SUPPLEMENTARY INFORMATION

Hydrophobic and membrane permeable polyethylenimine nanoparticles efficiently deliver nucleic acids in vitro and in vivo

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General

Branched polyethylenimine (bPEI, 25 kDa), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl suberimidate hydrochloride (DMS), Tris, ethidium bromide (EtBr), high retention dialysis tubing (cut off 12 kDa), xylene cyanol, bromophenol blue, GFP-specific siRNA and tetramethylrhodamine isothiocyanate (TRITC) were procured from Sigma-Aldrich Chemical Co., USA. Bradford reagent was purchased from Bio-Rad Inc., USA. Commercial transfection reagents used in the present study were purchased from their respective suppliers, viz., GenePORTER 2™ (Genlantis, USA), Superfect™ (Qiagen, France), Fugene™ (Roche Applied Science, USA) and Lipofectamine™ (Invitrogen, USA). YOYO-1 iodide (λ_{ex} 491nm and λ_{em} 509nm) was purchased from Invitrogen, USA. Cell culture products and plasmid isolation kit were obtained from Gibco-BRL-Life Technologies (UK) and Qiagen (France), respectively. Particle size and zeta potential of nanoparticles and their DNA complexes were determined on Zetasizer Nano-ZS (Malvern Instruments, UK). GFP reporter gene expression was observed under Nikon Eclipse TE 2000-S inverted microscope (Kanagwa, Japan). Green fluorescent protein (GFP) was analyzed spectrofluorometrically on NanoDrop® ND-3300 spectrofluorometer, USA, at an excitation wavelength of 488 nm and emission at 509 nm. Confocal imaging of labeled nanoparticles was carried out with a Zeiss LSM 510 Meta confocal microscope. The analysis of the transfected cells was performed by the CellQuest software (BD Biosciences, USA). Luciferase vector, pGL3, was used for in vivo delivery in Balb/c mice and the luciferase activity was measured on luminometer (Berthold Microplate Reader, Herts, UK). All the experiments were carried out using MilliQ (deionized) water, pH 7.2, filtered through 0.22 μm sterile filters (Millipore).

Cell cultures

HEK293 (Human embryonic kidney), HeLa (Human cervical adenocarcinoma) and HepG2 (Human hepatocellular liver carcinoma cell line) were maintained as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 50 mg/ml gentamicin.

Preparation of pDNA complexes

To prepare pDNA complexes with CLP NPs and bPEI, aqueous solutions of CLP nanoparticles and bPEI (1mg/ml) were mixed with 1μl of pDNA (0.3μg/μl) at various N/P ratios (2, 4 and 6 for CLP NPs and 1, 2 and 3 for bPEI). For transfection and cell viability assays, pDNA complexes were prepared at N/P ratios of 4, 8, 12, 16, 20, 24 and

30 in 5% dextrose solution (5µl) and final volume was made to 20 µl with water. The resulting complexes were vortexed and incubated for 30 min at ambient temperature prior to their use.

DNA mobility shift assay

pDNA complexes of CLP nanoparticles and bPEI were prepared as described above. These complexes were mixed with 2µl xylene cyanol (in 20% glycerol), electrophoresed (100V, 1h) in 0.8% agarose, stained with ethidium bromide (EtBr) and migration pattern was visualized on a UV transilluminator using a Gel Documentation System from Syngene (UK).

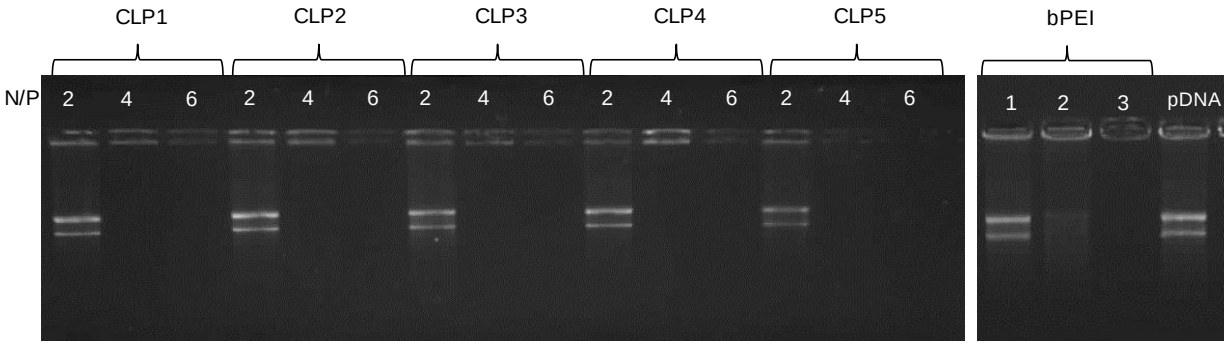

Fig. S1. DNA retardation assay of CLP/pDNA and bPEI/pDNA complexes.

DNase protection assay. In order to confirm the ability of CLP3 NPs to protect the condensed pDNA from endonucleases, DNase I protection assay was executed as reported earlier [1]. CLP3/pDNA nanoplex (N/P 20) was incubated with 1 µl of DNase I (1 Unit / µl) or 1 µl PBS, as a control, at 37 °C for different time intervals (15, 30, 60, and 120 min). The experiments were terminated by the addition of 5 µl of 0.1M EDTA followed by heating to 75 ^{o}C for 10 min to inactivate the enzyme and incubated for 60 min with 10 µl of heparin (5 mg/ml) to completely dissociate pDNA nanoplex. The analysis of released pDNA was performed by gel electrophoresis, as described above in the section, DNA release assay.

Table S1. Size and zeta potential measurement of CLP NPs in water and 10% FBS
(GP 2 : GenePORTER 2™; L'amine : Lipofectamine™)

Samples	Average particle size in nm ± S.D. (PDI)			Zeta potential in mV ±S.D.			Ratio of Nanoparticles : pDNA (N/P)
	Nanoparticles (in H_2O)	DNA loaded Nanoparticles (in H_2O)	DNA loaded Nanoparticles (in 10% FBS)	Nanoparticles (in H_2O) (+)	DNA loaded Nanoparticles (in H_2O) (+)	DNA loaded Nanoparticles (in 10% FBS)	
CLP1	210.2 ± 14.5 (0.28)	225.1 ± 11.1 (0.22)	134.5 ± 10.4 (0.18)	35.2 ± 1.27	30.6 ± 2.1	-10.6 ± 2.7	20
CLP2	198.4 ± 14.9 (0.24)	207.2 ± 14.5 (0.21)	110.4 ± 8.8 (0.2)	36.1 ± 2.21	32.3 ± 1.9	-10.3 ± 1.9	20
CLP3	167 ± 12.8 (0.25)	179.6 ± 15.9 (0.2)	91.8 ± 9.5 (0.17)	36.5 ± 1.61	31.9 ± 2.1	-9.8 ± 1.3	20
CLP4	154.2 ± 13.3 (0.29)	169.4 ± 15.2 (0.22)	88.9 ± 6.9 (0.19)	37.1 ± 2.61	31.2 ± 1.9	-9.6 ± 1.8	20
CLP5	151.6 ± 12.8 (0.22)	167.1 ± 15.7 (0.21)	80 ± 7.1 (0.18)	38.2 ± 2.33	30.8 ± 2.2	-9.5 ± 1.9	20
bPEI	--	346.5 ± 28.89 (0.38)	218.19 ± 5.17 (0.18)	39.5 ± 2.3	33.5 ± 1.6	-13.3 ± 1.3	12
Superfect	--	548.4 ± 26.49 (0.59)	--	--	38.4 ± 2.87	--	0.3µl/0.3µg
Fugene	--	387.5 ± 27.32 (0.53)	--	--	40.4 ± 1.8	--	1.2µl/0.3µg
GP 2	-	155.6 ± 22.31 (0.20)	--	--	19.2 ± 1.33	--	3.5µl/0.3µg
L'amine	--	475.6 ± 26.8 (0.49)	--	--	46.1 ± 1.78	--	2µl/0.3µg

Reference

1 R. Goyal, S. K. Tripathi, E. Vazquez, P. Kumar and K. C. Gupta, *Biomacromolecules*, 2012, **13**, 73.