Supplementary Information

Effective siRNA therapy of hepatoma mediated by a nonviral vector with MRI-visibility and biodegradability

Jin Wang[1], Linglan Ren[1], Jingguo Li [2], Jinsheng Huang[2], Du Cheng[2*], Xintao Shuai[2,3*]

[1]Department of Radiology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou 510630, China

[2]PCFM Lab of Ministry of Education, School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou 510275, China

[3]Center of Biomedical Engineering, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou 510080, China

* Correspondence should be addressed to:

Du Cheng

Tel.: +86-20-84112172

Fax: +86-20-84112245

E-mail: chengdu@mail.sysu.edu.cn

Xintao Shuai

Tel.: +86-20-84110365

Fax: +86-20-84112245

E-mail: shuaixt@mail.sysu.edu.cn

Synthesis of lPEI-COOH

DTDPA (2.1 g, 10 mmol) and NHS (1.15 g, 10 mmol) were dissolved in 20 mL of chloroform/DMSO (1:1) and placed in a flask equipped with a magnetic stirring bar. After the flask was cooled in an ice-water bath, DCC (2.05 g, 10 mmol) was added. The flask was then sealed off under argon. The reaction mixture was stirred at 0 °C for 1 h. Approximately 1.2 g of lPEI-NH_2 (2 mmol) was dissolved in 20 mL chloroform/DMSO (1:1), treated with TEA (1.2 eq.) at room temperature for 30 min, and then slowly added. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 24 h. The DCU precipitated was removed by filtration. The filtrate was then added into excessive cold ethanol. The precipitation was collected by centrifugation and washed with 2-propanol. The product lPEI-COOH was vacuum-dried at room temperature to obtain a light yellow powdery product (M_n = 0.8 kDa, calculated from the ^1H NMR spectrum).

Synthesis of *m*PEG-*b*-PLL (PPL)

A pre-determined amount of *m*PEG-*b*-PLL was synthesized according to literature [see: Dai J, Zou S, Pei Y, Cheng D, Ai H, Shuai X. Polyethylenimine-grafted copolymer of poly(L-lysine) and poly(ethylene glycol) for gene delivery. Biomaterials 2011;32:1694-1705]. CBZ-L-Lysine NCA was synthesized according to a previously described method [see: Zhang X, Li J, Li W, Zhang A. Synthesis and characterization of thermo- and pH-responsive double-hydrophilic diblock copolypeptides. Biomacromolecules 2007;8:3557-67]. Then, *m*PEG-b-PCBZLLys was synthesized by ring-opening polymerization of CBZLLys-NCA using *m*PEG-NH_2 as a macroinitiator. In brief, 1.0 g (0.5 mmol) of *m*PEG-NH_2 was vacuum-dried at 70 °C for 4 h in a 100 mL flask, and then 20 mL of anhydrous DMF was added to dissolve PEG. After 6.76 g (22 mmol) of CBZ-L-lysine NCA was dissolved in 30 mL of DMF and added to the

above solution, the reaction was allowed to proceed for 72 h at 35 °C. The mixture was dialyzed against DI water for 5 days to remove organic solvents and subsequently freeze-dried to achieve *m*PEG-*b*-PCBZLLys (denoted as PPCL) (M_n = 13.0 kDa, calculated from the ^{1}H NMR spectrum, *m*PEG$_{45}$-*b*-PCBZLLys$_{42}$).

Approximately 2.6 g of the copolymer *m*PEG-*b*-PCBZLLys (0.2 mmol) was dissolved in 30 mL trifluoroacetic acid. After stirring at room temperature, HBr/acetic acid (33%, 5 mL) was added. After stirring at room temperature for an additional 3 h, the copolymer was precipitated by adding an excess amount of diethyl ether. The copolymer was washed with ethyl ether at least four times. After evaporation of solvents in vacuum, the residue was vacuum-dried at room temperature. The polymer was dissolved in DI water, dialyzed against DI water for 5 days, and then freeze-dried to achieve *m*PEG-*b*-PLL (M_n = 7.4 kDa, calculated from the ^{1}H NMR spectrum).

Measurements of WSPIONs-loading contents and magnetization

The WSPIO loading contents of PLI-SPION@SCR were measured with a polarized Zeeman Atomic Absorption Spectrophtotometer (Model: Z-2000 series). A certain amount of PLI-SPION@SCR was suspended in 1 mol/mL HCl solution to completely degrade the PLI-SPION@SCR. The Fe absorption at 248.3 nm was determined, and the iron concentration was calculated by using a pre-established calibration curve (Y=0.00586+0.03594X, Y means absorbance value and X means Fe concentration). WSPIO loading content was calculated as the weight ratio of iron oxide to PLI-SPION@SCR. The magnetization of PLI-SPION@SCR was measured on an MPMS XL-7 Quantum Design SQUID magnetometer at 10 K and 300 K. The applied magnetic field varied from 2×10^4 Oe to -2×10^4 Oe in order to generate hysteresis loops.

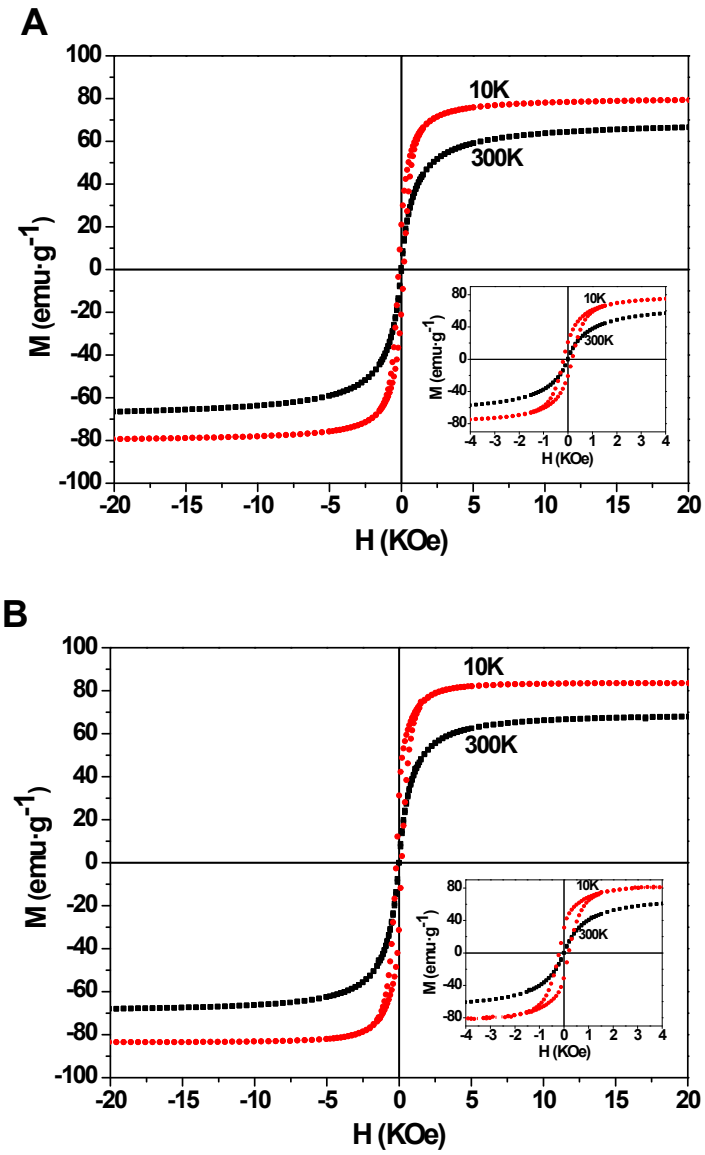

Fig.S1. Hysteresis loops of 6 nm WSPIONs (Fe_3O_4) nanoparticles (A) and PLI-SPION@SCR (B), measured at different temperatures.

Determination of siRNA complexation *via* gel retardation assay

The binding degree was recorded using agarose gel electrophoresis. The polyplexes of PLI (or PLI-SPION) and siRNA were loaded onto 1% agarose gels with ethidium bromide (0.5 mg/mL), and the electrophoresis was run with Tris-acetate buffer at 110 V for 15 min. The retarded siRNA mobility was detected *via* irradiation with UV light.

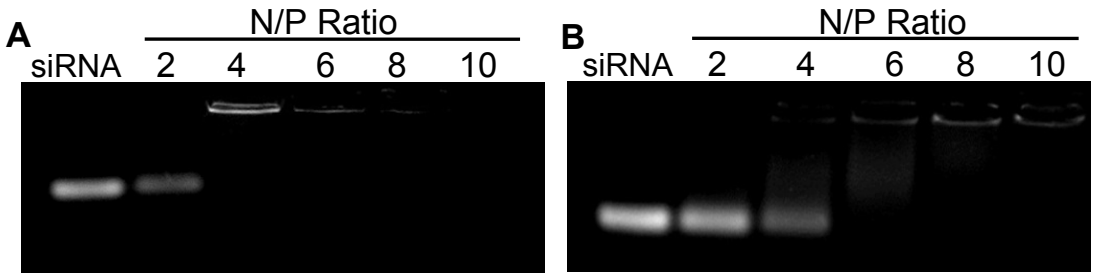

Fig.S2. Electrophoretic mobility of siRNA in agarose gel after complexing with PLI (A) and PLI-SPION (B) at various N/P ratios.

Cell viability

HCC cell line HepG2 cells were cultured in DMEM medium supplemented with 10% FBS (Gibco, USA) and incubated at 37 °C in a humidified atmosphere with 5% CO_2. The cells were trypsinized and seeded in 96-well plates at a density of 1×10^4 cells/well, and then cultured for 24 h in 100 μL of DMEM medium containing 10% FBS at 37 °C. Subsequently, the cells were incubated at 37 °C for 4 h in the presence of polymer or polyplex. The medium was replaced with 100 μL fresh DMEM medium and incubated for 24 h at 37 °C. The medium was replaced with fresh DMEM medium plus 10 μL of MTT solution (5 mg/mL) and then incubated for 4 h. The supernatant in the wells was discarded, and then 100 μL of DMSO was added to dissolve the formazan formed in living cells. After gentle agitation for 10 min, the

absorbance at 540 nm of each well was analyzed using a Tecan Infinite F200 multimode plate reader. All experiments were conducted in quintuplicate.

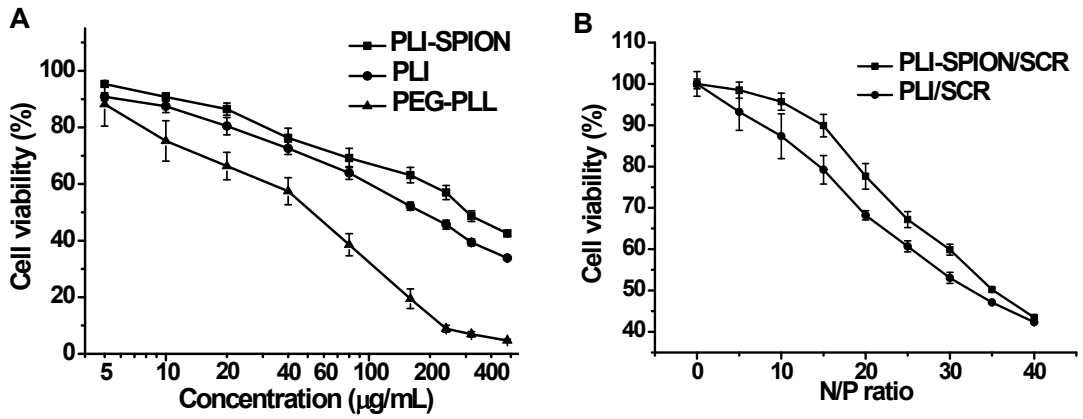

Fig.S3. Cytotoxicities of PEG-PLL, PLI and PLI-SPION without (A) and with (B) siRNA complexation.

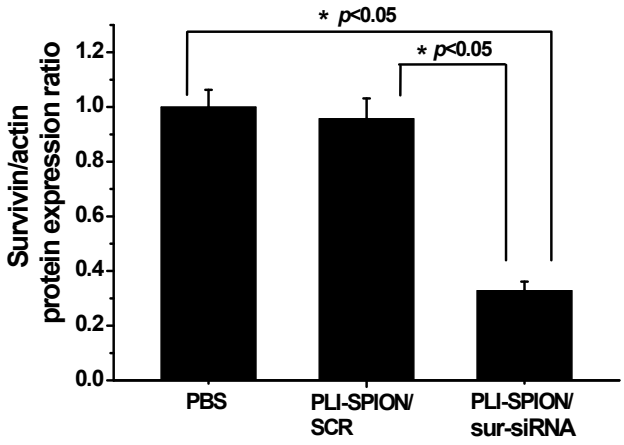

Fig.S4. Quantitative evaluation of survivin protein expression using western blotting assay.