Supporting Information for

Aerosol delivery of biocompatible dihydroergotamine-loaded PLGA-PSPE polymeric micelles for efficient lung cancer therapy

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Tab. S1. Mws of polyspermine with different reaction condition

Reaction Medium	Volume	Temperature	Time	Mw（Da）
Absolute Ethanol	9ml	45 °C	48h	2680
Absolute Ethanol	9ml	80 °C	48h	3378
Absolute Ethanol	9ml	80 °C	6d	18083
Absolute Methanol	15ml	60 °C	4d	17405
Absolute Methanol	6ml	60 °C	4d	39000
Absolute Methanol	6ml	60 °C	3d	21000
Absolute Methanol	6ml	60 °C	2d	13000
Absolute Methanol	6ml	60 °C	1d	5882
Absolute Methanol	6ml	60 °C	12h	5744

Tab. S2. Particle size of PLGA-PSPE with different Mws by different method

PLGA(Da)	PSPE(Da)	Solvent added to aqueous phase		Aqueous added to solvent phase	
		Particle size	PDI	Particle size	PDI
5K	2.6K	78.1±1.2	0.109	256.9±3.2	0.07
5K	5.8K	243.8±6.5	0.243	497.9±6.7	0.08
10K	5.8K	106±7.5	0.156	445.9±14.9	0.111
10K	13K	301.4±28.5	0.259	542±18.5	0.108
20K	5.8K	128.7±4.9	0.148	267.8±5.9	0.07
20K	21K	489.6±16.8	0.224	590±33.8	0.181
40K	21K	254.7±4.5	0.094	712.7±38.5	0.22

Tab. S3. Drug loading of PLGA-PSPE with different Mws loading DHE by different method

PLGA(Da)	PSPE(Da)	Drug loading	
		Solvent added to aqueous phase	Aqueous added to solvent phase
5k	2.6k	0.64±0.11%	1.7±0.4%
10K	5.8K	1.51±0.41%	4.21±1.4%
20K	5.8K	2.12±0.68%	11.65±2.1%

Tab. S4. Select of the optimal dosage forms of PLGA-PSPE/DHE

NO.	Water adding speed (ml/min)	Stirring rate (rpm)	Dosage of drug (mg)	Particle size (nm)	PDI	Kcps	Drug loading (100%)
1	0.5	200	5	299.3±1.0	0.045	372.0	2.46 ±0.3
2	0.5	400	10	339.6±9.2	0.058	296.6	2.30 ±0.2
3	0.5	800	20	297.6±1.5	0.089	337.6	11.65 ±2.1
4	1.5	200	10	220.1±0.8	0.064	350.5	4.39 ±0.9
5	1.5	400	20	254.5±0.7	0.038	458.4	6.41 ±1.2
6	1.5	800	5	239.7±7.4	0.050	458.6	1.92 ±0.4
7	3.0	200	20	224.7±8.1	0.050	480.5	5.38 ±1.3
8	3.0	400	5	211.0±3.7	0.073	455.2	1.83 ±0.3
9	3.0	800	10	218.7±1.9	0.037	456.6	4.784 ±0.6

The mass of PLGA-PSPE is 20mg.

Tab. S5. Serum biochemical analysis

Parameter (reference range)	Cont.	PLGA-PSPE	0.5mg/kg PLGA-PSPE/DHE	1mg/kg PLGA-PSPE/DHE	DHE
Aspartate aminotransferase (U/L) (99.5 ± 33.4)	56.7±13.8	47.2±13.5	42.6±6.0$^{\$\$}$	48.9±10.5	76.5±31.7
Alanine aminotransferase (U/L) (41.4 ± 16.4)	29.9±13.1	28.7±6.9	28.6±6.9	33.7±8.0	38.7±10.9
Total Protein(g/dL) (5.4 ± 0.8)	4.7±0.2	4.1±0.3*	4.3±0.1**	4.1±0.1$^{***,++}$	4.4±0.4
Albumin/Globulin (ratio)	1.1±0.0	1.1±0.1	1.3±0.1	1.3±0.1$^{**,\#}$	1.3±0.1
Blood urea nitrogen (mg/dL) (32.7 ± 3.5)	23.0±4.0	20.4±2.1	23.8±4.7	22.7±3.4	24.3±2.9$^{\#}$
Creatinine (mg/dL) (0.5 ± 0.1)	0.4±0.1	0.4±0.0	0.4±0.1	0.4±0.0	0.5±0.0

Biochemical analysis of serum after in vivo treatment. The treated groups have no meaningful changes in levels of liver damage indicators, alanine aminotransferase, alkaline phosphatase, total protein, and kidney damage indicators, blood urea nitrogen, creatinine in serum. Results are represented as mean ±SD. *p < 0.05, **p < 0.01, and ***p < 0.001 versus Cont., #p < 0.05 versus PLGA-PSPE, $$p < 0.01 versus DHE only, ++p < 0.01 versus 1mg/kg PLGA-PSPE/DHE.

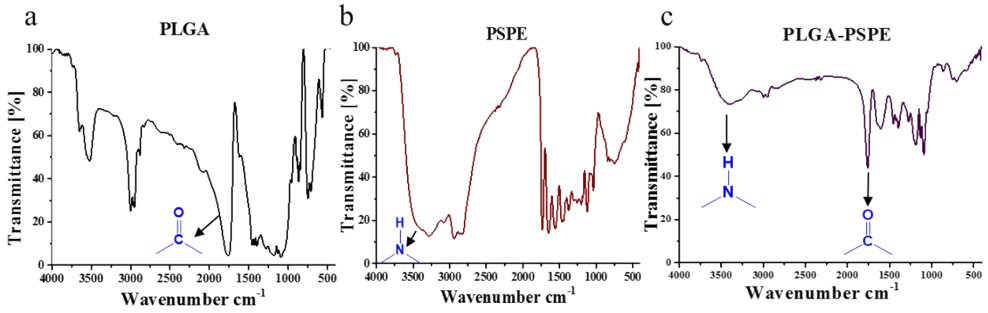

Fig. S1. Characterizations of PLGA, PSPE and PLGA-PSPE via IR

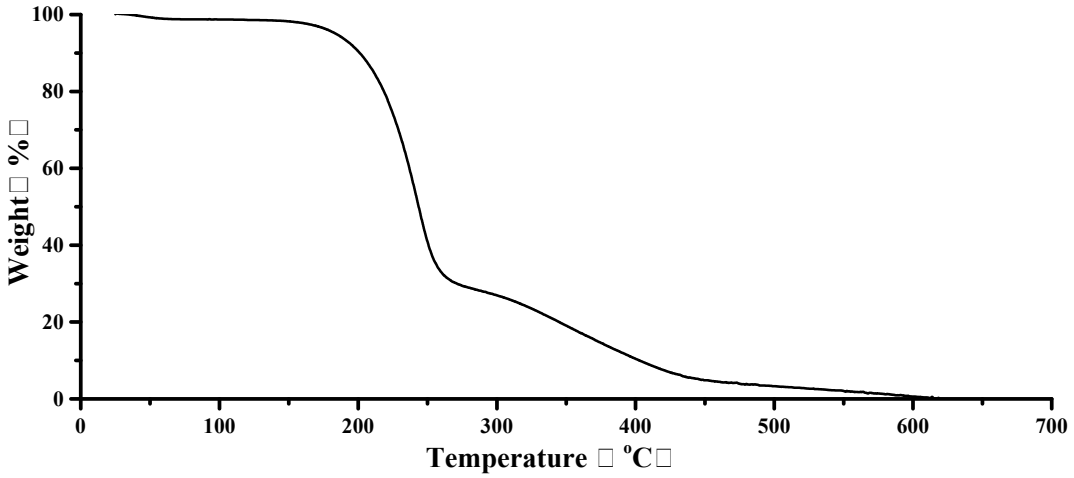

Fig. S2. TGA of PLGA-PSPE.

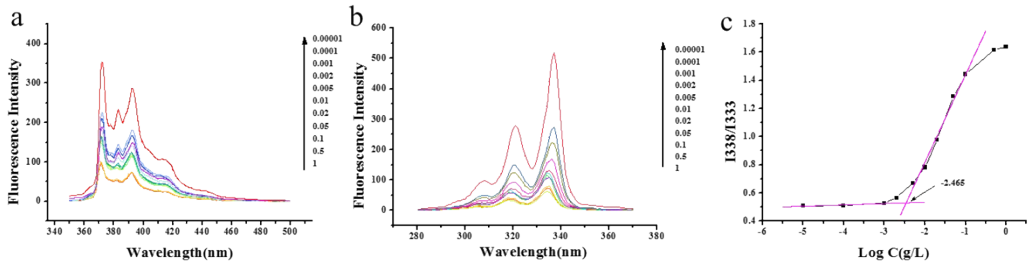

Fig. S3. CMC of PLGA-PSPE. (a) emission wavelength. (b) excitation wavelength. (c) Intensity ratios (I338/I333) of the emission spectra of pyrene-loaded PLGA-PSPE at with different concentrations of PLGA-PSPE.

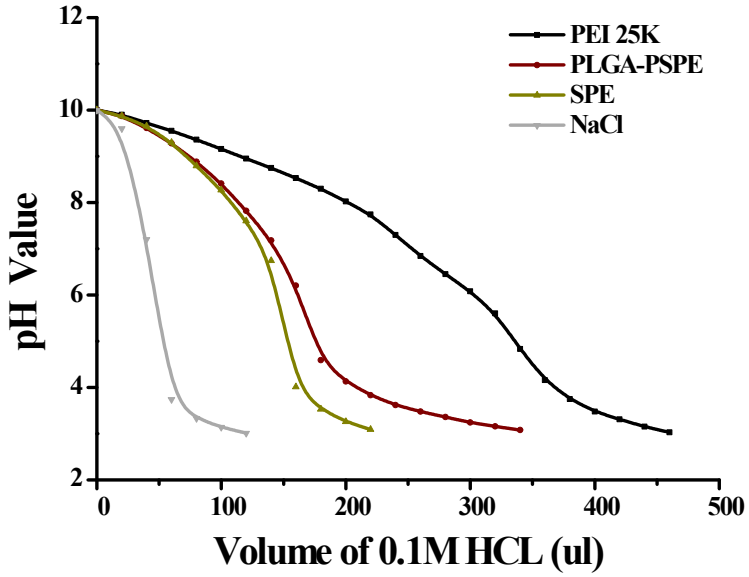

Fig. S4. Buffering capability assay of HPSPE, PEI 25 K. All samples were dissolved in 150 mM NaCl solution at a concentration of 0.2 mg/mL. The solution was brought to a starting pH of 10.0 with 0.1 M NaOH and then was titrated with 0.1 M HCl from 10.0 to 4.0.

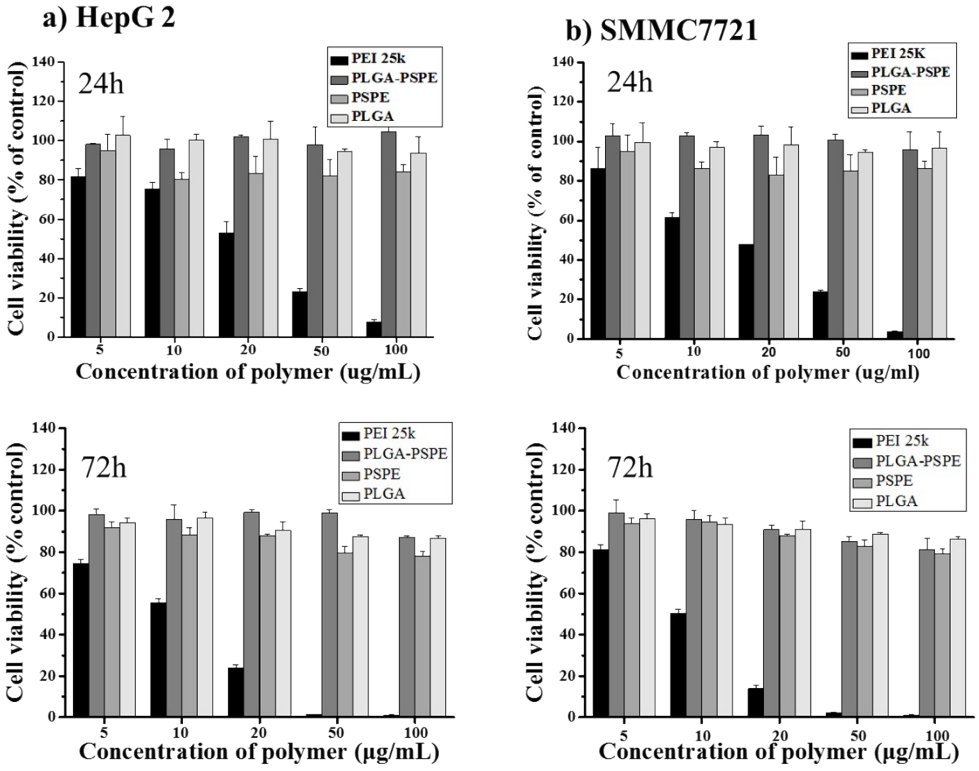

Fig. S5. Cytotoxicity of PLGA-PSPE at various concentrations in different cell (a) HepG2 cells and (b) SMMC7721 cells at 24 and 72 h. Results are represented as mean ±SD, n=3.

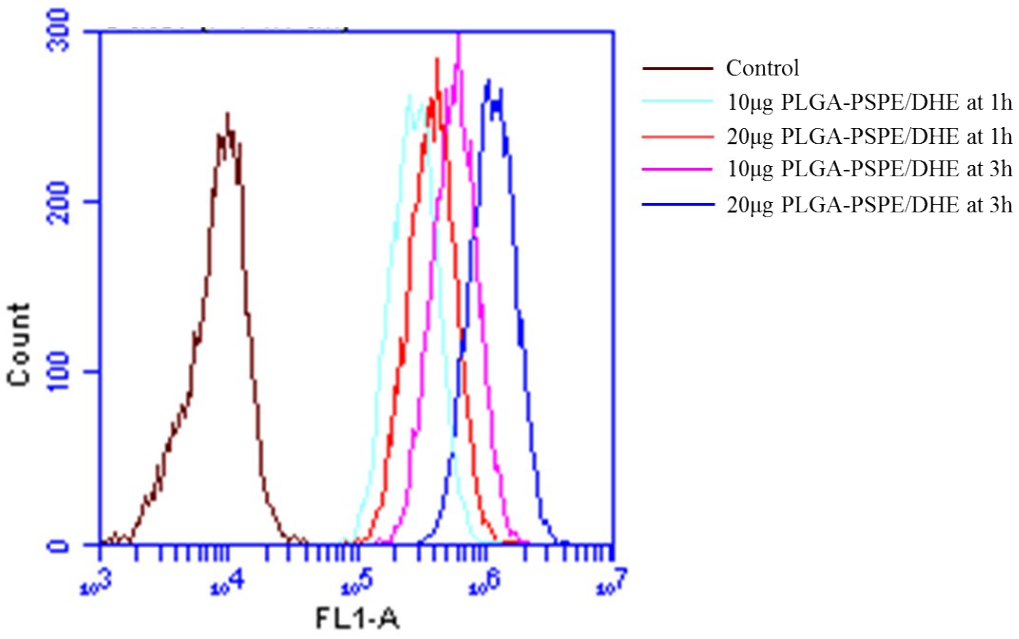

Fig. S6. Cellular uptake of PLGA-PSPE/DHE by FACS in A549 cells**.**

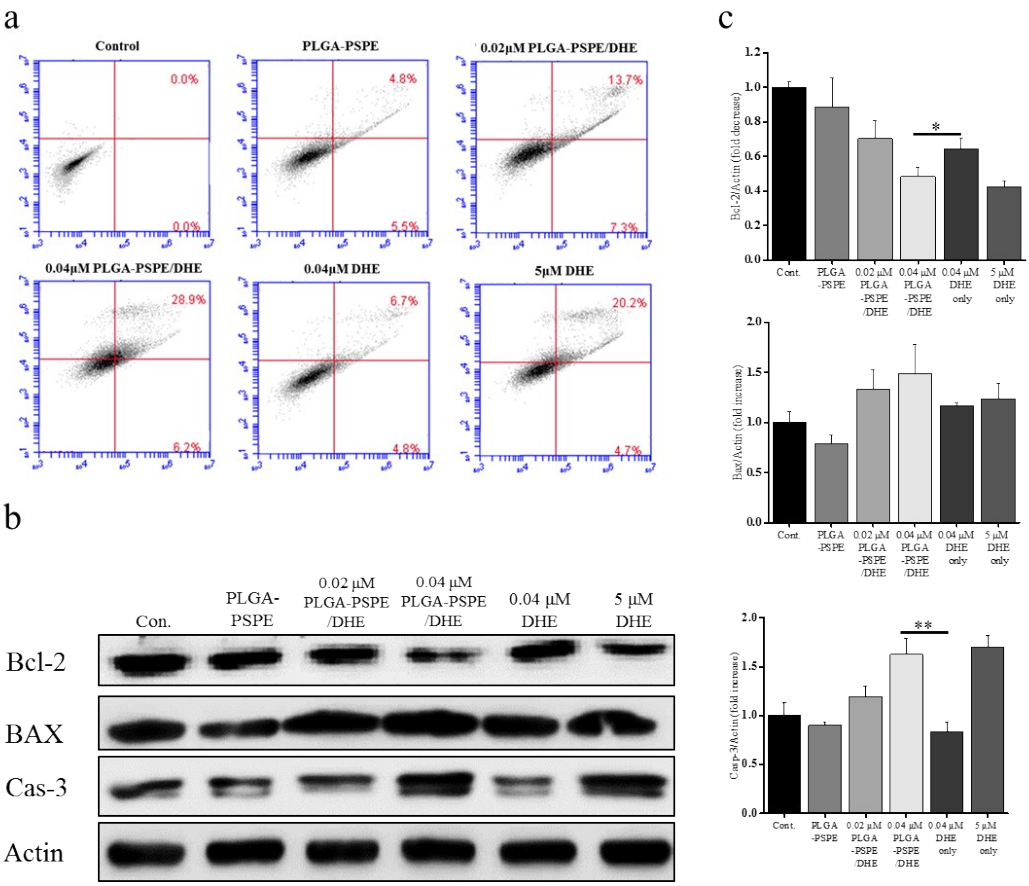

Fig. S7. In vitro apoptosis assay. (a) Flow cytometric analysis of apoptosis in A549 cells induced by various formulations. The lower-right and upper-right quadrants indicate the early apoptotic cells and the late apoptotic cells, respectively. (b) Western blot analyses of apoptosis-related proteins in A549 cells. (c) Densitometric analysis of Western blotting bands of apoptosis-related proteins in A549 cells. Results are represented as mean ± SD. *$p < 0.05$, **$p < 0.01$.

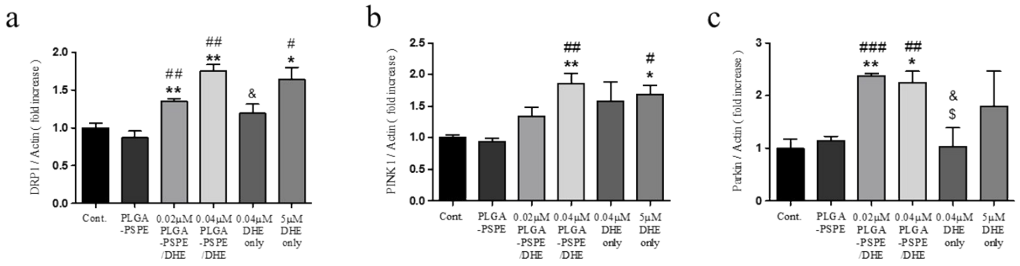

Fig. S8. Densitometric analysis of Western blotting bands of mitophagy-related proteins in A549 cells. Results are represented as mean ± SD. *$p < 0.05$, and **$p < 0.01$ versus Cont., $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ versus PLGA-PSPE, $^{\$}p < 0.05$ versus 0.02 μM PLGA-PSPE/DHE, $^{\&}p < 0.05$ versus 0.04 μM PLGA-PSPE/DHE.