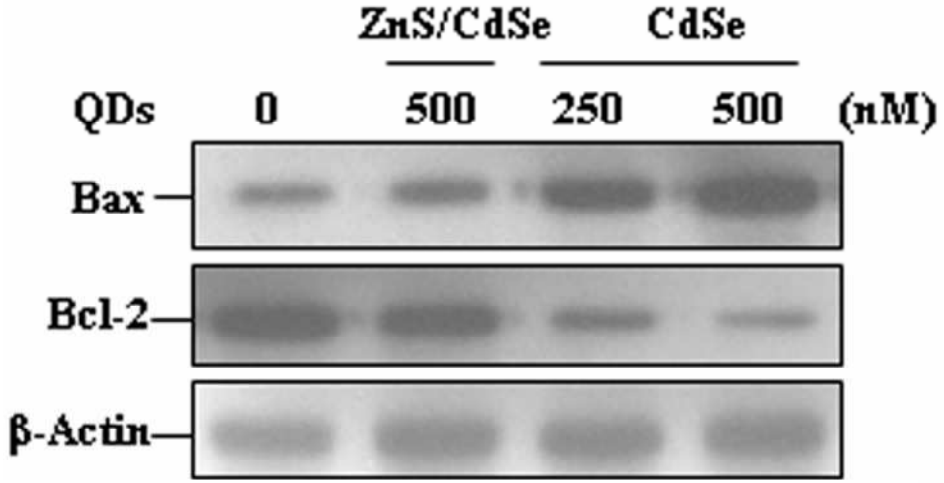
Expression of Bcl-2 family were modulated in CdSe-core QDs-treated osteoblasts

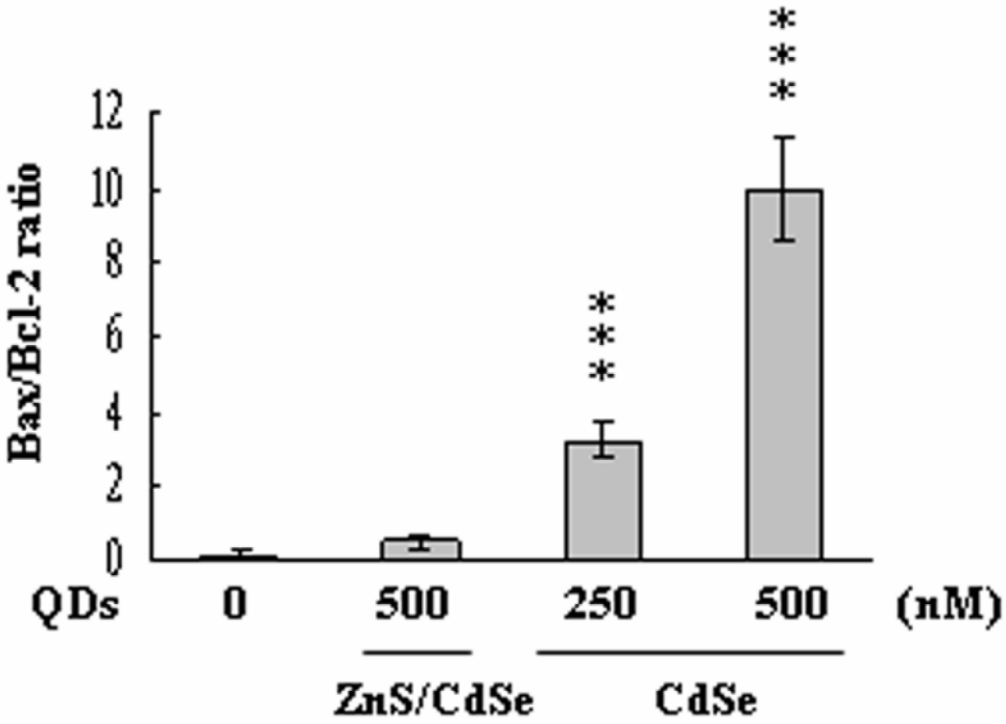
Bax and Bcl-2 in protein level was analyzed by immunoblot analysis.

The ratio of Bax and Bcl-2 determined by densitometer was then caculated and graphed.

Values are presented as means ± SD of three to five determinations.

Expression of Bcl-2 family in quantum dots-treated osteoblast cells





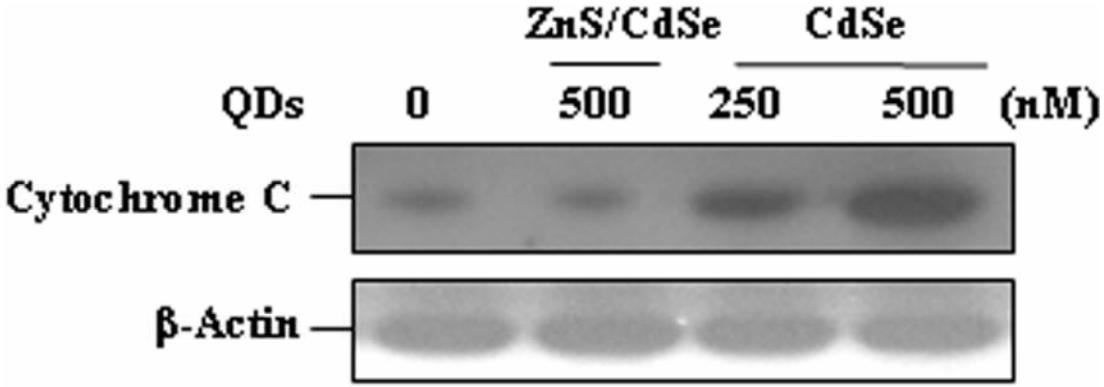
*P<0.05 versus control (untreated) group

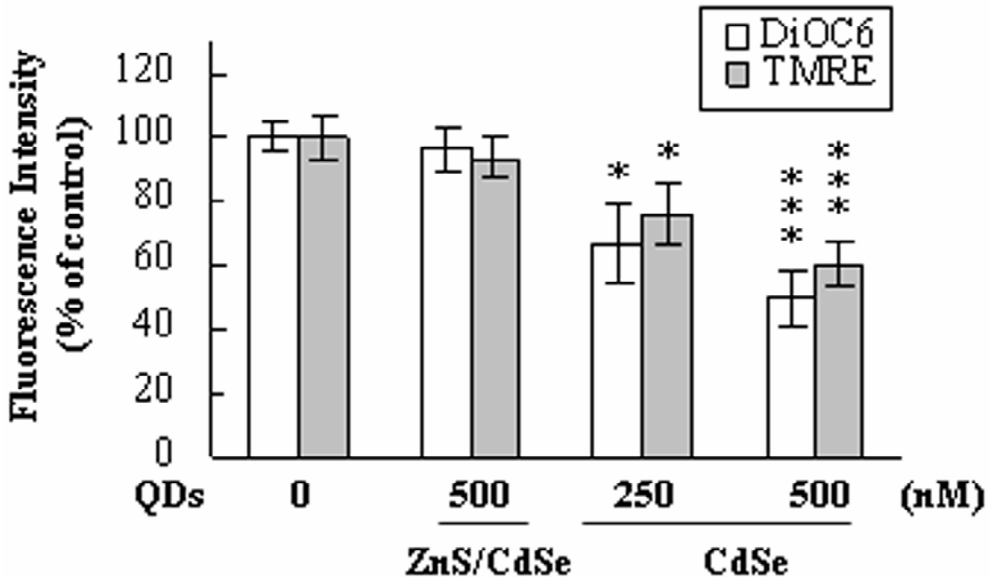
Analysis of mitochondrial membrane potential and cytochrome C release from mitochondria in CdSe-core QDs-treated osteoblasts.

Cells were then incubated with 40 nM DiOC6(3) or 1 μM TMRE at 37 ℃ for 1 h, and analyzed by spectrofluorometry. Values are presented as means ± SD of three to five determinations.

Cytosolic and mitochondrial fractions were separated, and cytosol aliquots (40 mg) were resolved by 15% SDS-PAGE then immunoblotted using anti-cytochrome C antibody. The data are representative of three independent experiments.

Mitochondrial membrane potential and cytochrome c release from mitochondria in quantum dots-treated osteoblast cells





**P<0.05 versus control (untreated) group*

Effects of CdSe-core QDs treatment on the components of the survival signaling and heat shock protein 90 (HSP-90)

Immunoblot assay for the expression of the

total ras protein (A),

Raf-1 protein (B), and

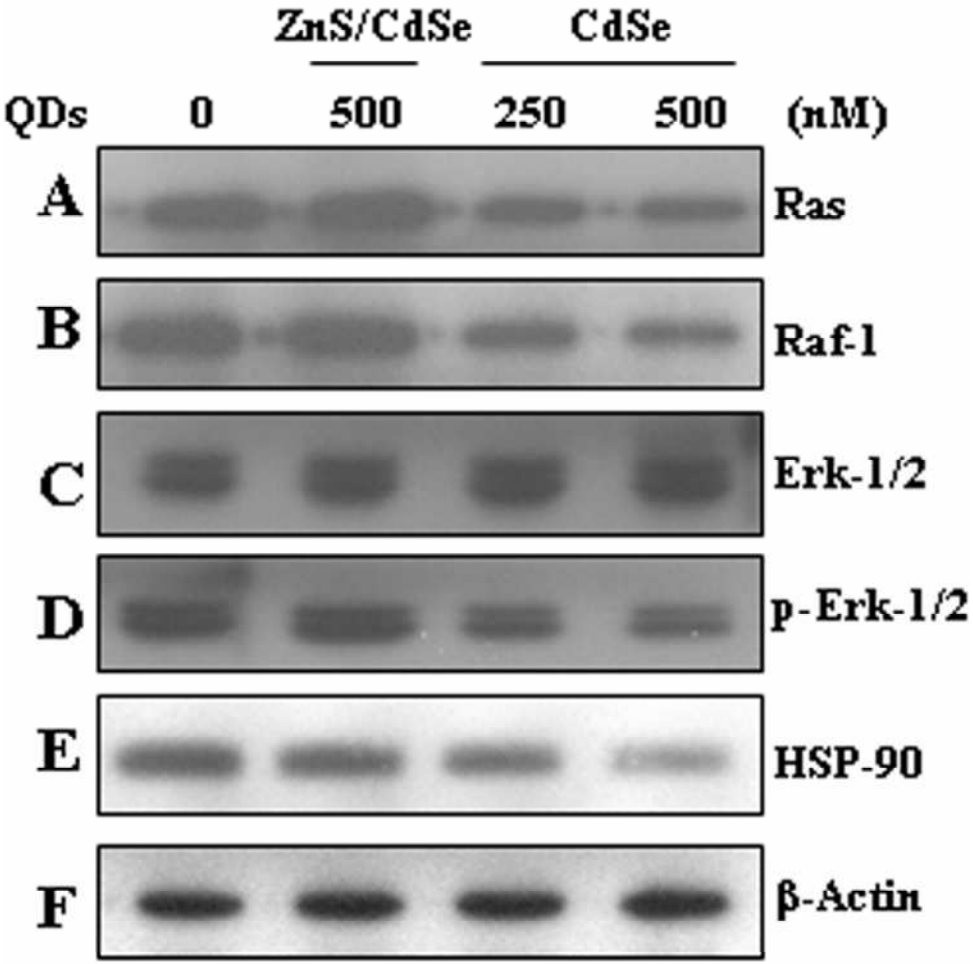
ERK-1 and 2 proteins (C).

(D) The phosphorylation of ERK-1 and 2 were evaluated with immunoblotting using anti-pMAPK antibody.

(E) Immunoblot assay for the expression of the HSP90

(F) Expression of β-actin, a house-keeping protein, was using the loading control.

Activation of survival signaling and heat shock protein 90 in quantum dots-treated osteoblast cells

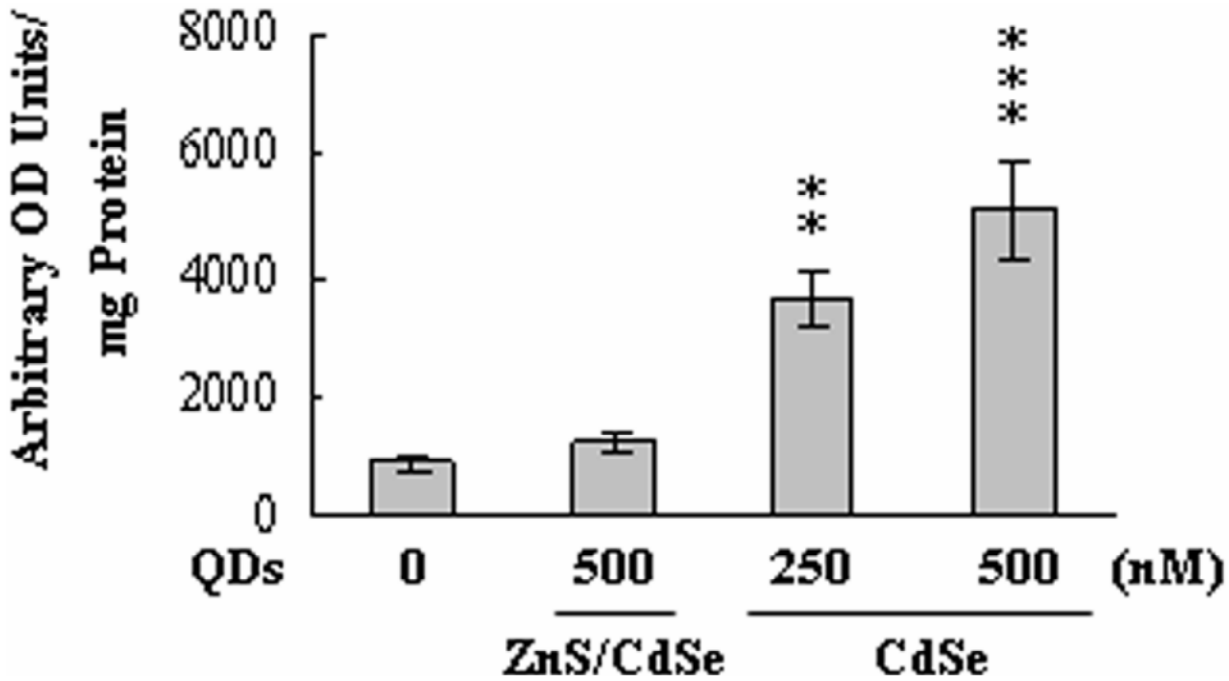


ROS generation in CdSe-core QDs-treated osteoblast cells.

Osteoblasts were preloaded with 10 mM DCF-DA for 1 h. Cells were then left untreated or treated with various concentrations CdSe-core QDs or ZnS-coated CdSe QDs (ZnS) as indicated for 24 h.

The generation of ROS was assayed by DCF-DA and is expressed as absorbance/mg of protein.

ROS generation in quantum dots-treated osteoblast cells



***P<0.01 versus control (untreated) group*

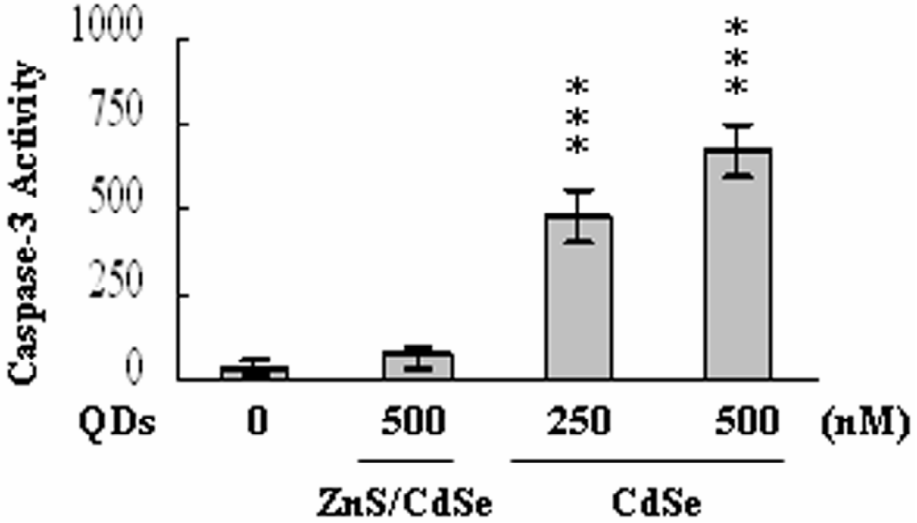
activation of caspase-9 and caspase-3 in CdSe-core QDs-treated osteoblast cells.

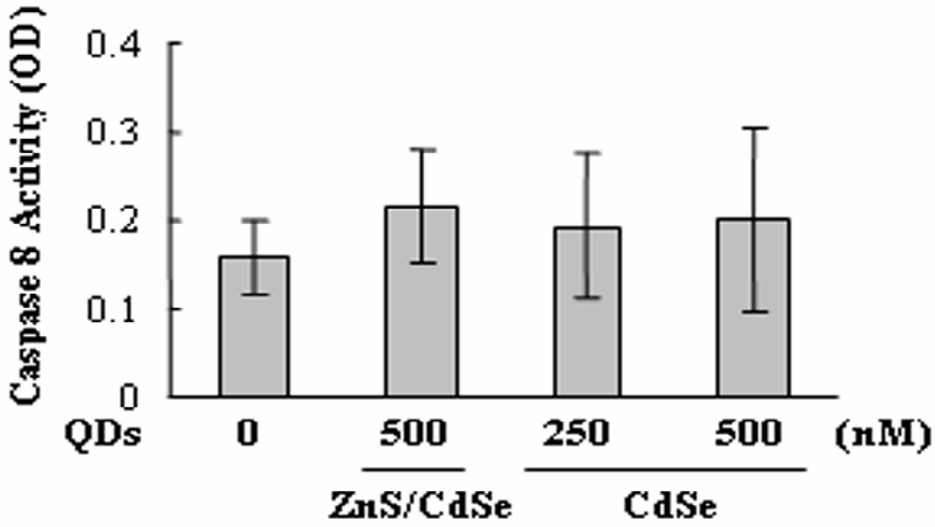
Caspase-9 activities were assayed using the colorimetric caspase-9 Assay Kit.

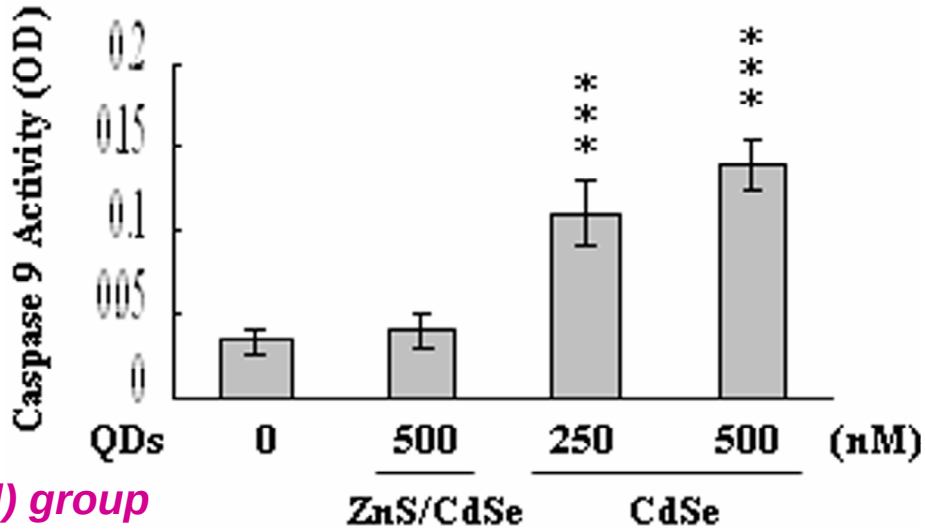
Cell extracts (60 µg) were analyzed for caspase-3 activity using Z-DEVD-AFC as the substrate.

Caspase-8 activities were assayed using the colorimetric caspase-8 Assay Kit.

Activation of caspase-3, caspase-8 and caspase-9 in quantum dots-treated osteoblast cells







**P<0.01 versus control (untreated) group

For determining the relationship between JNK and caspase-3 activities during CdSe core QDs-induced apoptosis, Osteoblast cells were pre-incubated with various concentrations of JNK-specific inhibitor SP600125 at 37ºC for 1 h and then incubated with various μM concentrations of CdSe-core QDs for another 24 hrs.

Cell extracts (60 mg) were immunoblotted with anti-p-JNK antibody. The lower panel shows an immunoblot of JNK1 protein from 60 mg cell extract.

JNK/AP-1 activity was evaluated by ELISA detection of phosphorylated c-Jun.

Cell extracts (60 μg) were analyzed for caspase-3 activity using Z-DEVD-AFC as the substrate.

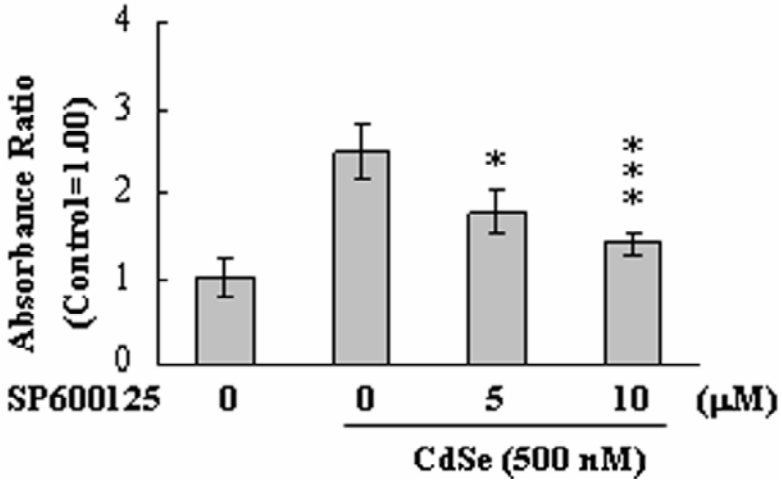
Apoptosis was detected with the Cell Death Detection ELISA kit.

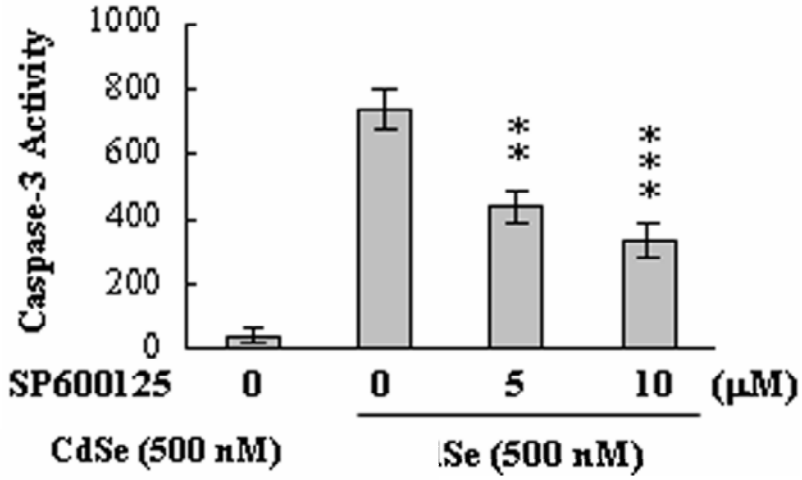
The generation of ROS was assayed by DCF-DA and is expressed as absorbance/mg of protein.

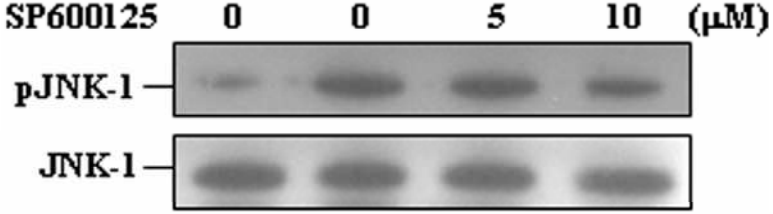
SP600125 actually reduces the JNK activity stimulated by CdSe core QDs to about 56% of that in inhibitor untreated cells.

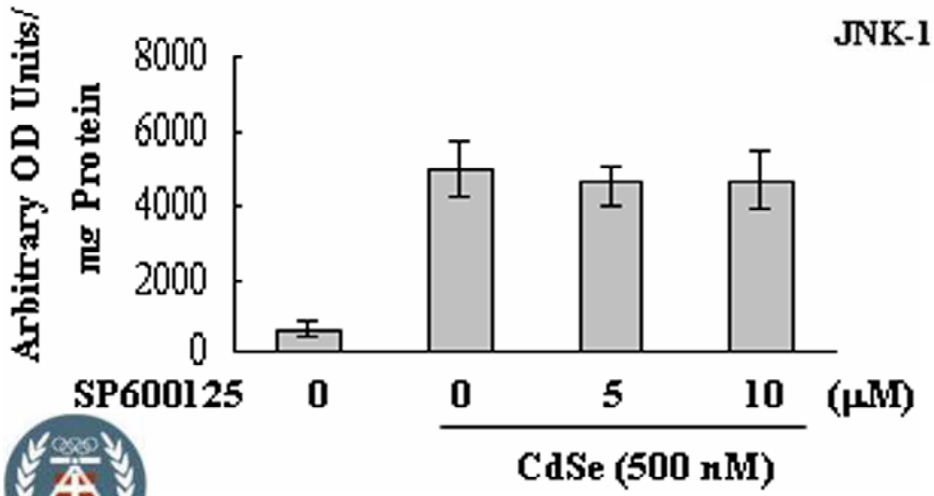
We also found that SP600125 inhibition of JNK activity further reduces caspase-3 activation clearly indicating that JNK activity is essential for caspase-3 activation and subsequent apoptotic biochemical changes during CdSe core QDs-induced apoptosis.

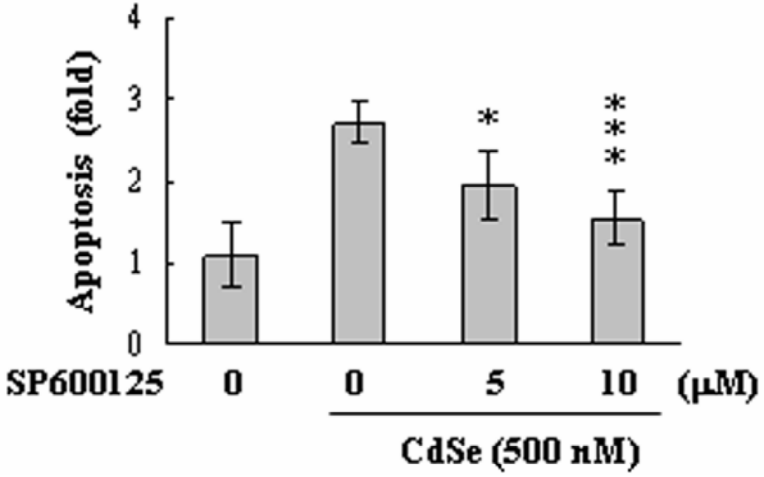
Effect of JNK-specific inhibitor SP600125 on quantum dots-induced caspase-3 and JNK activation and apoptosis in osteoblast cells











***P<0.05 versus control (untreated) group

Summary

curcumin induced osteoblast cell apoptosis were detected using CdSe/ZnS core shell QDs

cytotoxic effects of CdSe-core and CdSe/ZnS core shell QDs on osteoblast cells were analyzed.

JNK, caspase-9, and caspase-3 mediated apoptotic pathway by CdSe-core QDs.

these apoptotic biochemical events could not be detected in CdSe/ZnS core-shell QDs, as they have minimal cytotoxicity.

CdSe-core QDs-induced caspase-3 activation and apoptosis in osteoblast cells are mediated by JNK activity as shown by JNK specific inhibitor (SP600125)

Acknowledgments

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