Electronic Supplementary Material (ESI) for RSC Advances

Supplementary Information

Radio-opaque Theranostic Nanoemulsions with Synergistic Anti-cancer Activity of Paclitaxel and Bcl-2 siRNA

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Table S1. The nanoemulsion formulations prepared with different amounts of LPEI-*g*-chol.

Composition		Weight (mg)			
		Formula 1	Formula 2	Formula 3	Formula 4
Core	Lipiodol	22.5	22.5	22.5	22.5
Shell	Cholesterol	5.0	5.0	5.0	5.0
	LPEI-*g*-chol	1.0	2.0	5.0	10.0
	$DSPE\text{-}PEG_{2000}$	0.05	0.05	0.05	0.05

Table S2. The weight and molar compositions of the prepared nanoemulsions.

	Composition	Weight (mg)	Mole number (μmoles)
Core	Lipiodol	22.5	46.3
	Paclitaxel	0.5	0.6
Shell	Cholesterol	5.0	12.9
	LPEI-*g*-chol	10.0	2.1
	$DSPE\text{-}PEG_{2000}$	0.05	0.02
Total		38.05	

Calculation of the number of siRNA loaded on the surface of the nanoemulsions

The loading number of siRNA per nanoemusion was calculated by dividing the total number of siRNA by the total number of the nanoemulsions. The total number of nanoemulsions was calculated by dividing the total volume of materials used for the preparation of the nanoemulsions (V_t) by the average volume of each nanoemulsions (V_n), which was obtained from the hydrodynamic diameter of the nanoemulsions (d). The total volume of the nanoemulsions was $V_t = 3.26 \times 10^{19}$ nm^3, and the average volume of individual nanoemulsions was $V_n = 1.46 \times 10^6$ nm^3. The calculated number of the nanoemulsions was 2.23×10^{13}. The total number of siRNA was 8.6×10^{16}. Therefore, the average number of siRNA bound to the surface of the nanoemulsions was 3,857.

***In vitro* release test of paclitaxel**

Two milliliters of *ptx*-NE and *sr*-*ptx*-NE containing 90 μg were placed in a dialysis bag (molecular weight cutoff = 25 kDa). The bags were immersed in 30 mL PBS at pH7.4 containing 0.1 wt-% tween 80 and incubated in a shaking incubator at 150 rpm and 37 ^{o}C. At pre-determined time intervals, 1 mL of the medium was collected and replaced with the same volume of fresh medium. The amount of paclitaxel released into medium was measured by high performance liquid chromatography as described the experimental section in the main text.

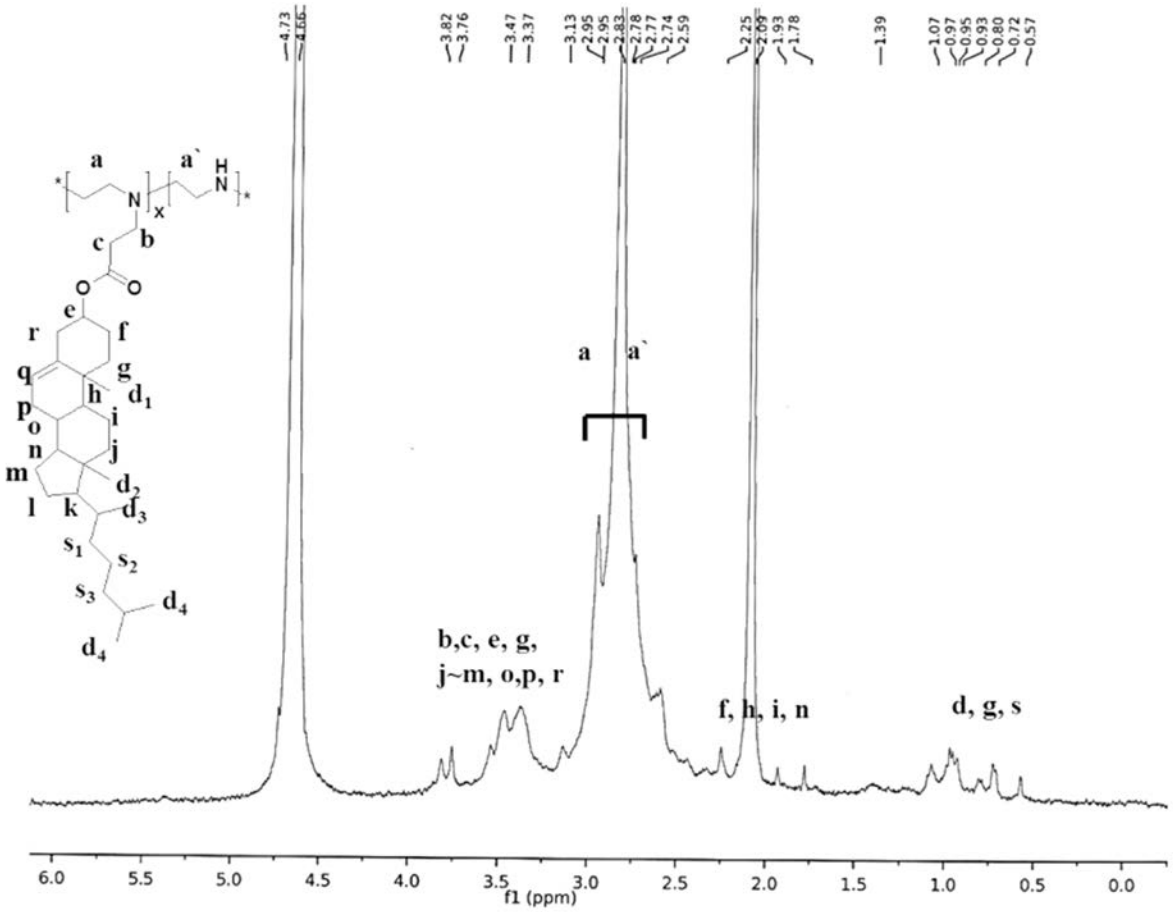

Figure S1. ^{1}H NMR analysis of LPEI-*g*-chol with a grafting percentage of 3.8 %.

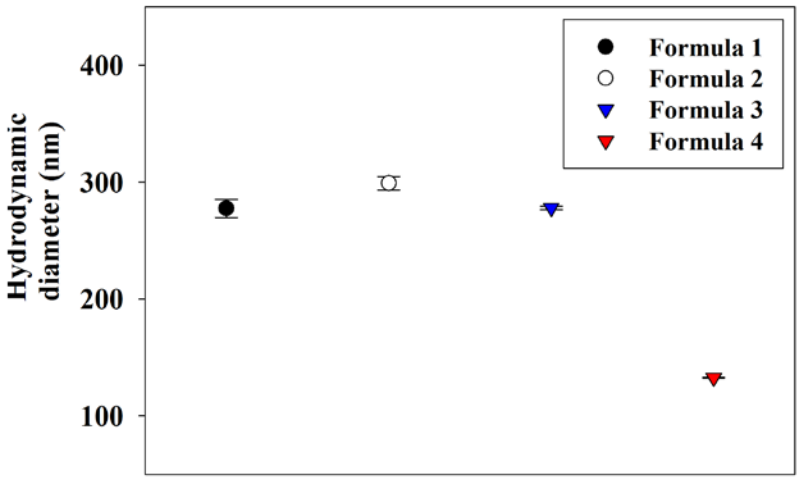

Figure S2. Hydrodynamic diameters of different nanoemulsions. Hydrodynamic diameters have shown no significant difference among formula 1 to 3. However, the size of nanoemulsion formulation containing 10 mg LPEI-*g*-chol was decreased to 132.3 nm. Therefore, formula 4 was utilized to prepare multifunctional nanoemulsions for further studies

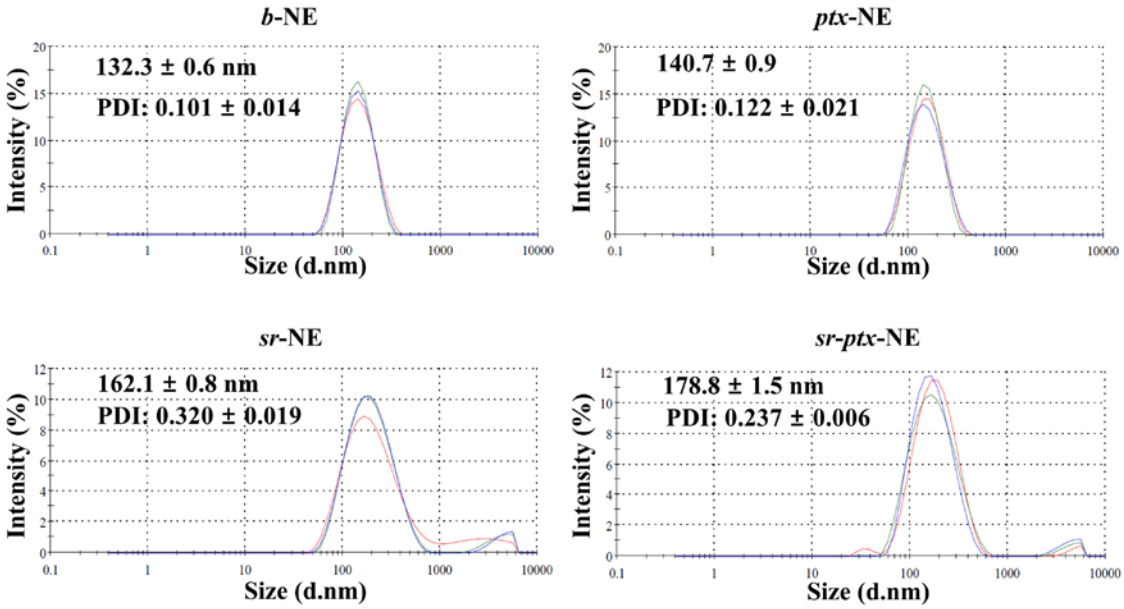

Figure S3. Particle size distribution and polydispersity index (PDI) of different nanoemulsion formulations. Hydrodynamic diameters of each sample were determined by repeating the measurement three times (the number of runs is 11 per measurements).

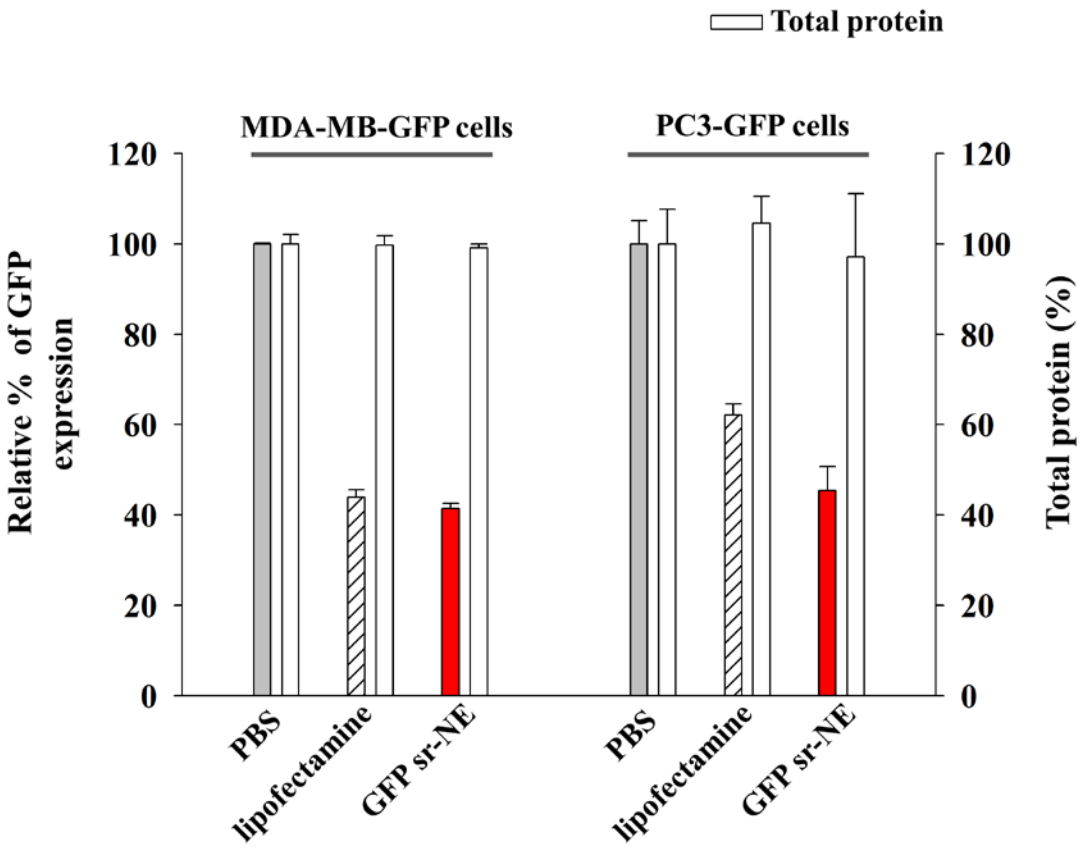

Figure S4. GFP gene silencing efficiency and relative total protein level in MDA-MB-GFP cells and PC3-GFP cells transfected with GFP *sr*-NE.

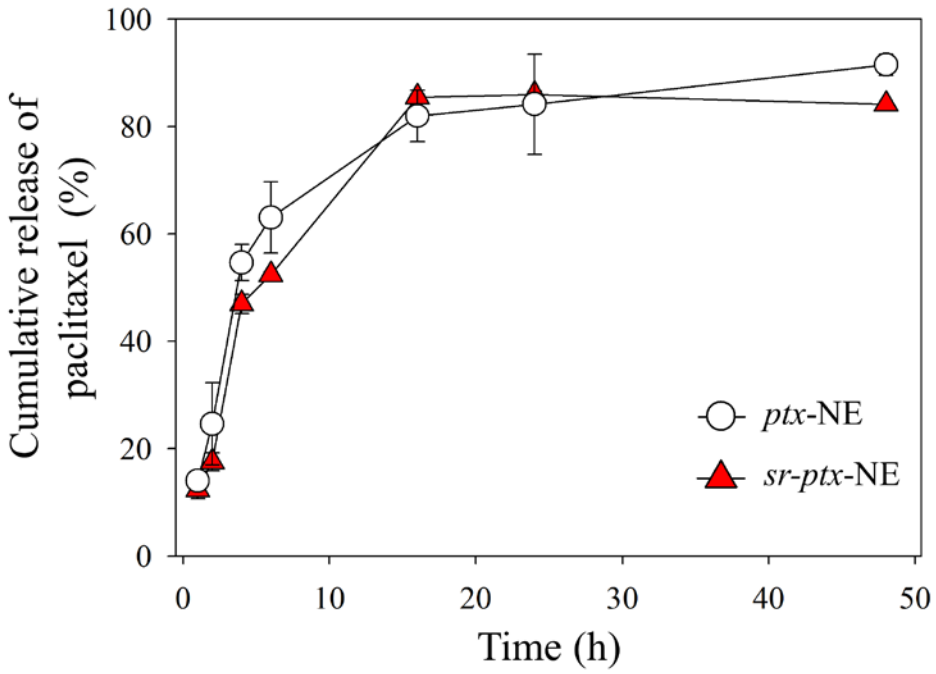

Figure S5. *In vitro* release profiles of paclitaxel from *ptx*-NE and *sr-ptx*-NE.

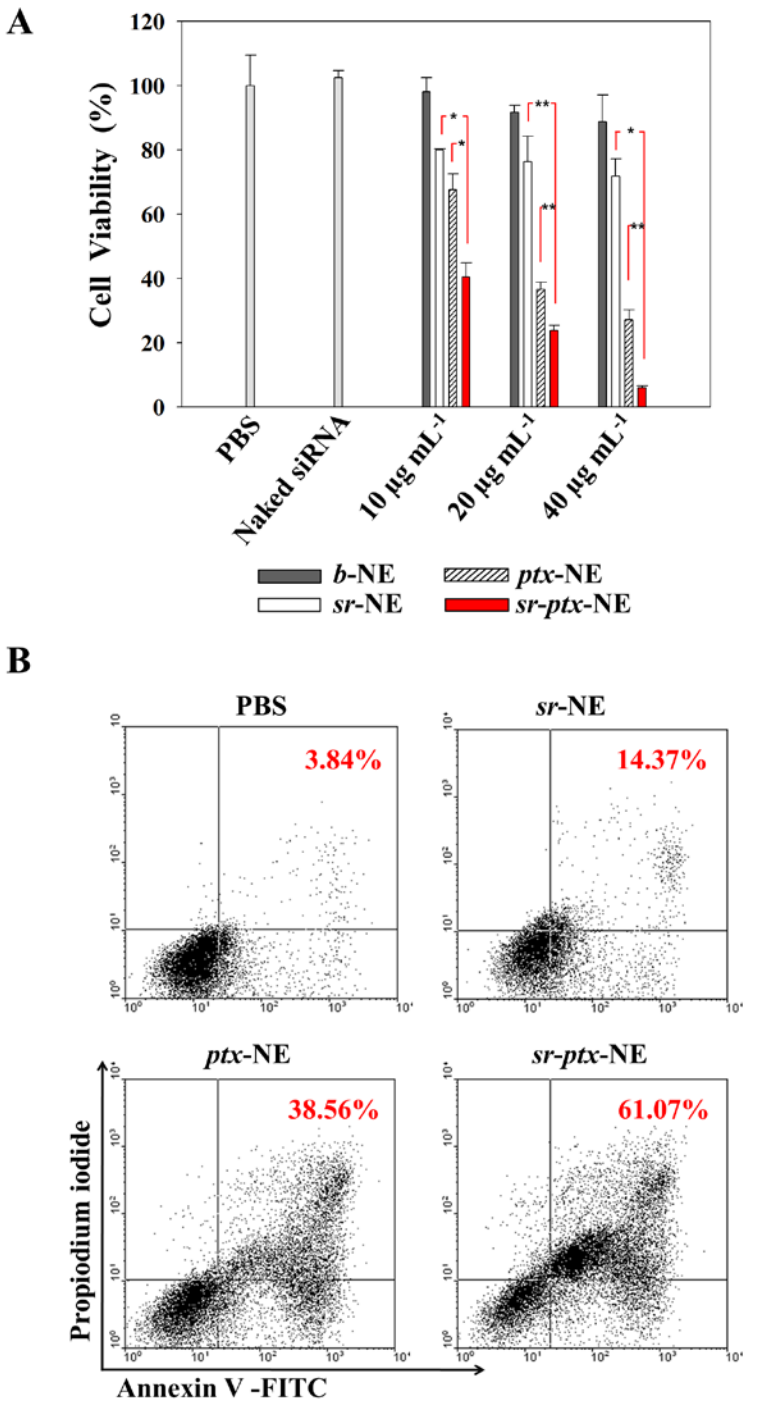

Figure S6. Effect of the co-delivery of paclitaxel and Bcl-2 siRNA in serum-free condition. A. Cytotoxic effect of co-delivery by Bcl-2 *sr-ptx*-NE in MCF7 cells. B. Induction of apoptosis on MCF7 cells by Bcl-2 *sr-ptx*-NE (*p < 0.005, **p < 0.001)