

Electronic Supplementary Information

Preparation of optimized lipid-coated calcium phosphate nanoparticles for enhanced in vitro gene delivery to breast cancer cells

Jie Tang, Li Li, Christopher B. Howard, Stephen M. Mahler, Leaf Huang, Zhi Ping Xu

Table S1. Polydispersity index (PDI) value of LCP nanoparticles synthesised at different Ca/P ratios; the data is presented as the average size \pm standard error (n= 3).

Ca/P ratio	25	50	100	200	400
PDI	0.605 \pm 0.086	0.532 \pm 0.021	0.263 \pm 0.014	0.310 \pm 0.055	0.441 \pm 0.141
Size (nm)	194.4 \pm 78.0	73.6 \pm 11.2	48.4 \pm 3.9	45.4 \pm 2.0	47.8 \pm 1.9
Zeta potential (mV)	-7.5 \pm 1.8	-7.8 \pm 0.1	-11.7 \pm 1.2	-11.5 \pm 0.9	-14.7 \pm 1.0

Table S2. Encapsulation efficiency and loading capacity of LCP; the data is presented as average size \pm standard error (n= 3).

Ca/P molar ratio	Mass of LCP NPs (μ g)	Adding amount (Cy3-dsDNA, μ g)	Loading efficiency (%)	Loading capacity (μ g/mg)
50	907	40	72.8 \pm 4.9	32.1 \pm 2.2
100	454	40	66.6 \pm 2.4	58.8 \pm 2.1
200	227	40	36.9 \pm 6.4	65.0 \pm 11.3
400	113	40	32.9 \pm 5.2	116.1 \pm 18.2

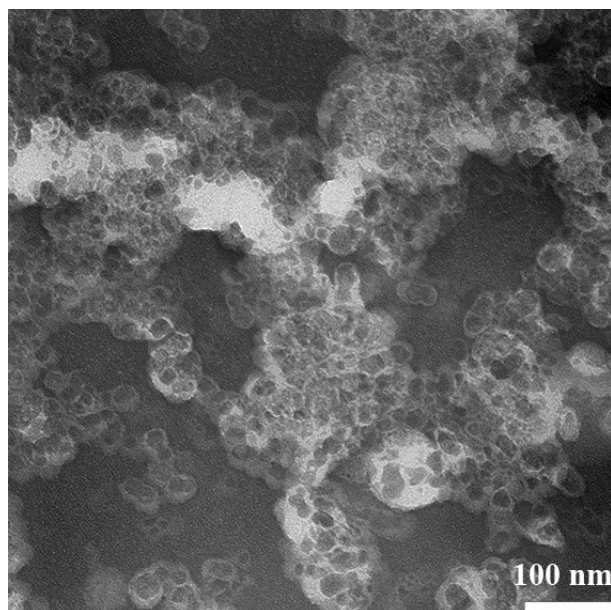


Fig. S1 TEM image of LCP prepared at Ca/P of 25 after negative staining.

In the TEM image of LCP NPs synthesised at the Ca/P ratio of 25, aggregates were clearly observed.

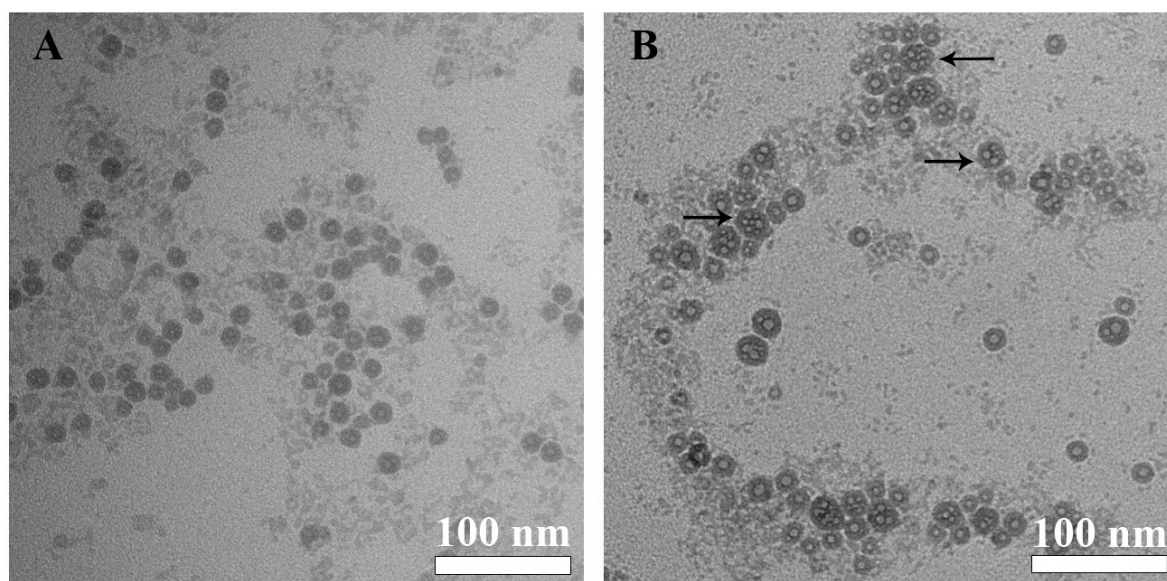


Fig. S2 TEM image of CaP cores prepared at Ca/P of 100 (A) and 400 (B). Arrows in B indicate the porous structure of CaP cores.

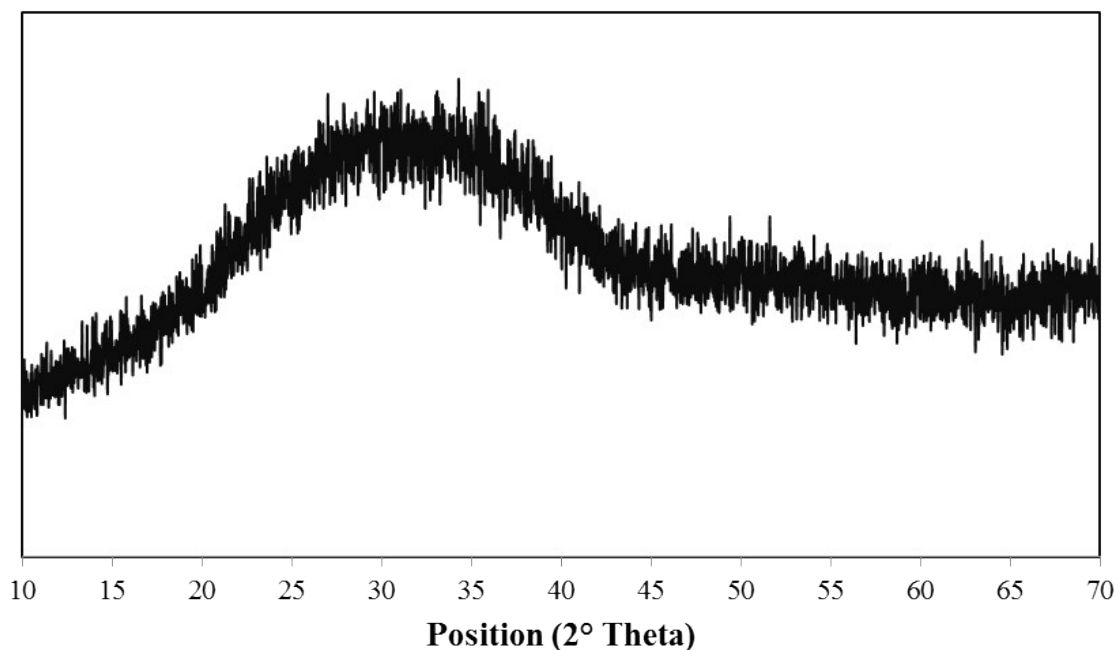


Fig. S3 The XRD pattern of as-prepared LCP NPs at the Ca/P ratio of 100.

The X-ray powder diffraction measurements were taken in a Philips PW 3040/60 X'Pert PRO (PANalytical) diffractometer (Netherlands) using nickel filtered $\text{CuK}\alpha$ radiation at 1.54 Å. The resultant intensity data was processed using in-built PC-APD diffraction software to monitor the peak position and its corresponding intensity data correctly. The LCP NP suspension was dropped on a glass slide and dried, and repeated dropping-drying two more times. Then the measurement was taken from $2\theta = 10^\circ$ to 70° at 0.02° interval. The XRD pattern of LCP NPs prepared at the Ca/P molar ratio of 100 shows no obvious diffraction peaks, indicating the nature of an amorphous calcium phosphate (ACP) phase

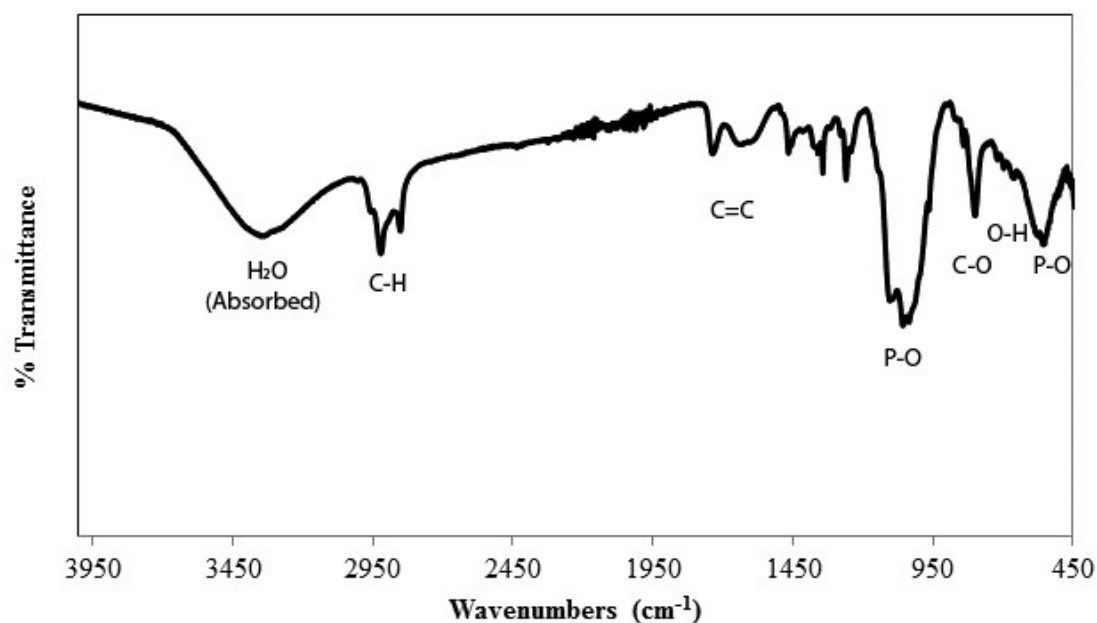


Fig. S4 FT-IR spectrum of LCP NPs.

A NEXUS 670 FT-IR spectrometer (Thermo Nicolet, Madison, WI) was used to record the infrared spectra of the LCP powders at a resolution of 4 cm^{-1} in the range of $4000\text{--}400\text{ cm}^{-1}$ after 40 scans. In Fig. S4, most characteristic chemical groups in the FTIR spectrum of ACP are PO_4^{3-} , CO_3^{2-} , as well as HPO_4^{2-} , and the broad band between $3400\text{--}3500\text{ cm}^{-1}$ (OH-) have been observed, and confirm the formation of CaP precipitates, and the peaks at around 2900 cm^{-1} (C-H bonds) indicate the presence of lipids.

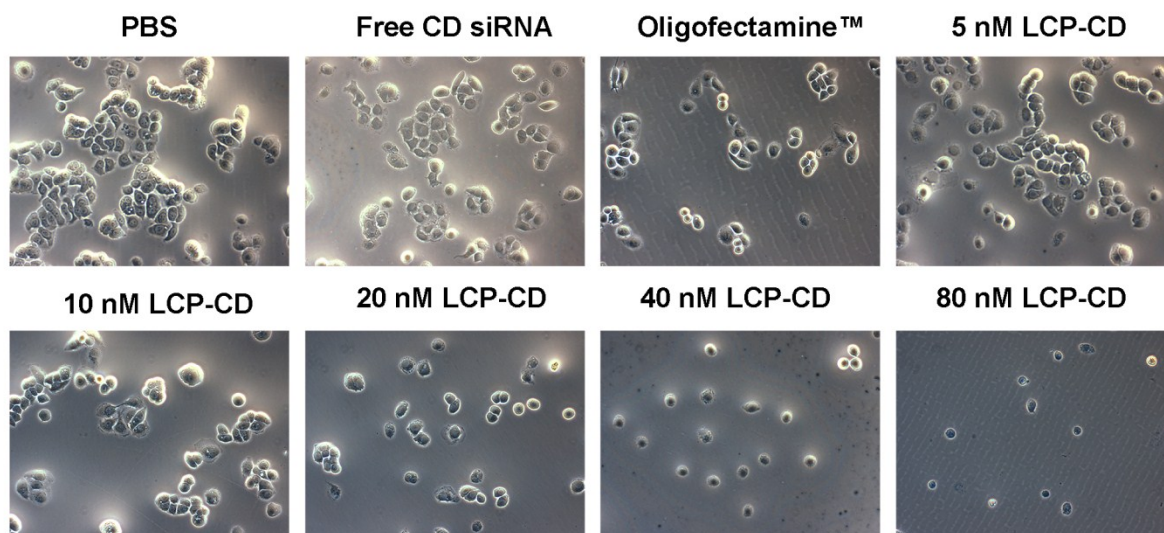


Fig. S5 The morphology of MDA-MB-468 cells in vitro after treatment with LCP-CD siRNA NPs. Oligofectamine™ loaded with 80 nM CD siRNA was used as a positive control.

To evaluate the viability of MDA-MB-468 cells after LCP-CD siRNA NP treatment, after 48 h CD siRNA transfection, the morphology of MDA-MB-468 cells was further measured. As shown in Fig. S5, the morphology changes were observed in the LCP-CD NP-treated groups. Consistent with previous MTT assay results, the change of cell morphology becomes more severe with the increase of CD siRNA concentration in LCP NPs. Treatment with LCP-CD siRNA NPs at low CD siRNA concentrations (5 and 10 nM) caused only small amount of MDA-MB-468 cells to become round or oval-shaped, and the majority of cells were attached well to the plate. With increasing the CD siRNA concentration in LCP NPs, more cells became around with a small cell size. Particularly, LCP-CD siRNA NPs with the siRNA concentration of 40 and 80 nM induced more severe cell morphology changes than Oligofectamine™. Most cells died, floated and clustered in the complete medium at 80 nM of CD siRNA in LCP NPs.

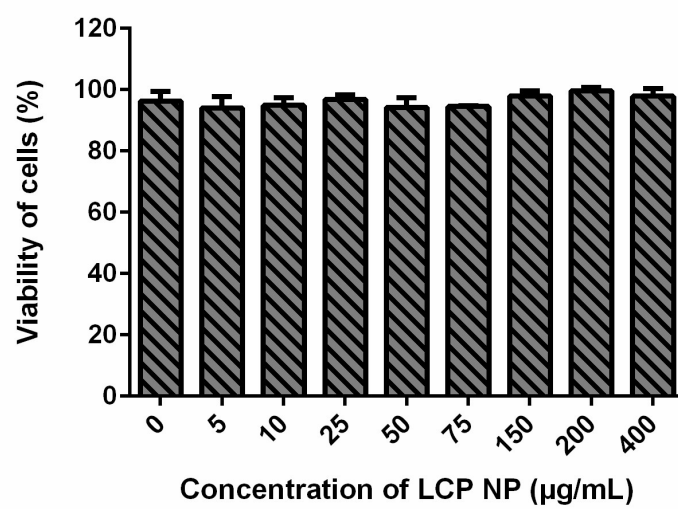


Fig. S6 Viability of MDA-MB-468 cells in the presence of LCP NPs.