A TRAIL-Delivered Lipoprotein-Bioinspired Nanovector Engineering Stem Cell-Based Platform for Inhibition of Lung Metastasis of Melanoma

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1. Synthesis and Characterization of PEI-LA.

LA-substituted PEI (Mw = 25 kDa, PEI 25 K) with fatty acid: ethylenimine unit molar ratios of 1:40 was prepared as previously described procedure with a little optimization. Briefly, 5.82 mg of lauric acid, 16.66 mg of EDCI and 10.03 mg of NHS (molar ratio of LA/EDCI/NHS = 1:3:3) were dissolved in the dimethylsulfoxide (DMSO). Then the mixture was stirred at 25 °C for 1.5 h to active the carboxyl groups of LA. Subsequently, 50 mg of PEI 25 K dissolved in DMSO was added into the activated mixture dropwise under N2 protection at room temperature with magnetic stirring for 24 h. As for the termination of reaction, an excess of cold diethyl ether (3 times volume of reaction system) was added to remove unreacted LA and the precipitation was further purified by dialysis in deionized water (MWCO = 3500 Da, 2 L × 3) to remove residual EDCI and NHS. The resulting solution was lyophilized to obtain PEI-LA. The structure of product was determined by using a 300 MHz 1H-NMR spectroscope (AV-500, Bruker, Switzerland) and an infrared spectrometer (TENSOR 27, Bruker, Switzerland) respectively. The degree of LA to PEI unit was determined based on the relative proton peak area of correlated groups in ^{1}H-NMR.

2. Determination of buffering capacity of PEI-LA.

The buffering capacity of PEI-LA, PEI 25 K and PEI 1.8 K was tested by acid-base titration. Briefly, 6.28 mg of PEI-LA, 6 mg PEI 1.8 K and 6 mg PEI 25 K (containing 6 mg PEI 25 unit equally) were dissolved in 30 mL 0.1 M NaCl, and the solution was adjusted to pH 10 with 1 M NaOH or HCl. Subsequently, the solution was titrated with 0.1 M HCl stepwise in increments of 10 μL and the change in pH value was recorded by a pH meter (pHS-25B,

Dapu, China). 0.1 M NaCl solution was set as control.

3. DNase I protection assay of rHDL/PEI-LA/pDNA nanoparticles.

The rHDL/PEI-LA/pDNA nanoparticles were incubated with 3.5 μL of DNase I solution (1 U/μL in 10× reaction buffer containing 100 mM Tris-HCl, 1 mM $CaCl_2$ and 25 mM $MgCl_2$) for different time (0 h, 1 h, 3 h, 6 h, 12 h and 24 h) at 37 °C, then DNase I was inactivated with EDTA (3 μL, 50 mM) for 10 min at 80 °C. The samples were treated with heparin (10 mg/mL) for 1 h at 37 °C to dissociate pDNA from nanoparticles. Finally, the resulting samples were detected by a 0.6% agarose gel electrophoresis assay. Naked pDNA with DNase I incubation for different time was set as control.

4. Safety test of MSCs-TRAIL (rHDL).

To evaluate the potential side effects of MSCs-TRAIL (rHDL), healthy female C57BL/6 mice (six week-old) were randomly divided into 2 groups followed by administrated by 200 μL of MSCs-TRAIL (rHDL) and saline, respectively. After 12 days of administration, the blood of each mice in different groups was collected and centrifuged to obtain plasma, which was used to quantify the serum levels of aspartate amino transferase (AST), alanine amino transferase (ALT) and blood urea nitrogen (BUN) to assess hepatic and renal damage; while the major organs (heart, liver, spleen and kidney) were fixed in 4% paraformaldehyde for histopathology analysis.

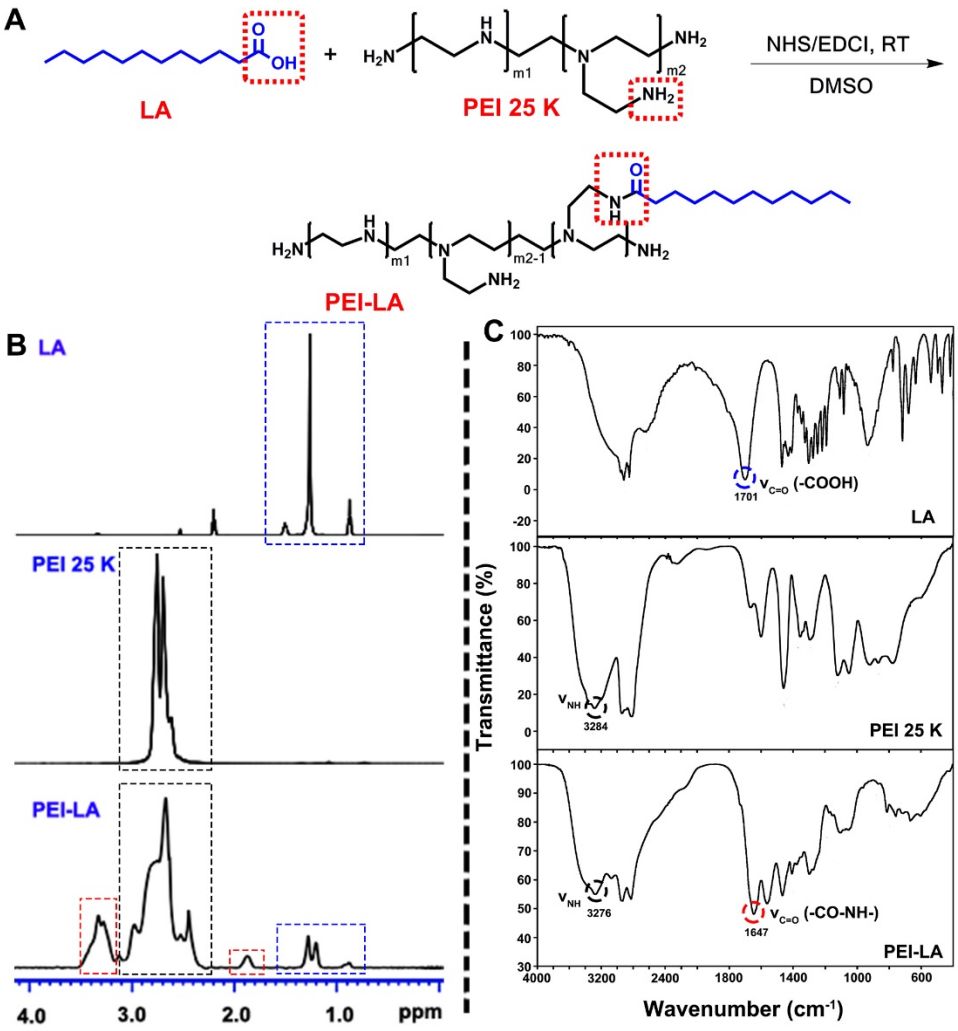

Figure S1. (A) Synthesis scheme of PEI-LA. (B) ^{1}H NMR spectra of LA, PEI 25 K and PEI-LA. (C) FT-IR spectra of LA, PEI 25 K and PEI-LA.

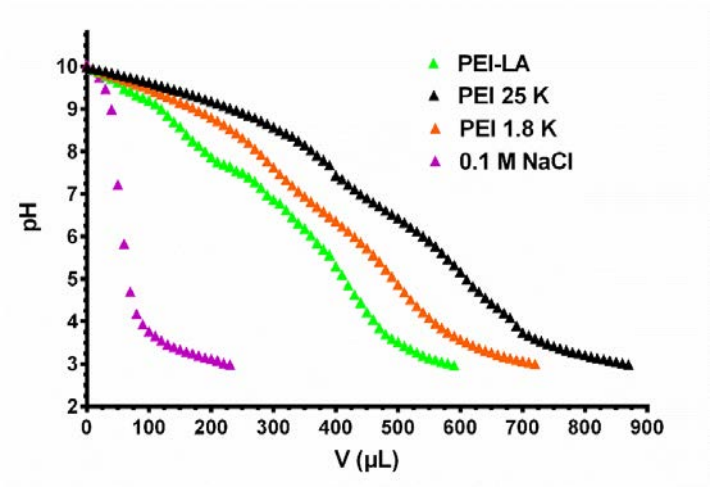

Figure S2. Acid–base titration profiles of each rate of charge of PEI-LA, PEI 25 K, PEI 1.8 K and 0.1 M NaCl.

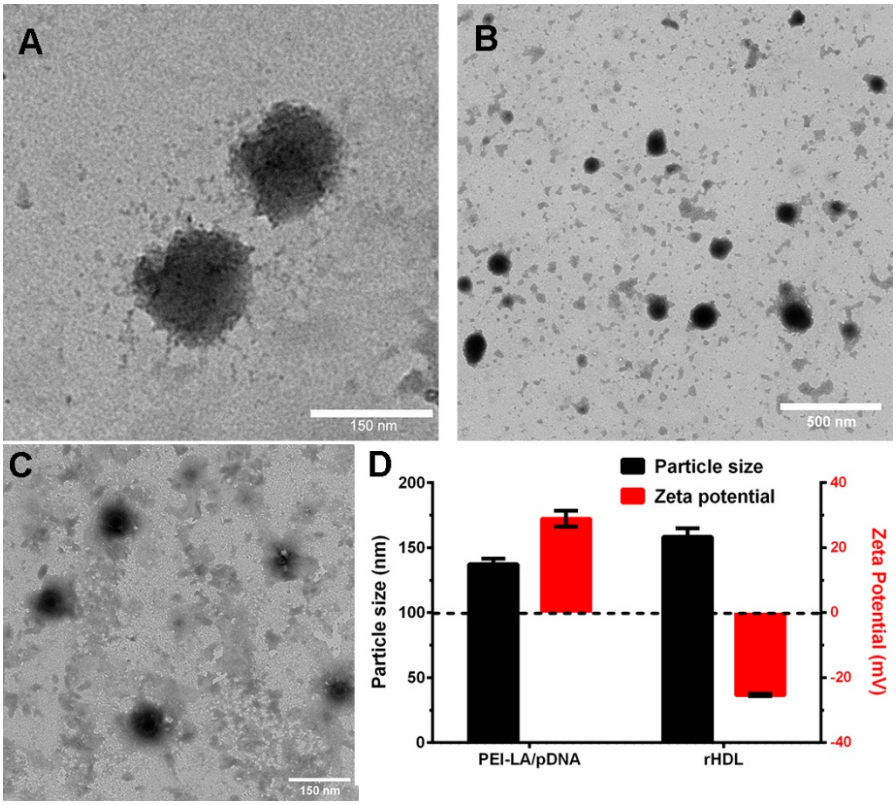

Figure S3. TEM image of PEI-LA/pDNA(A), rHDL(B), rHDL/PEI-LA/pDNA(C) and size/zeta potential of PEI-LA/pDNA and rHDL(D).

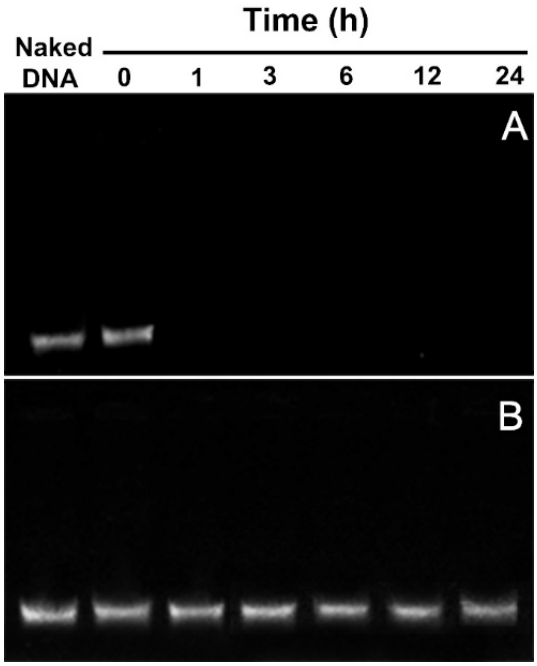

Figure S4. Protection assay of rHDL/PEI-LA/pDNA nanoparticles against DNase I for different time. (A) Naked pDNA as control. (B) rHDL/PEI-LA/pDNA nanoparticles.

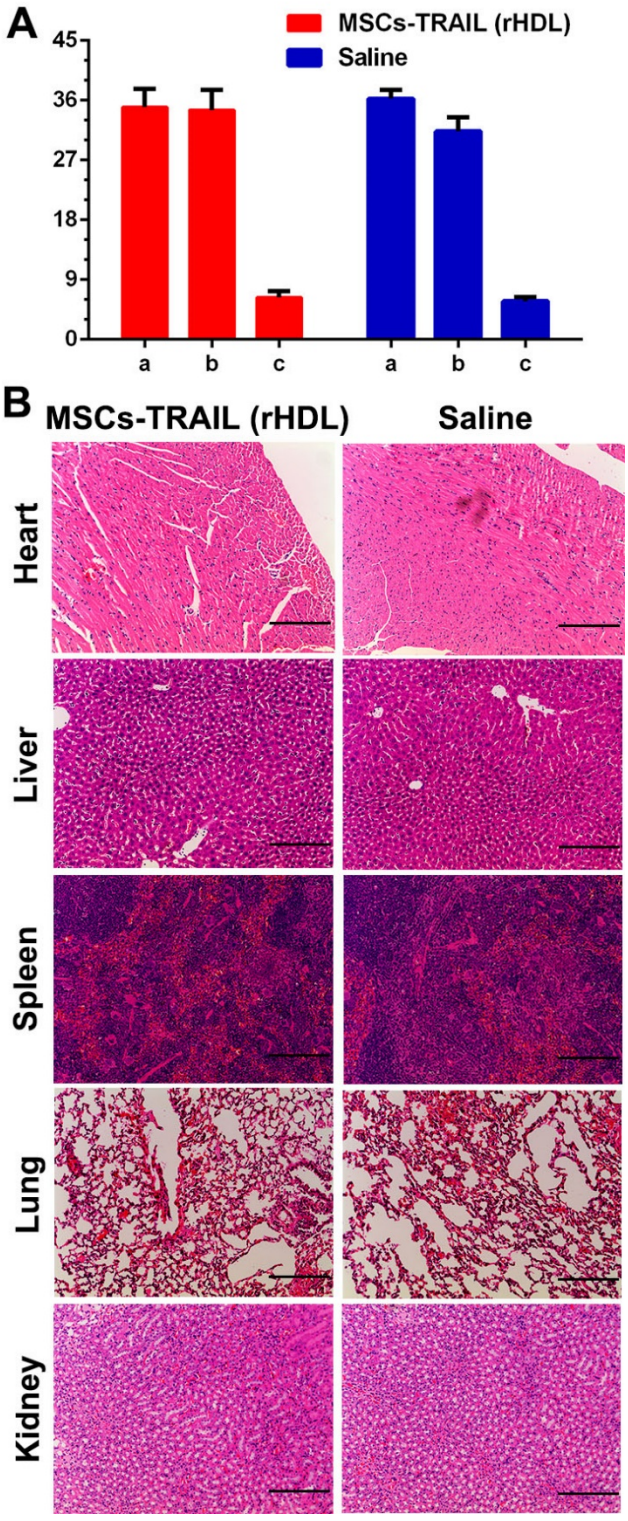

Figure S5. (A) Serum levels of (a) ALT (IU/L), (b) AST (IU/L) and (c) BUN (mmol/L) at 12 days after intravenous injections in C57BL/6 mice (n = 5). (B) Representative histological images of the H&E-stained heart, liver, spleen, lung, and kidney harvested from the mice treated by MSCs-TRAIL (rHDL) and saline (Scale bar: 200 μm).