Electronic Supplementary Material (ESI) for Polymer Chemistry.

Mannosylated PEI-CPP Hybrid for TRAIL Gene Targeting Delivery for Colorectal Cancer Therapy

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Supporting Information

Synthesis of PEI_{5k}-CPP

The synthetic step of PEI_{5k}-CPP was similar to Man-PEI_{5k}-CPP, using SPDP as a crosslinker. SPDP in methanol was added dropwise into the PEI_{5k} solution at a reaction ratio of 3:1 (mol/mol). The mixture was stirred under N_2 protection until SPDP was not detected in TLC, and the solvent was then removed by evaporation. The residue was dissolved in 5 mL of water, to which LMWP (3 equiv) in 1.0 mL of water was subsequently added dropwise. The mixture was stirred overnight at room temperature under N_2. The resultant was purified by dialysis (MWCO 2000) in de-ionized water. The final solution was freeze dried and confirmed by IR and ^{1}H NMR spectroscopy. 1H NMR spectra and IR spectra of PEI_{5k}-CPP are shown in **Fig. S1**.

Cell Uptake Study in U87 Cells

The U87 cells were seeded in the 24-well plates at a density of 5 × 10^4 cells per well and were incubated for 24 h before use. The Man-PEI_{5k}-CPP/Yoyo-1-labeled DNA complexes were added to the plates and incubated with the cells for 4 h. The cells were then collected after thorough wash with PBS. The cellular uptake efficiency was determined by flow cytometry analysis (Becton Dickinson, USA). Fluorescence images were obtained by fluorescence microscope (CARL ZEISS, Germany).

Cell Transfection in U87 Cells

The U87 cells were seeded into the 24-well plates at a density of 5 × 10^4 cells/well. After 24 h, the cells were treated with the nanocomplexes (equivalent to 1 μg of pEGFP/well) in 0.5 mL of the fresh medium containing 10% FBS for 4 h at 37°C, and then the transfection medium was replaced with the fresh medium containing 10% FBS.

After an additional 44 h of incubation, the medium was removed, and the cells were rinsed twice with cold PBS. The EGFP expression was photographed using a fluorescence microscope. In addition, the EGFP expression efficiency was quantified by a flow cytometer with the emission wavelength of 530 nm. The analysis was performed by FlowJo 7.6 software.

***In vitro* Cytotoxicity in 293T cells**

The cytotoxicity of the Man-PEI_{5k}-CPP/pTRAIL and PEI_{25k}/pTRAIL nanocomplexes on the 293T cells was evaluated with a standard MTT assay. The blank genetic vector pUC-19 was used to assess the biocompatibility of the materials. Briefly, the 293T cells were seeded onto a 96-well plate. After 24 h, the medium was replaced with 200 μl of fresh medium containing a series of concentrations of PEI_{25k}, the PEI_{25k}/pUC-19, or the Man-PEI_{5k}-CPP/pUC-19 nanocomplexes, respectively. For the antitumor efficacy test, the cells were treated with a series of concentrations of PEI_{25k}, PEI_{25k}/pTRAIL, and the Man-PEI_{5k}-CPP/pTRAIL nanocomplexes, respectively. After 48 h of incubation, 20 μl of MTT solution (5 mg/mL in PBS) was added to each well and the cells were incubated for another 4 h. Subsequently, the medium was removed and the cells were dissolved in 200 μl of DMSO per well. The absorbance was measured by a microplate reader (Multiskan GO, Thermo, USA) at 490 nm. Cell viability was calculated using the formula:

$$Cell\ Viability\ (\%) = [(A_T - A_B) / (A_C - A_B)] \times 100\%$$

A_T, A_C, and A_B represent the absorbance of the treated cells, the untreated cells, and the blank culture medium, respectively.

Supplementary Figures:

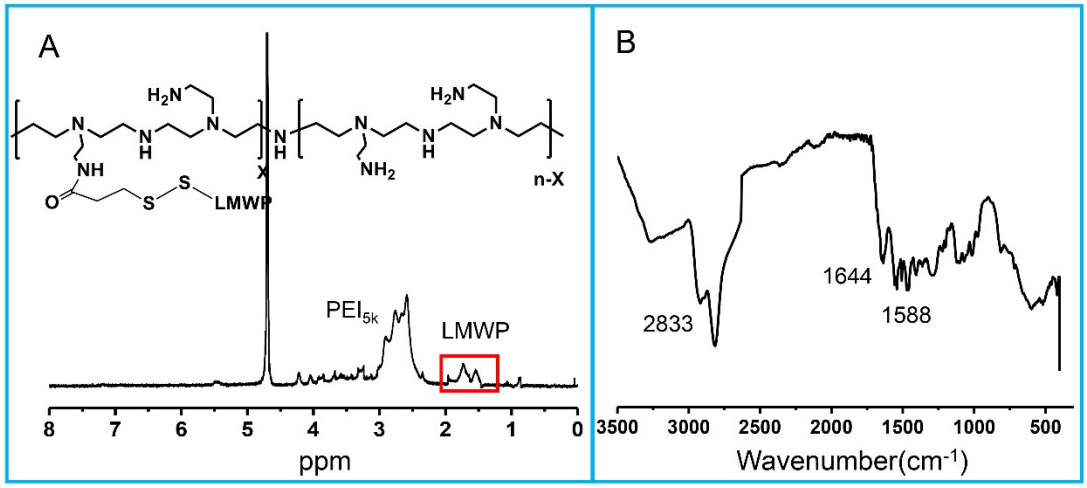

Fig. S1 (A) ^{1}H NMR spectrum of PEI_{5k}-CPP in D_2O and (B) IR spectrum of PEI_{5k}-CPP.

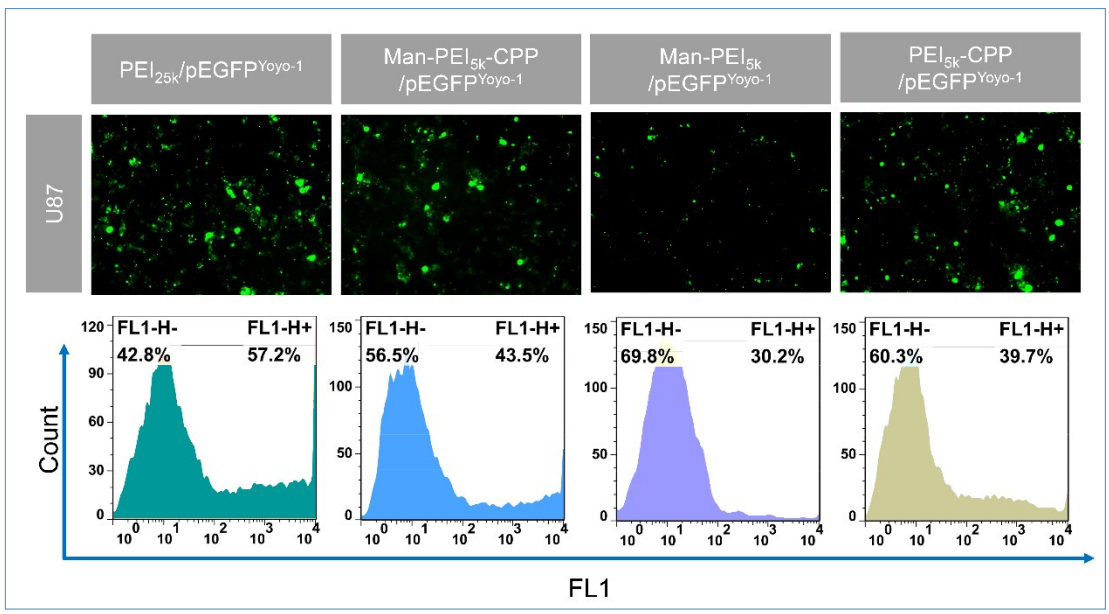

Fig. S2 Cellular uptake in U87 cells.

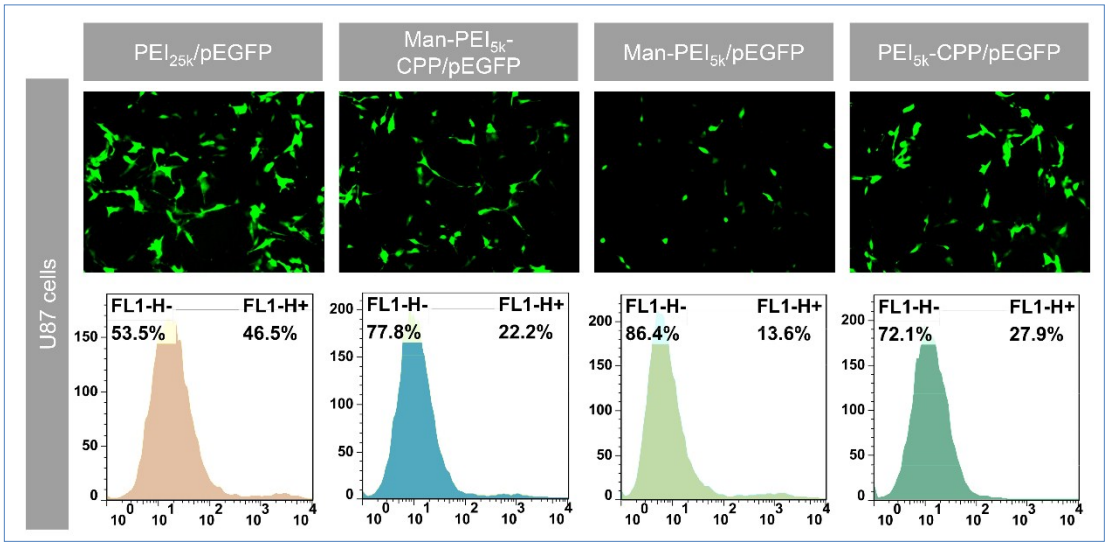

Fig. S3 *In vitro* transfection study in U87 cells.

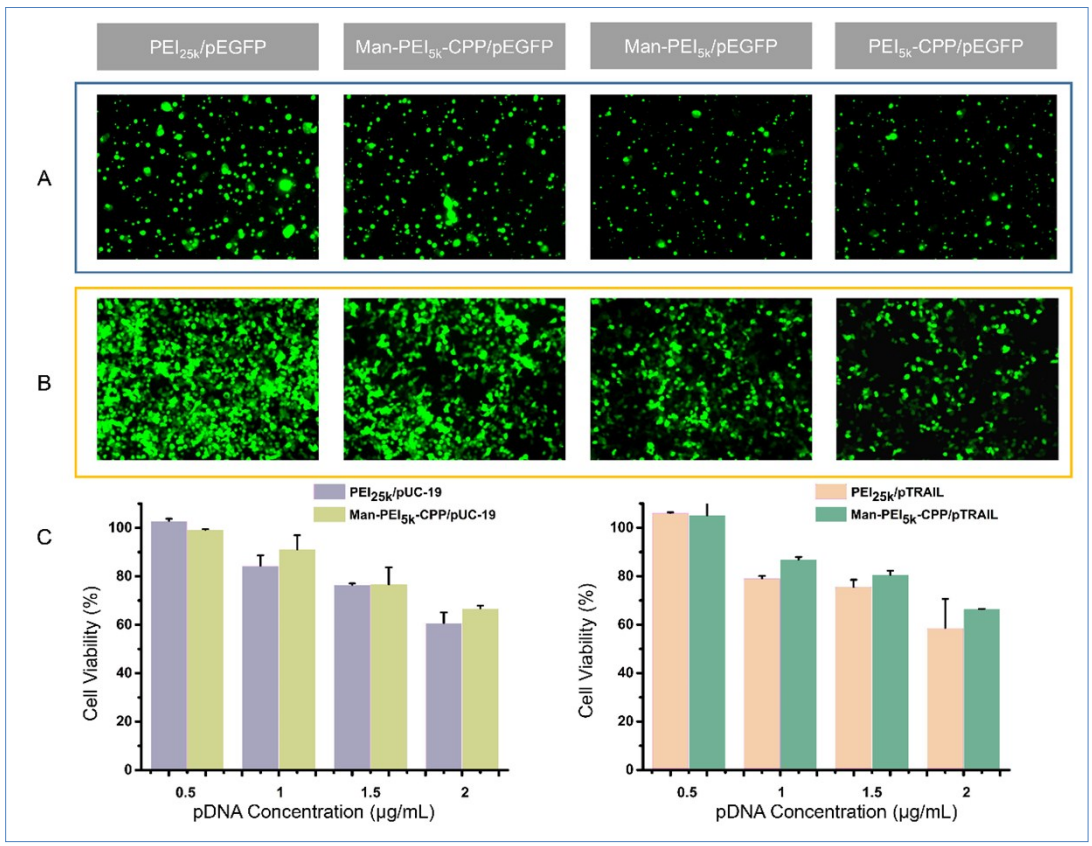

Fig. S4 (A) Cellular uptake in 293T cells. (B) *In vitro* transfection in 293T cells. (C) Cytotoxicity of the polymer/blank vector and polymer/pTRAIL complexes in 293T cells.