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Calcium phosphate nanoparticles primarily induce cell necrosis through lysosomal rupture: the origination of material cytotoxicity

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Supporting Information

1. Characterization of calcium phosphate (CaP) nanoparticles (NPs): The diameter of CaP NPs were sizes by dynamic light scattering (DLS) photometer (ZEN 3600, Malvern, England), 200 μL 140 μg/mL, 300 μg/mL, 360 μg/mL, 440 μg/mL CaP suspensions were dispersed in 1 mL ethanol with 10 min ultrasonic treatment. Then diluted the suspension for 300-fold in ethanol for measurement. 300 μg/ml CaP suspension were washed twice and dispersed in ethanol, after that the samples were loaded on the silica specimen stubs and the energy dispersive X-ray spectroscopy (EDX) analyses was performed by SEM (S-4800, Hitachi, Japan). Other samples were used for XRD (X'Pert PRO, PANalytical B.V., Netherlands) and FT-IR (IRAffinity-1, Shimadzu, Japan) analyses.

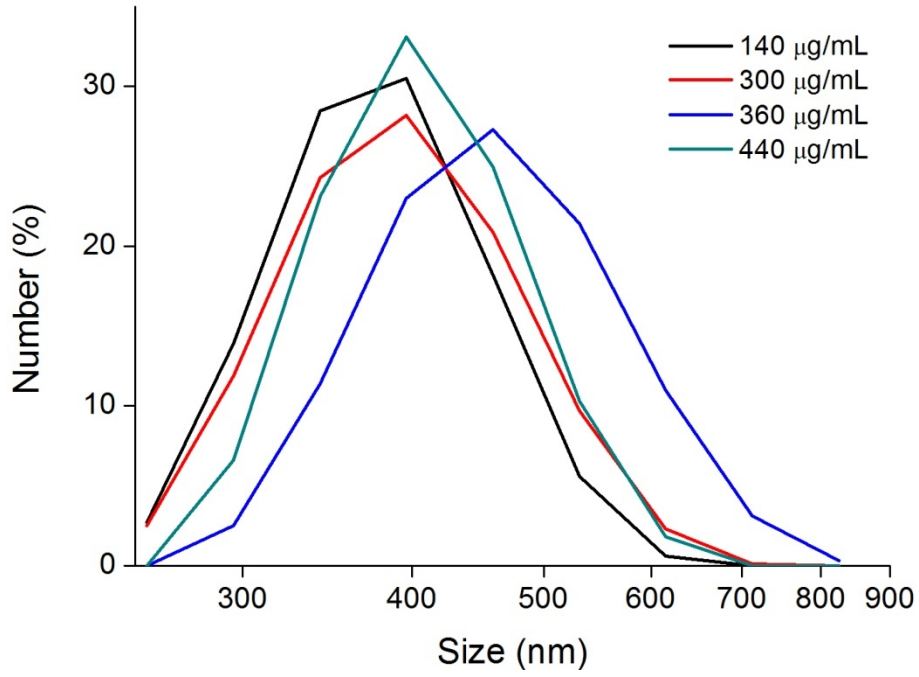

Fig. S1. Diameter of different concentrations of CaP suspension.

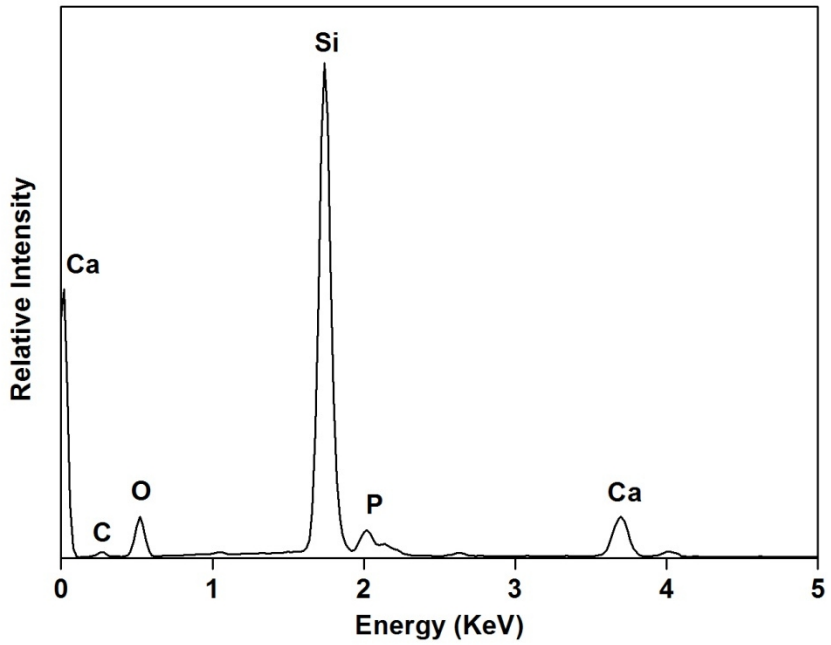

Fig. S2. EDX analyses of 300 μg/ml CaP suspensions.

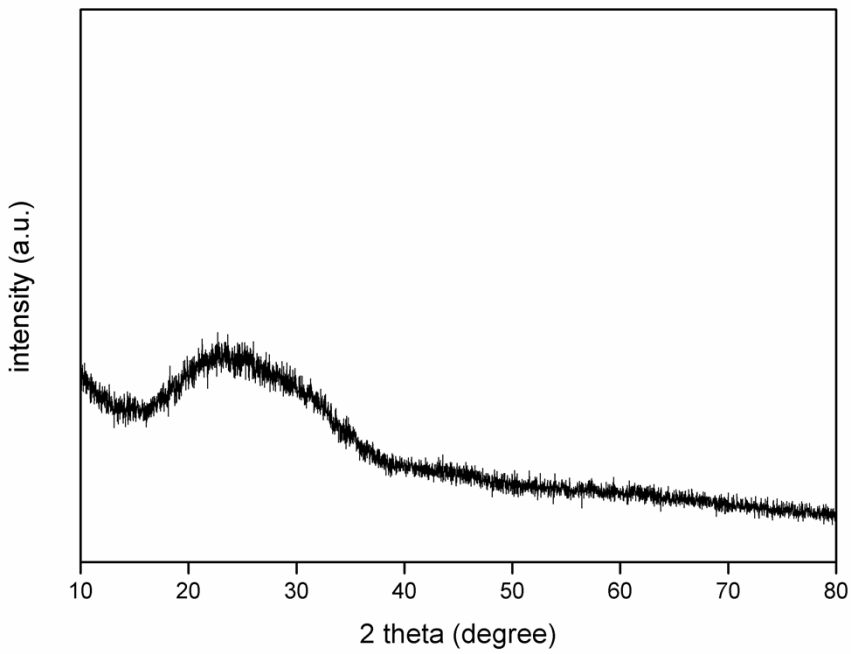

Fig. S3. XRD analyses of 300 μg/ml CaP suspensions, which matches with the broad XRD patterns of amorphous calcium phosphate.[1]

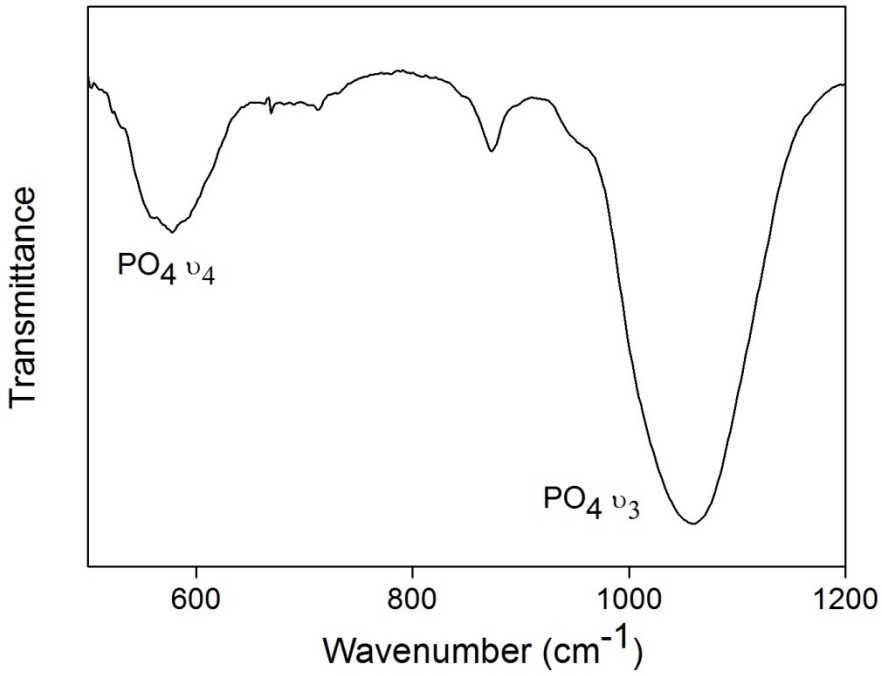

Fig. S4. IR analyses of 300 μg/ml CaP suspensions. The broad absorption bands at about 1055 cm^{-1} presents for phosphate υ_3 vibrations and 570 cm^{-1} presents for phosphate υ_4 bending. This spectrum matches with ACP phase.[2]

Table S1. Elemental analyses of 300 µg/ml CaP suspensions.

Elements	Weight Percent (%)	Atoms Percent (%)
C	10.36	18.02
O	30.85	40.27
Si	47.15	35.06
P	3.81	2.57
Ca	7.82	4.07

2. Stability of CaP NPs: 20 mL of 300 µg/ml CaP suspension were used and the pH was decreased by adding 1 M HCl and 3 ml suspension was taken at each pH. The optical density at 320 nm for CaP was measured with UV spectrophotometer (T6, Puxi, China) to test the solubility of CaP suspension in acidic solution.[3]

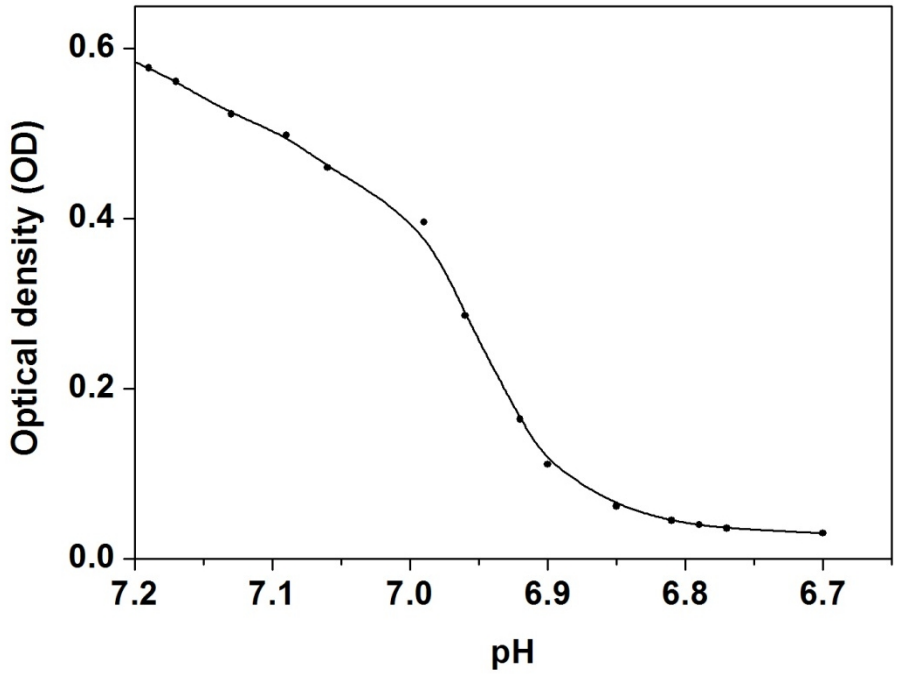

Fig. S5. Optical density of CaP against pH values. The Result indicates that CaP dissolve at slight acid environment.

3. Treatment by using SiO_2 NPs: The apoptotic and necrotic cells treated with 300 μg/ml CaP or SiO_2 suspensions separately were detected according to the instruction of Hochest33342/PI apoptosis detection kit (Beyotime Institute of Biotechnoligy, China). 2.0×10^5 cells were seeded in 6 well plates per well, cultured for 18 h and mixed with different concentrations of CaP suspension for 4 h. Then the cells were stained with Hochest33342 and PI and immediately observed with fluorescent microscope (TE2000-5, Nikon). Normal cells (Hochest 33342 (-), PI (-)); Necrotic cells (Hochest 33342 (+), PI (+)); Apoptotic cells (Hochest 33342 (+), PI (-)).[4]

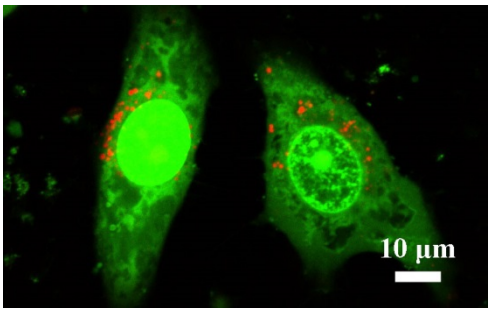

Fig. S6. 300 μg/ml SiO_2 suspension treated HepG2 stained by AO (bar = 10 um). Red fluorescent presents for lysosomes, green fluorescent presents for cytoplasm and nucleolus. This figure exhibits an apoptotic process of HepG2.

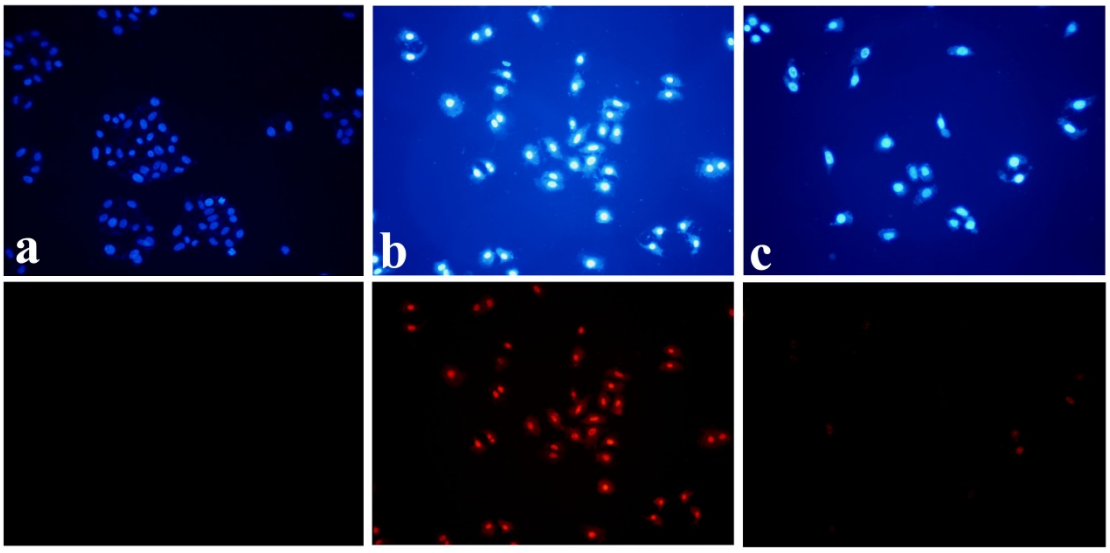

Fig. S7. Live cell imaging stained with Hochest 33342/PI. Blue fluorescent presents for Hochest 33342, red fluorescent presents for PI. (a) Normal cells. (Hochest 33342 (-), PI (-)). (b) Cells treated with 300 μg/ml CaP suspensions exhibits necrotic process. (Hochest 33342 (+), PI (+)). (c) Cells treated with 300 μg/ml SiO_2 suspension exhibits apoptotic process. (Hochest 33342 (+), PI (-)).

4. Changing of lysosomal pH and rupture: 1.0×10^6 HepG2 were incubated with 0.1 mg/mL FITC-dextran (40 kDa, Sigma, USA) for 3 days prior to the exposure to CaP NPs. 1.0×10^4 HepG2 cells per well were cultured on cover glass for 18 h in the presence of 0.1 mg/mL FITC-dextran and then exposed to 140 μg/mL CaP NPs for 0.5, 1.0, 1.5 and 2 h. Under the microscope, the green fluorescence intensity of FITC-dextran was measured by using the software of NIS-Element D (Nikon, Japan). The average fluorescence intensity = (total fluorescence intensity) / (number of cells). During the fluorescence intensity examination, each group contained at least 50 cells for the statistics. Since the lysosomal pH is proportionate to the fluorescent intensity,[5] the relative lysosomal pH could be estimated by the ratio of the average fluorescence intensity to that in control group.

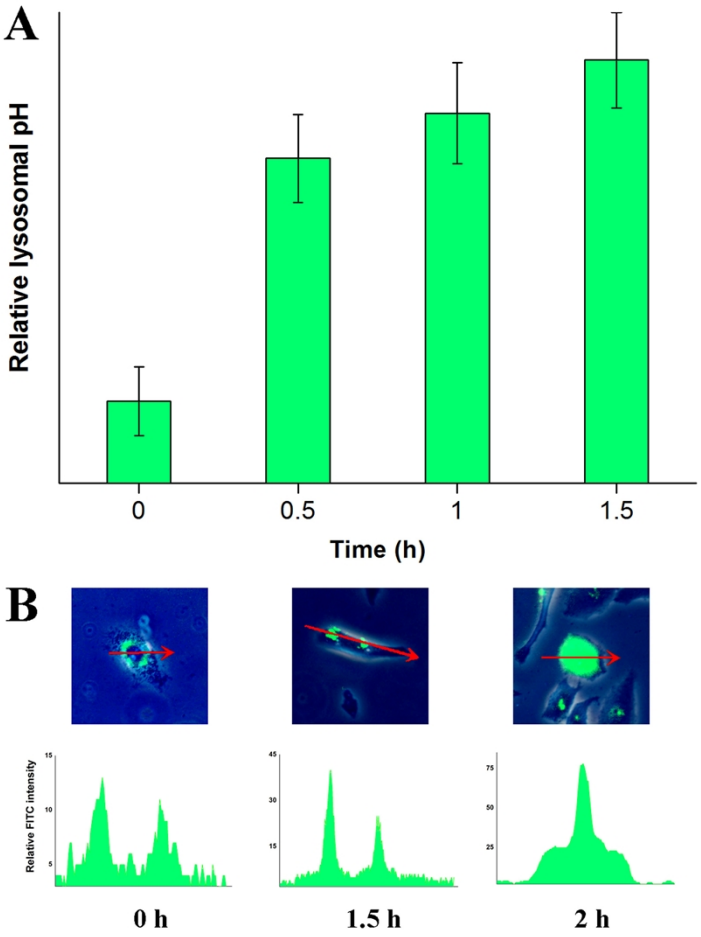

Fig. S8. HepG2 were incubated with 40 kDa FITC-dextran for lysosomal pH measurement and lysosomal rupture imaging. (A) Time dependent relative lysosomal pH. The results indicated that lysosomal pH increased after incubating with CaP NPs. (B) The fluorescence intensity and distribution of FITC-dextran at different incubation period with CaP NPs. FITC-dextran was distributed in cytoplasm with low fluorescence intensity at 0 h; the fluorescent intensity increased at 1.5 h, which was attributed to the CaP dissolution-induced pH increasing in lysosomes. After 2 h, the lysosomes disrupted so that FITC-dextran leaked to nucleus; moreover, the fluorescence intensity was further increased due to the enhanced fluorescence of fluorescein under non-acidic conditions.

Reference

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