Supplementary Information

Stabilized calcium phosphate hybrid nanocomposite using a benzoxaborole-containing polymer for pH-responsive siRNA delivery

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Synthesis of benzoboroxole (BOB). The compound was synthesized according to the paper reported[1]. In brief, To a cooled (0 °C) suspension of 2-Formylbenzeneboronic acid (5 g, 0.033 mol) in 50 mL CH_3OH, $NaBH_4$ (6.3 g, 5 eqs, 0.165 mol) was added slowly and stirred for 1 h. The progress of the reaction was monitored by TLC. Water was added to the mixture at 0°C to stop the reaction and MeOH was evaporated afterward. The mixture was extracted with ethyl acetate (50 mL×3) and washed with brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated and precipitated with hexane. Purification using silica gel column chromatography (ethyl acetate/hexane=1:1) to get pure benzoboroxole (4 g) with a yield of 75%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.77 (d, 1H), 7.50 (ddd, 1H), 7.44 - 7.31 (m, 2H), 5.13 (s, 2H).

Synthesis of 6-nitrobenzoboroxole. To a mixture of concentrated HNO_3 and concentrated H_2SO_4 (v/v=3:2) maintained at precooled ethyal alcohol (-45 °C), BOB (1 g, 7.2 mmol) was added dropwise with fast stirring. The reaction was stirred and maintained at -20°C for 0.5 h. The mixture was poured into ice cube and stirred for another 10 min, the mixture was then extracted with ethyl acetate (50 mL×3) and washed with brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, concentrated and precipitated with hexane to get a yellow power (0.6g, 60% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.60 (d, 1H), 8.37 (dd, 1H), 7.51 (d, 1H), 5.19 (s, 2H).

Synthesis of 6-aminobenzoboroxole: The compound was synthesized by a modified procedure in literature. To a solution of 6-nitrobenzoboroxole (0.5 g, 2.8 mmol) dissolved in in a 1:1 mixture of MeOH (10 mL) and 1N HCl (10 mL). 1.826 g (28 mmol) of zinc power was added in portions with vigorous stirring. The reaction was stirred at r.t. for 2 h. When TLC indicated the complete conversation of the starting materials, the precipitate was filtered off and MeOH was evaporated afterward. The mixture was basified using saturated $NaHCO_3$ and extracted with ethyl acetate (50 mL×3) and washed with brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated and precipitated with hexane to obtain pure 6-aminobenzoboroxole as a white solid with a yield of 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, 1H), 7.02 (t, 1H), 6.85 (dd, 1H), 5.02 (s, 2H), 4.73 (s, 1H), 3.71 (s, 2H).

Synthesis of 5-acrylamidobenzoboroxole (BOB monomer): To a solution of 6-aminobenzoboroxole (0.3 g, 2.0 mmol) dissolved in 5 ml THF was added a solution of $NaHCO_3$ (0.68 g, 8.0 mmol) in 5 mL of water. Acryloyl chloride (200 μL, 2.4 mmol) was added to the solution dropwisely at 0 °C. The reaction was stirred at r.t. for 2 h and THF was evaporated. The suspension was extraction with ethyl acetate followed by washed with saturated NaCl-solution, dried over $MgSO_4$ and finally evaporated and precipitated to get the BOB monomer (0.35g, 1.7 mmol) as a white power. ^{1}H NMR (400 MHz, DMSO) δ 10.13 (d, 1H), 9.23 (d, 1H), 8.05 (d, 1H), 7.87 – 7.55 (m, 1H), 7.36 (d, 1H), 6.59 – 6.35 (m, 1H), 6.26 (dd, 1H), 5.76 (dd, 1H), 4.95 (s, 2H).

RAFT polymerization of PEG-PBO. $mPEG_{5k}$-CTA was synthesized as the paper reported[2]. $mPEG_{5k}$-CTA (0.3 g, 0.06 mmol), BOB monomer (0.3 g, 25 eqs), AIBN (5 mg, 0.03 mmol) was dissolved in DMF/water (v/v=95/5, 2 mL) in a sealed vial and bubbled with dry N_2 for 30 min. The reaction was carried out at 80 °C for 12 h and quenched by cooling in liquid nitrogen immediately. The reaction mixture was precipitated with cold diethyl ether for three times and the polymer was obtained as a lemon yellow power after drying under vacuum (80% yield).

Supporting Figures and Tables

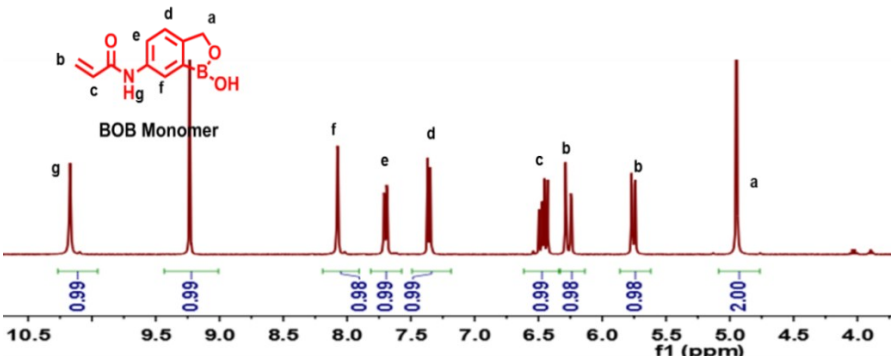

Figure S1. ^{1}H-NMR spectra of BOB monomer in DMSO-D6

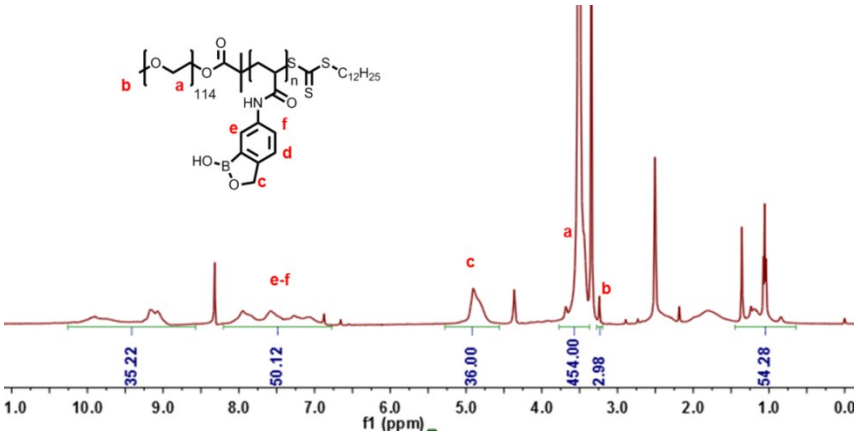

Figure S2. ^{1}H-NMR spectra of PEG-PBO in DMSO-D6

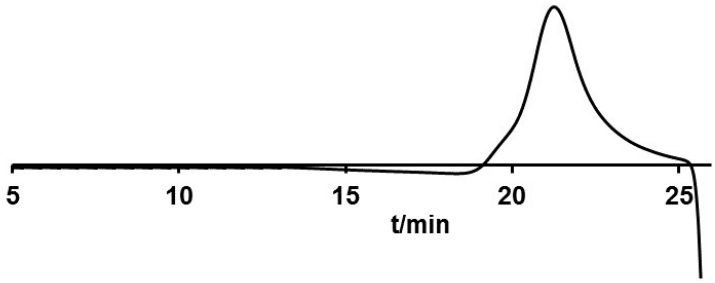

Figure S3. GPC trace of PEG-PBO in DMF

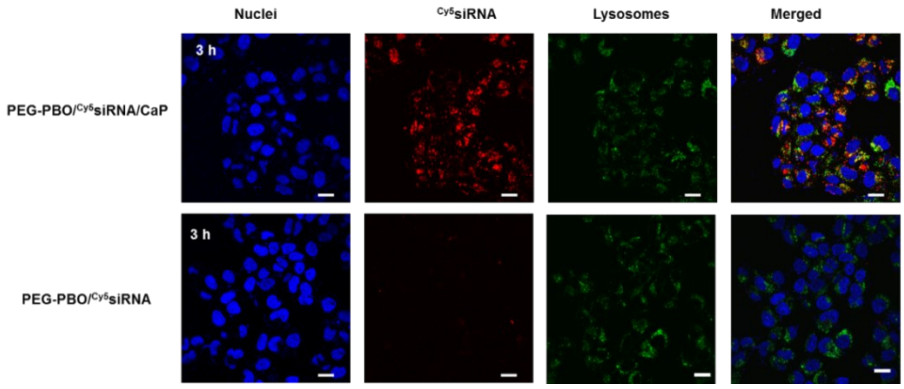

Figure S4. Confocal laser scanning microscopy images of cells cultured with PEG-PBO/^{Cy5}siRNA and PEG-PBO/^{Cy5}siRNA/CaP hybrid nanoparticles in DMEM medium for 3 h. ^{Cy5}siRNA is shown in red, lysosomes dyed with LysoTracker red are shown in green, and the cell nuclei stained with Hoechst 33342 are in blue. All scale bars are 20 μm.

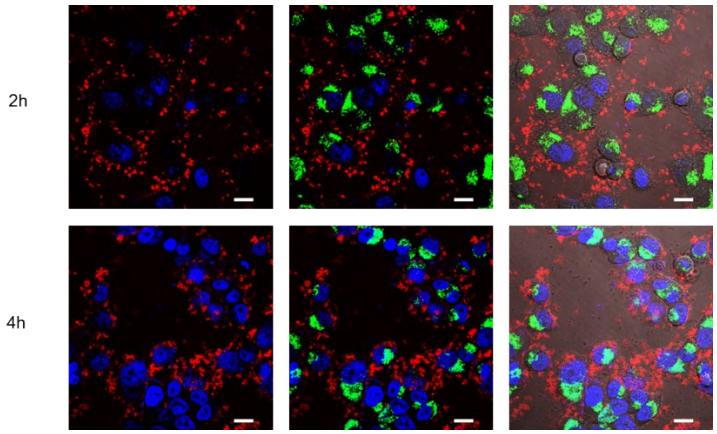

Figure S5**.** Confocal laser scanning microscopy images of Hela-luc cells cultured with CaP/^{Cy5}siRNA polyplexes in DMEM medium for 2 h or 4 h. ^{Cy5}siRNA is shown in red, lysosomes dyed with LysoTracker red are shown in green, and the cell nuclei stained with Hoechst 33342 are in blue. All scale bars are 20 μm.

Reference:

1. M. A. Alam, K. Arora, S. Gurrapu, S. K. Jonnalagadda, G. L. Nelson, P. Kiprof, S. C. Jonnalagadda and V. R. Mereddy, *Tetrahedron*, 2016, **72**, 3795-3801.
2. Y. C. Zhou, F. T. Huang, Y. Yang, P. L. Wang, Z. Zhang, Y. N. Tang, Y. Q. Shen and K. Wang, *Small*, 2018, **14**, 12.