Supporting information

Controlled Synthesis and Transformation of nano-Hydroxyapatite with Tailored Morphologies for Biomedical Applications

Ma Xiaoyu[a,b], Li Yuanyuan[c], Wang Chengwei[a,b], Sun Yi[c], Ma Yifan[a,b], Dong Xiuling[c], Qian Jiangchao[c], Yuan Yuan*[a,b,c]and Liu Changsheng*[a,b,c]

a. Key Laboratory for Ultrafine Materials of Ministry of Education, East China University of Science and Technolo-gy, Shanghai 200237, P R China

b. Engineering Research Center for Biomedical Materials of Ministry of Education, East China University of Sci-ence and Technology, Shanghai 200237, P R China

c. The State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shang-hai 200237, P R China

E-mail: yyuan@ecust.edu.cn; liucs@ecust.edu.cn

Experimental:

Drug-loading and releasing profile

The drug-loaded nanoparticles were prepared by mixing DOX aqueous solution (1 mg/mL, 10 mL) with 20 mg dried particles for 48 hr at room temperature under continuous stirring. After the loading procedure, the particles were washed with deionized water twice and centrifuged at 8000 rpm for 10 min. The supernatants were all collected and the concentration of the free DOX in the supernatant was measured by UV-vis spectroscopy at 480 nm and compared to the standard curve. The drug loading content and entrapment efficiency can be calculated from the difference in DOX amount.

As for the release profile of DOX, 10 mg DOX-loaded nanoparticles were dispersed in 10 mL PBS with different pH values (7.4 and 5.4) with gentle stirring. At various time intervals, the suspensions were centrifuged at 8000 rpm for 10 min and the concentration of released DOX in the supernatant was withdrawn and determined by UV-vis spectroscopy. Meanwhile, 10 mL fresh PBS was added as the replacement.

Cell culture

Different cells were used for the determination of cell viability. Hela and L02 were purchased directly from the American Type Culture Collection (ATCC), and incubated in Dulbecco's modified Eagle's medium (α-MEM) supplemented with 10% fetal bovine serum (FBS) at 37 ºC and 100% humidity with 5% CO2. The cell density was calculated by cytometer after detached with 0.25% trypsin/0.03% ethylene diamine

tetraacetic acid (EDTA). All the cell culture reagents were obtained from Sigma-Aldrich, Saint Louis, MO, USA.

Cytotoxicity study in vitro

The cytotoxicity of DOX/CaP was assessed by the MTT assay. Hela and L02 cells were seeded in 96-well plates with a density of 5×103 cells/well and incubated for 24 h. Then, cells were treated with blank CaP, free DOX, DOX/CaP at equivalent DOX concentration and incubated for another 72 h. MTT solution (5 mg.mL-1) was added to each well at a volume of 30 μL and incubated for another 4 h at 37 °C. The formed formazan crystals were dissolved with 200 μL DMSO for 10 min. The absorbance was examined at 570 nm using an automated plate reader. The experiments were performed in three replicates. Cell viability (%) was calculated according to the following formula:

Cell viability (%) = (OD test /OD control) ×100 %

Intracellular distribution of DOX/CaP

For confocal observation, Hela/DOX cells were seeded in 6-cm culture dishes (4×104 cells per dish) and incubated for overnight. Then, DOX and DOX/CaP were added into the above dishes at same DOX concentration and incubated for 4, 24 h. After incubation, cells were washed with PBS and fixed by 3.7% paraformaldehyde for 15 min and stained with DAPI for 15 min at 37°C respectively. The localization of DOX and DOX/CaP was observed by CLSM.

Hemolysis test

To evaluate the safety of the nanoparticles for further in vivo experiment, firstly the hemolysis test was performed by utilizing the rabbit blood cells from New Zealand rabbit. 4 mL blood was obtained through auricular vein, centrifuged for 10 min under 1500 rpm and washed by saline(0.9% sodium chloride solution) for three times. The red blood cells obtained were diluted to 2% suspension by addition of saline. 0.5 mL 2% red blood cell suspensions were added into 0.5 mL DOX and DOX-loaded particles suspensions(without solvothermal treatment and with solvothermal treatment) with different concentrations and incubated at 37 °C for various hours. At the selected time intervals, the samples were centrifuged for 5 min under 5000 rpm and the supernatants were collected for the determination of OD value at 570 nm via UV-vis spectroscopy. 0.5 mL saline and 0.5 mL distilled water were also mixed with 0.5 mL 2% red blood cell suspensions respectively, to serve as negative and positive controls. The hemolysis percentage could be calculated by using the following formula:

$$\text{Hemolysis percentage}\ (\%) = \frac{\left[OD_{sample} - OD_{negative} \right] \times 100}{OD_{positive} - OD_{negative}}$$

Mechanical Test of HA incorporated PLA

Different amount of HA (5%, 10%, 25%, 40% and 50%) was incorporated into PLA (7-8 KDa, purchased from) through solvent evaporation. PLA was firstly dissolved into trichloromethane to form a homogeneous solution. HA was then added into the solution under stirring. The PLA/HA solution was then transferred into a dish and dried to remove the remaining trichloromethane. After the film of PLA/HA was formed, the

samples were cut by standard cut-off knife (sample length*width: 25mm*4mm). The samples were tested by Universal Tensile Testing Machine (Shanghai Hengyi Equipment Co. Ltd).

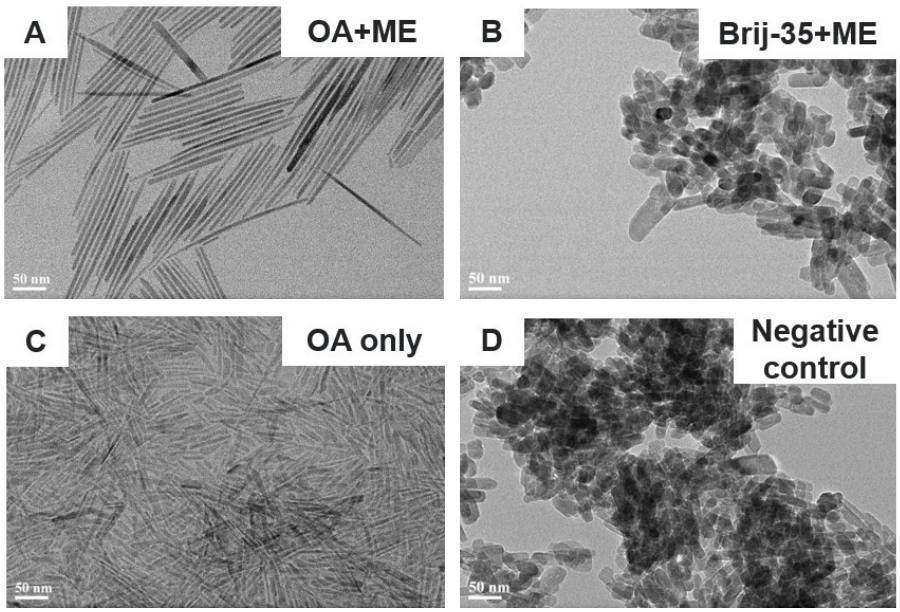

Figure S1 Particles obtained under different conditions after solvothermal treatment at 140°C A: oleic acid and microemulsion B: Brij35 and microemulsion C: Oleic acid without microemulsion D: without oleic acid and microemulsion

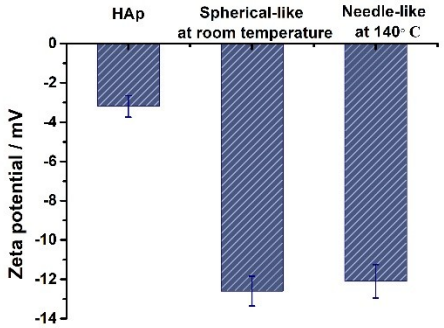

Figure S2 Zeta potential of HA from traditional chemical precipitation as negative control, spherical-like particles obtained at room temperature for 2 hrs and needle-like ones at 140 °C for 2 hr

	Loading amount / %	Entrapment efficiency / %	Surface Area / m^2/g
Spherical Morphology	25.42%	98.03%	57.1796
Needle-like Morphology	23.46%	87.31%	32.8704

Table1. Loading profile and surface area of nano-particles

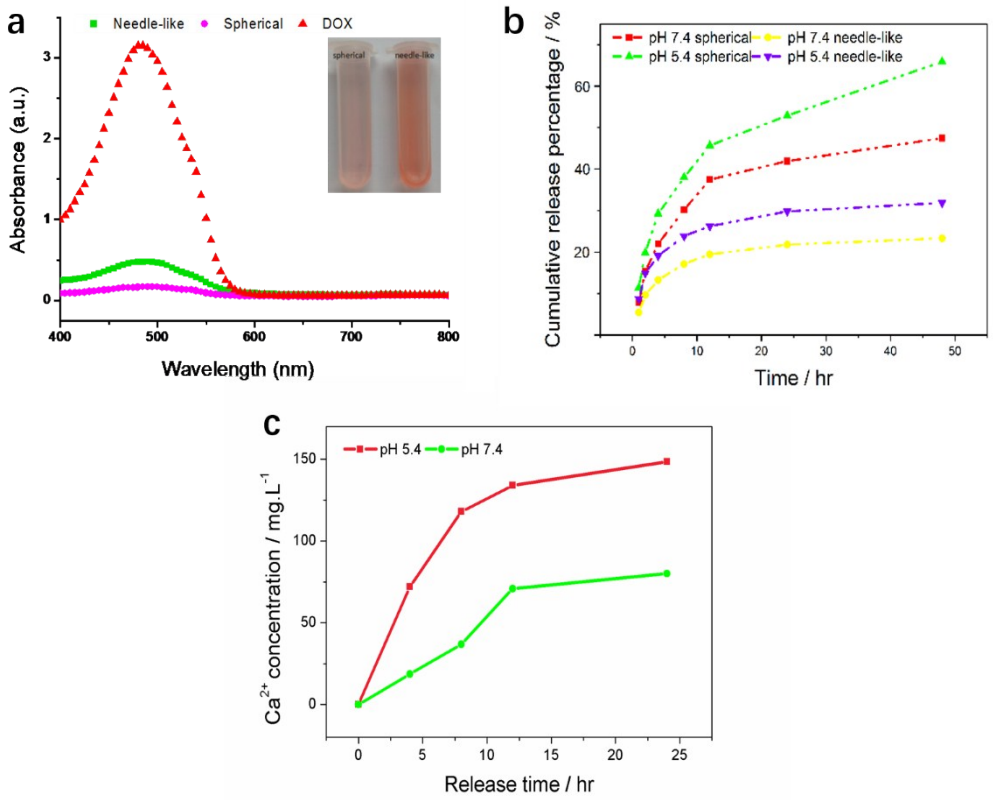

Figure S3 (a) UV-vis absorption spectra of DOX solution before and after interaction with both spherical and needle-like NPs (Inset: photos of supernatants containing unloaded DOX after absorption) (b) Cumulative release percentage of DOX by spherical and needle-like particles at different pH (c) Ca^{2+} dissolution of spherical particles at different pH

The supernatants after centrifugation and collection exhibited lower peaks at 480 nm in UV-vis absorption spectra, indicating the low amount of unloaded DOX remaining in the solution. The drug release profile was studied in PBS solutions at pH 7.4 and pH 5.4, mimicking physiological conditions at normal and tumor site in Fig 10(b), where the cumulative release amount was up to 65% (nearly 1000 μg) at pH 5.4 while drastically decreased to 44% (about 600μg) at pH 7.4. The pH dependent behavior for drug release was mainly due to the difference in electrostatic interactions and the solubility of CaP at neutral and acidic environment, confirmed by the uplifted amount of Ca2+ dissolution in acidic environment than that at pH 7.4 in S3(c).

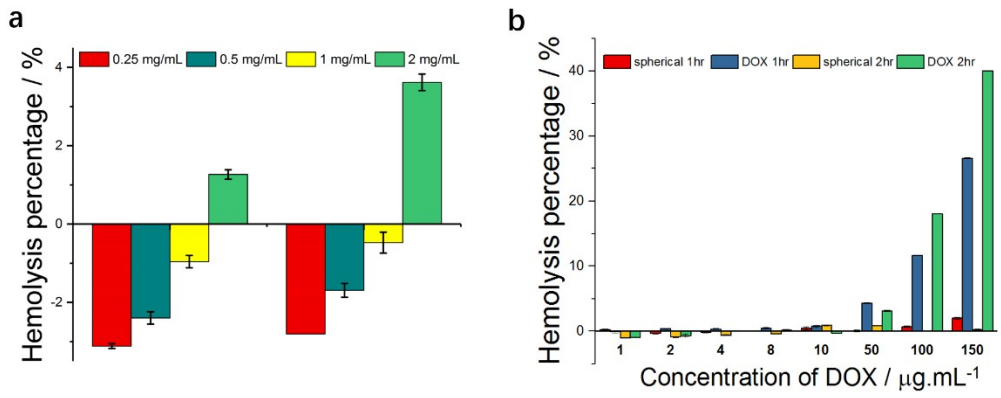

Figure S4. Hemolysis percentage of (a) spherical particles and (b) DOX-loaded particles

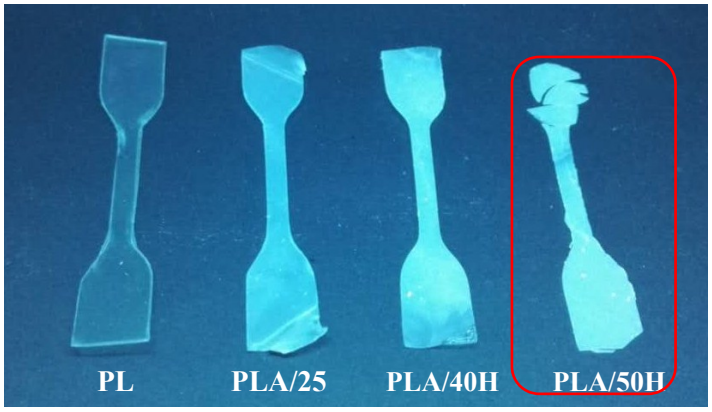

Figure S5. Samples with different amount of HA incorporation and easy break of samples at 50% content

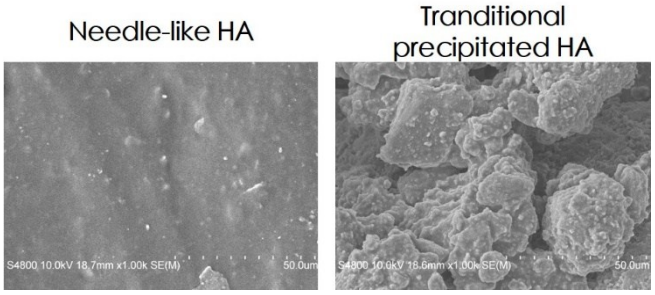

Figure S6. Samples of composite with needle-like HA and traditional precipitated HA,

Figure S6 showed that a clear agglomeration of particles and a clear phase separation between the samples at 10% incorporation. As for normal HA particles, the poor compatibility with polymer leads to heterogeneous distribution in the matrix while the current system showed no obvious agglomeration of HAps.