SUPPORTING INFORMATION FOR

pH Dependent Activity of Dextran Coated Cerium Oxide Nanoparticles on Prohibiting Osteosarcoma Cell Proliferation

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Supporting information consists of 6 pages, 6 figures, and 1 table.

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X-ray photoelectron spectroscopy (XPS) measurements were performed to determine the relative atomic composition and the bonding state of the Ce on the surface of the nanoparticles. Around 1 atomic% Ce was detected in the 0.1 M dextran coated cerium oxide sample. The sampling depth for the XPS is approximately 10 nm. Assuming a uniform particle sample, the ability to detect a small amount of Ce suggests a layered structure with a dextran coating thickness of close to, but less than, 10 nm surrounding each particle. Figure S1 shows the four peaks of the Ce3d spectrum corresponding to the pairs of spin orbit doublets identified as Ce(III) oxides [**Ce^{3+}** state][1, 2].

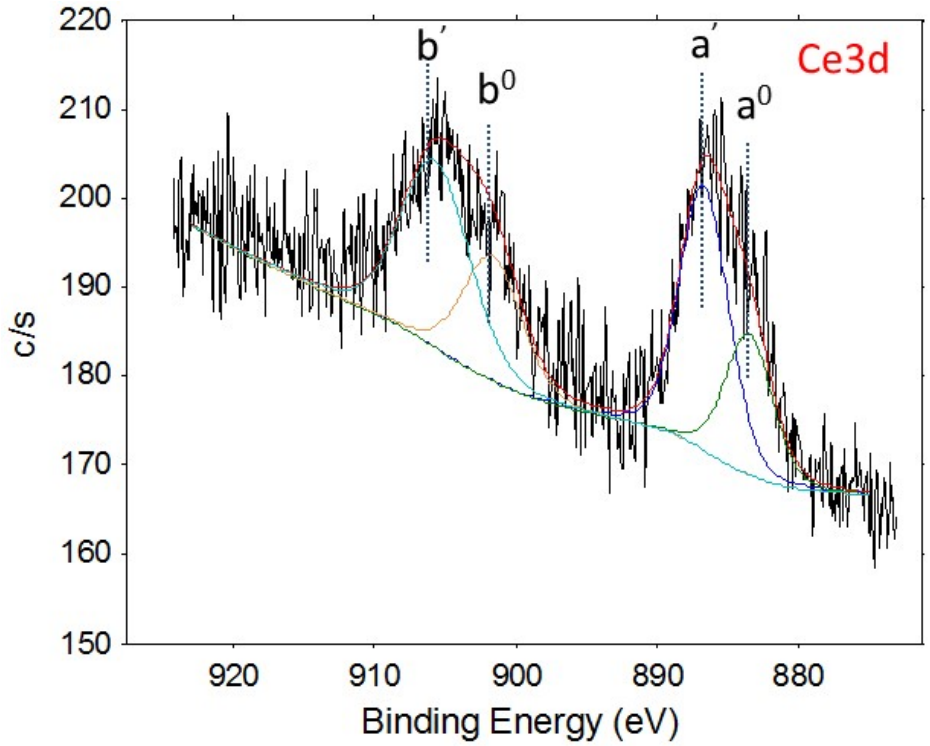

Figure S1: XPS Ce 3d peaks of cerium oxide nanoparticles. The Ce 3d spectra are composed of two core electrons $3d_{5/2}$ and $3d_{3/2}$ (labeled a and b) with multiplets (a' and a^0; b' and b^0) corresponding to the spin-orbits.

UV-visible spectroscopy measurements were performed in order to elucidate the oxidation state of cerium oxide nanoparticles at different pH values in solution. Cerium can strongly absorb the ultraviolet light at both oxidation states. Cerium (III) absorbance occurs between the 230 to 260 nm range, whereas cerium (IV) absorbs the light between the 300 to 400 nm range. [3] In contrary to the XPS results performed on dried particles immediately after synthesis, at pH 6 and pH 7 in solution cerium oxide nanoparticles were identified as Ce (IV) oxides as a single peak was obtained around 300 nm (Figure S2).

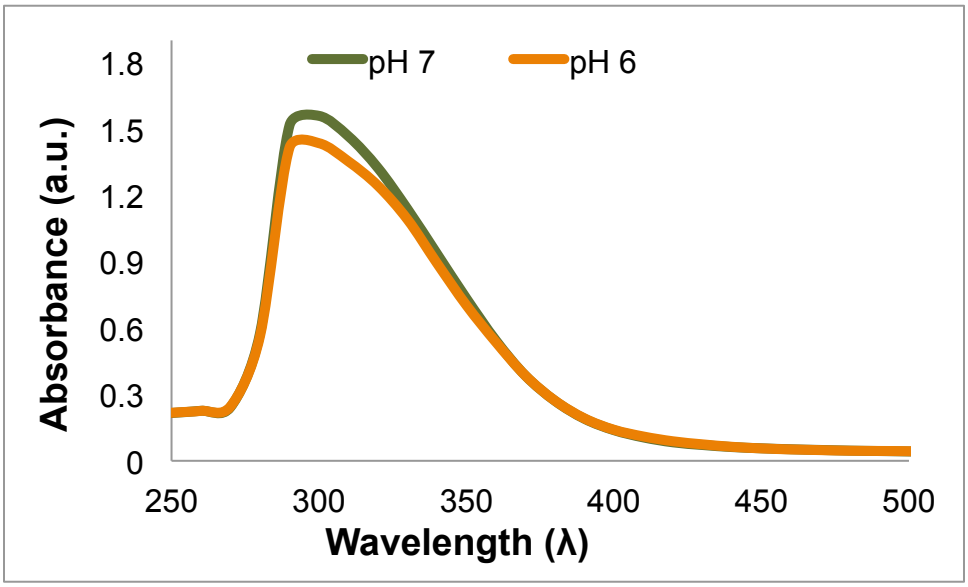

Figure S2: UV-visible absorption spectroscopy of cerium oxide nanoparticles in solution at different pHs.

Viability assay results in terms of cell density were shown below.

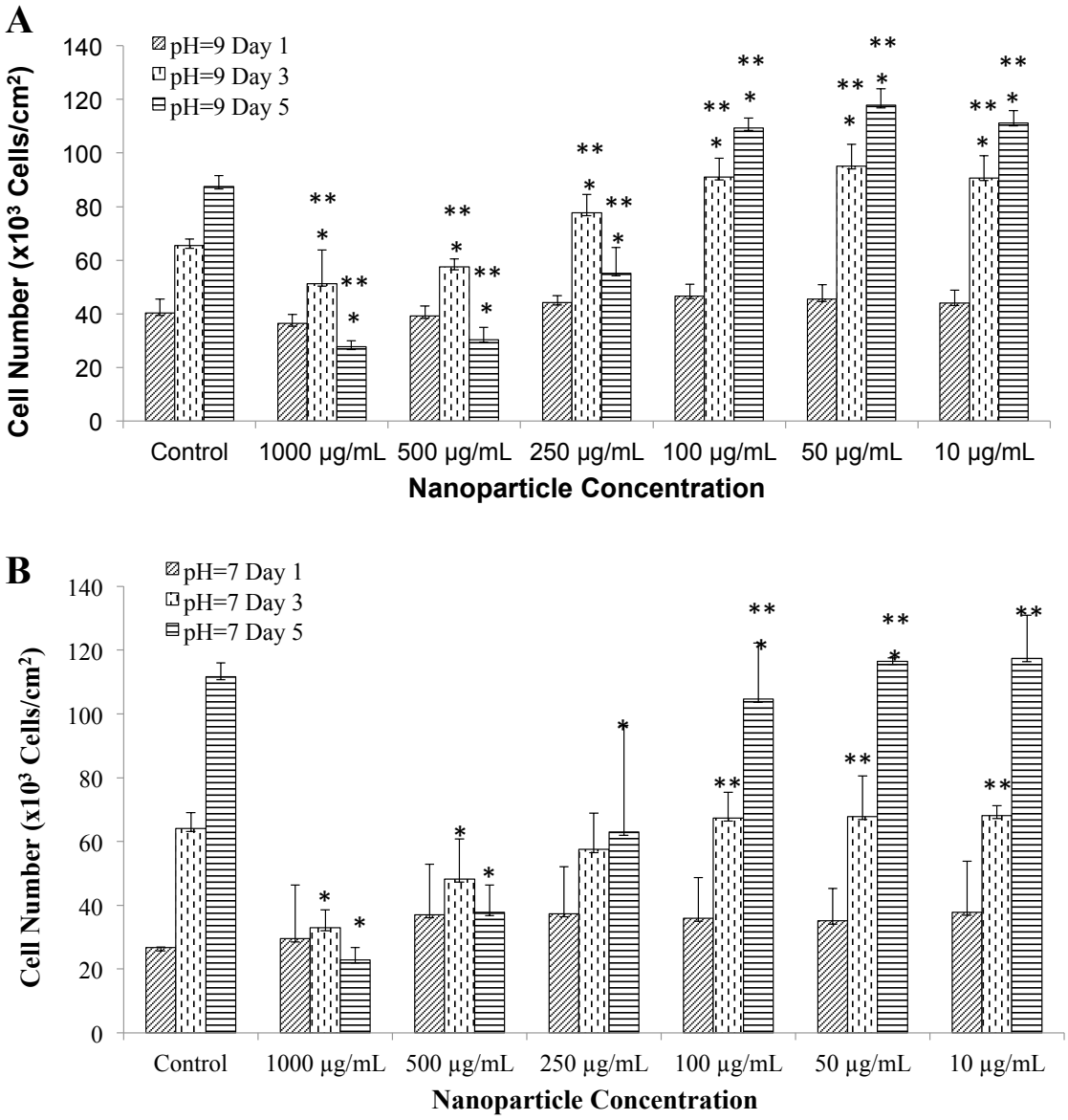

Figure S3: Toxicity effect of 0.1 M DCN against osteosarcoma cells at various concentrations after 1, 3 and 5 days treatment at (A) pH 9 and (B) pH 7. Data = mean +/- SEM and *$p < 0.05$ compared to control at same time period and **$p<0.05$ at the same DCN concentration at the same time period. Control groups are cells treated with media at (A) pH 9 and (B) pH 7.

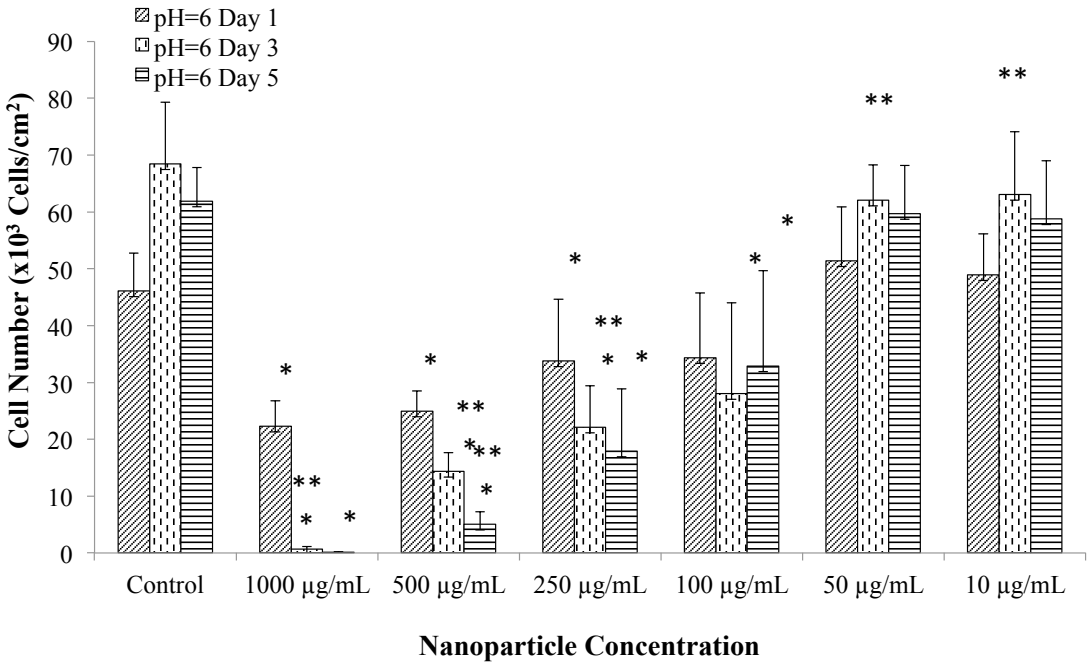

Figure S4: Toxicity effect of 0.1 M DCN against osteosarcoma cells at various concentrations after 1, 3 and 5 days treatment at pH 6. Data = mean +/- SEM and *p < 0.05 compared to control at the same time period and **p<0.05 at the same DCN concentration and the same time period. Control groups are cells treated with media at pH 6.

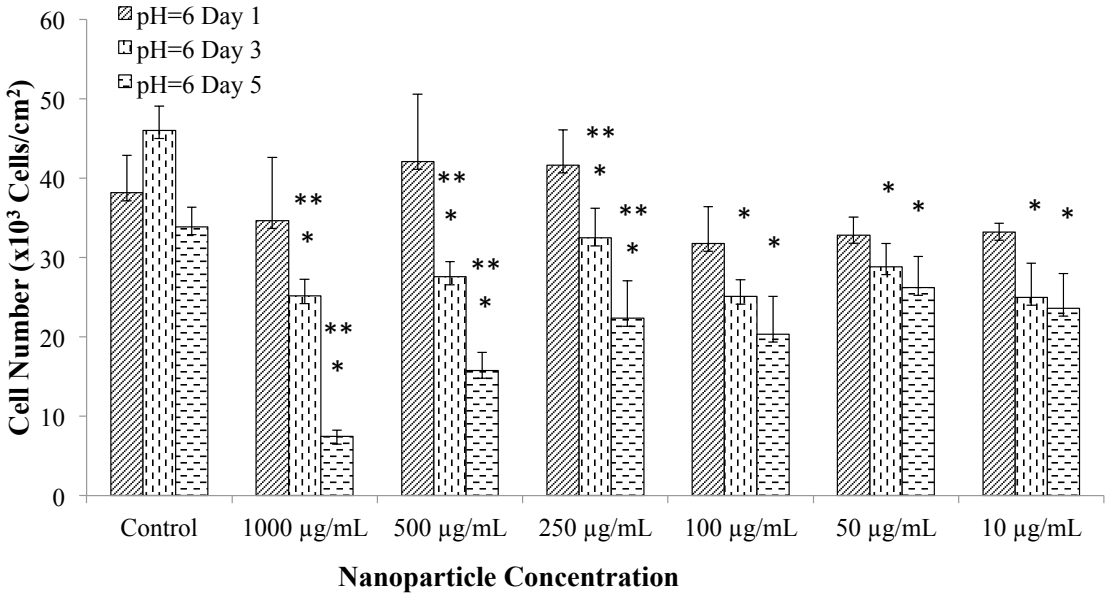

Figure S5: Toxicity effect of 0.1 M dextran DCN against healthy osteoblast cells at various concentrations after 1, 3 and 5 days treatment at pH 6. Data = mean +/- SEM and *$p < 0.05$ compared to control at the same time period and **$p<0.05$ at the same DCN concentration and time period. Control groups are cells treated with media at pH 6.

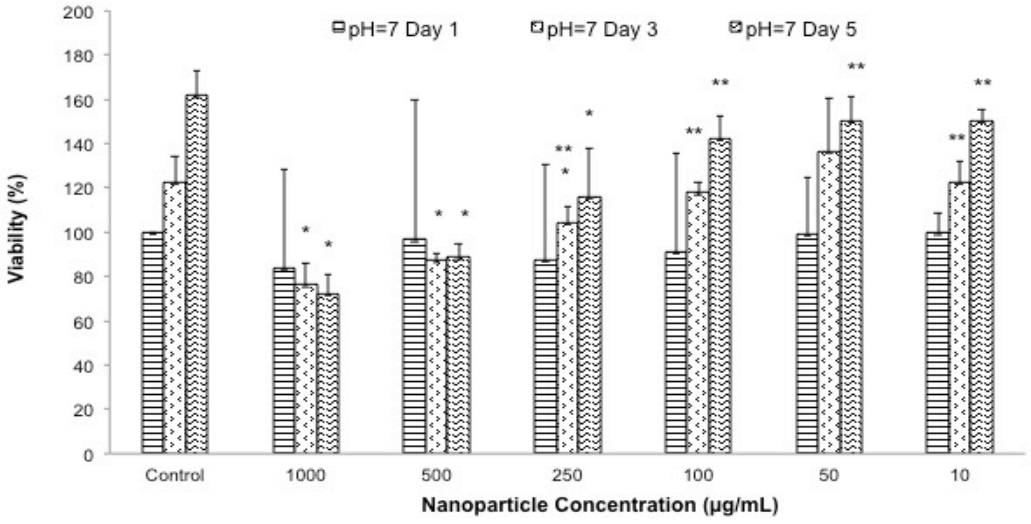

Figure S6: Toxicity effect of 0.1 M dextran DCN against healthy osteoblast cells at various concentrations after 1, 3 and 5 days treatment at pH 7. Data = mean +/- SEM and *$p < 0.05$ compared to control at the same time period and **$p<0.05$ at the same DCN concentration and the same time period. Control groups are cells treated with media at pH 7.

0.1 M dextran coated nanoparticles were synthesized in 30 % ammonium hydroxide solution and used in cell culture without washing them. Thus, the resulting pH values of particles varied once the particles were dispersed in cell culture media at various concentrations. Below, Table S1 shows the concentration versus pH variation for dextran coated nanoceria particles.

Nanoparticle Concentration (µg/mL)	pH
10	7.8
50	8.3
100	8.7
250	9.2
500	9.5
1000	9.8

Table S1: Concentration dependent DCN pH values

References

(1) Naganuma, T., Traversa, E. Stability of the ce 3+ valence state in cerium oxide nanoparticle layers. *Nanoscale.* **2012**, *4*, 4950-3.
(2) Bêche, E.; Charvin, P.; Perarnau, D.; Abanades, S., Flamant, G. Ce 3d xps investigation of cerium oxides and mixed cerium oxide (cextiyoz). *Surface and Interface Analysis.* **2008**, *40*, 264-7.
(3) Heckert, E. G.; Karakoti, A. S.; Seal, S., Self, W. T. The role of cerium redox state in the sod mimetic activity of nanoceria. *Biomaterials.* **2008**, *29*, 2705-9.