Supporting Information

Alkylated branched poly(β-amino esters) demonstrate strong DNA encapsulation, high nanoparticle stability and robust gene transfection efficacy

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1. Materials and Methods

1.1. Materials

Trimethylolpropane triacrylate (TMPTA), 1,4-butanediol diacrylate (B4), undecylamine (UDA), 3-morpholinopropylamine (MPA) and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich. Diethyl ether was purchased from Fisher Chemical. The commercial transfection reagents branched PEI (Mw~25 kDa, Sigma-Aldrich) was used at a w/w ratio of 3:1. Lipofectamine 2000 (Lipo2000, Life Technologies) was used as per manufacturers protocols. The DNA used here included green fluorescent protein coding plasmid (pCMV-GFP) and Gaussia princeps secreted

luciferase coding plasmid (pCMV-GLuc), and their expansion, isolation and purification were conducted using the EndoFree Plasmid Kit (Tiangen, China) as per protocols.

1.2. Instrumentation

Chemical composition was verified by ^{1}H NMR spectra on a Varian UNITY-plus 400 spectrometer.

The molecular weight, polydispersity index (PDI), and alpha-value of the Mark-Houwink plots of polymers were measured by gel permeation chromatography (GPC) (Agilent Technologies, PL-GPC 50) with dimethylformamide (DMF) as elution. The GPC was equipped with a refractive index detector (RI), a dual angle light scattering detector (LS 15° and LS 90°) and a viscometer detector (VS DP). The machine was calibrated with linear poly(methyl methacrylate) (PMMA) standards.

1.3. Synthesis of branched poly(β-amino esters) with alkyl chains (BPA)

BPA was synthesized *via* Michael addition reaction. The monomer feed ratio of TMPTA:B4:UDA was set as 0.13:1:1. Briefly, B4 (1.98 g, 10mmol), TMPTA (0.394 g, 1.33 mmol) and UDA (1.71 g, 10 mmol) were dissolved in DMSO at a concentration of 50%. The reaction was conducted at 90°C. For its linear counterpart (LPA) synthesis, the feed ratio of B4:UDA was set as 1.2:1, and B4 (2.376 g, 12 mmol) and UDA (1.71 g, 10mmol) were used for polymerization. Reactions were terminated when the polymer reached the desired molecular weight (~10 kDa), using gel permeation chromatography (GPC) to monitor molecular weight. Afterwards, the acrylate terminated polymer was mixed with excess MPA for end-capping, and the mixture was stirred for 48 h at room temperature. The products were precipitated in diethyl ether three times and dried under vacuum for 24 h to give brown sticky solid. BPA, ^{1}H NMR ($CDCl_3$): 0.8 (bs, C***H***$_3$CH$_2$-), 1.2-1.3 (m, CH$_3$C***H***$_2$-), 1.7 (m, -CH2C***H***2CH2CO-), 3.7 (t, -NCH$_2$C***H***$_2$O-), 4.0 (s, -COOC***H***$_2$C-); ^{13}C NMR ($CDCl_3$): 7.3 (***C***H3CH2C-), 14.0 (***C***H$_3$CH$_2$CH$_2$-), 49.1 (-NH-***C***H$_2$CH$_2$OOC-), 66.8 (-COO***C***H$_2$C-), 172.5 (-***C***OO-). LPA, ^{1}H NMR ($CDCl_3$): 0.8 (bs, C***H***$_3$CH$_2$-), 1.2-1.3 (m, CH$_3$C***H***$_2$-), 1.7 (m, -CH2C***H***2CH2CO-), 3.7 (t, -NCH$_2$C***H***$_2$O-); ^{13}C NMR ($CDCl_3$): 14.0 (***C***H$_3$CH$_2$CH$_2$-), 49.1 (-NH-***C***H$_2$CH$_2$OOC-), 172.4 (-***C***OO-).

1.4. DNA binding affinity assays

DNA binding affinity of polymers was evaluated by Picogreen assays. Calculated polymers and DNA were diluted in 25 mM sodium acetate and then mixed at a 1:1 volume ratio for 15 min incubation and 0.5 μg DNA was used for each sample. Then picogreen solution was mixed with the polyplex solution, after 5 minutes 200 μL DMEM media was added into the mixture and transferred to a black 96-well plate. Fluorescence of the solution was measured at a 490 nm excitation wavelength and a 535 nm emission wavelength. The mixture of DMEM, DNA and picogreen was used as control.

1.5. Particle size and zeta potential assays

The polyplexes were prepared as mentioned above. For particle size assays, the polyplexes were added to 1mL of DMEM media with 10% fetal bovine serum (FBS). For zeta potential assays, the polyplexes were added to 1 mL of phosphate buffered saline (PBS). Particle sizes and zeta potentials were recorded on a Malvern Zetasizer. Sizes were measured by the dynamic light scattering (DLS) and zeta potentials were determined by the electrophoretic light scattering.

1.6. Transmission electron microscopy (TEM)

To characterize the polyplex morphology, LPA/DNA and BPA/DNA polyplexes were prepared as mentioned above at a w/w ratio of 10 : 1. After 15 min of incubation, the polyplexes were centrifugated and washed with deionized water twice. After the polyplexes were resuspended in deionized water, 3 μL of polyplex solution was dropped on holey carbon films on 200 mesh copper grids and freeze dried immediately. TEM assays were operated at 120 kV.

2. Biological studies

2.1. Cell Culture

The human cervical cancer cell line (HeLa) and rat adipose-derived stem cells (rADSC) were purchased from ATCC (Teddington, UK) and maintained in DMEM containing 10% FBS at 37 °C, 5% CO_2 atmosphere.

2.2. *In vitro* transfection

Transfection efficiency of polymer/DNA polyplexes was assessed by Gluciferase activity and green fluorescence protein (GFP) expression. Hela and rADSC cells were

seeded at a density of $1x10^4$ cells/well in a 96-well plate 24 h before polyplex addition. The polyplexes were prepared as mentioned above and 250 ng DNA per well was used. After incubation for 15 min, polyplexes were diluted with DMEM containing 10% FBS, and replace the previous cell culture media. After 48 h, the transfection efficiency was analyzed for Gluciferase activity and GFP expression using Gaussia Luciferase Assay Kit (BioLux®) and fluorescence microscope (Olympus), respectively.

2.3. Cell viability assays

Cell viability was evaluated by alamarBlue assay. Briefly, 48 h post transfection, cell viability was assessed following the alamarBlue assay manufacturers' protocol. Cells were washed with HBSS, followed by the addition of alamarBlue® solution (100 μL, 10% in HBSS). After 2 h incubation, absorbance was read at 570 nm and 600 nm on a plate reader (SpectraMax® M3) and cell viability percentage was calculated.

2.4. Cellular uptake of polyplexes

HeLa cells were seeded in 96 well plates at a density of 3000 cells per well. DNA was labeled by Cy3 (a red fluorescent dye). Polyplexes were prepared as montioned above using DNA-Cy3 at w/w = 10: 1. Transfection procedure was carried out as mentioned above. 4 h post transfection, the cell culture media was removed, cells were washed three times with PBS and fixed with 4% paraformaldehyde for 15 min. 4',6-diamidino-2-phenylindole (DAPI) was utilized to stain the cell nucleus and cells were visualized with an Olympus fluorescence microscopy.

2.5. Transfection at high serum concentrations and low DNA doses

To assess the serum resistance of LPA, BPA and commercial PEI and Lipo2000, Hela cells were seeded at a density of $1x10^4$ cells/well in a 96-well plate 24 h before polyplex addition. The polyplexes were prepared as mentioned above and 250 ng DNA per well was used. After incubation for 15 min, polyplexes were diluted with DMEM containing 10% FBS, 30% FBS and 50% FBS respectively, and replace the previous cell culture media. After 48 h, the transfection efficiency was analyzed for Gluciferase activity using Gaussia Luciferase Assay Kit (BioLux®). To investigate the influence of DNA doses on transfection efficiency, transfection was conducted in Hela cells at 250 ng, 120 ng and 60 ng DNA per well, respectively. Serum concentration was fixed at 10%.

2.6. Statistical analysis

Data are shown as mean ±standard deviations (SD). Statistical analysis was performed using Student'st-tests. Mean values and SD were calculated from at least three independent experiments. Statistical significances were set at $P < 0.05$ (*).

3. Figures

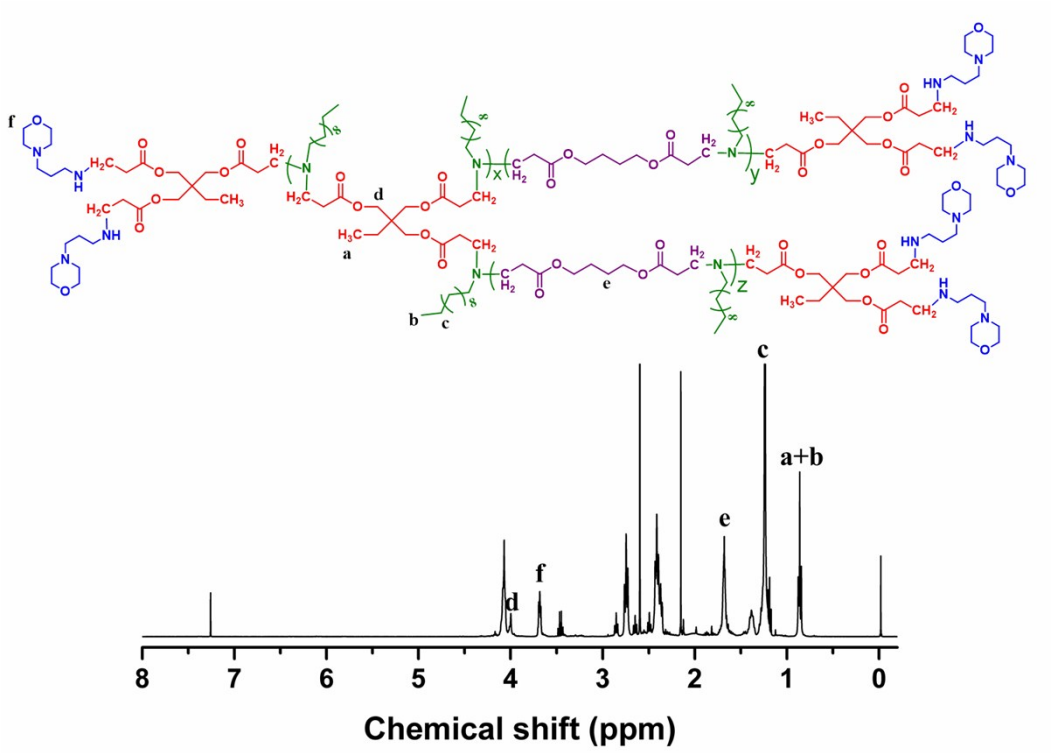

Fig. S1 ^{1}H NMR spectra of BPA. The peaks at 4.0 ppm are attributed to methylene of TMPTA, the peaks at 1.68 ppm are assigned to methylene of B4, and the peaks at 1.25 are attributed to methylene of UDA. The characteristic peaks of MPA can be identified at 3.69 ppm.

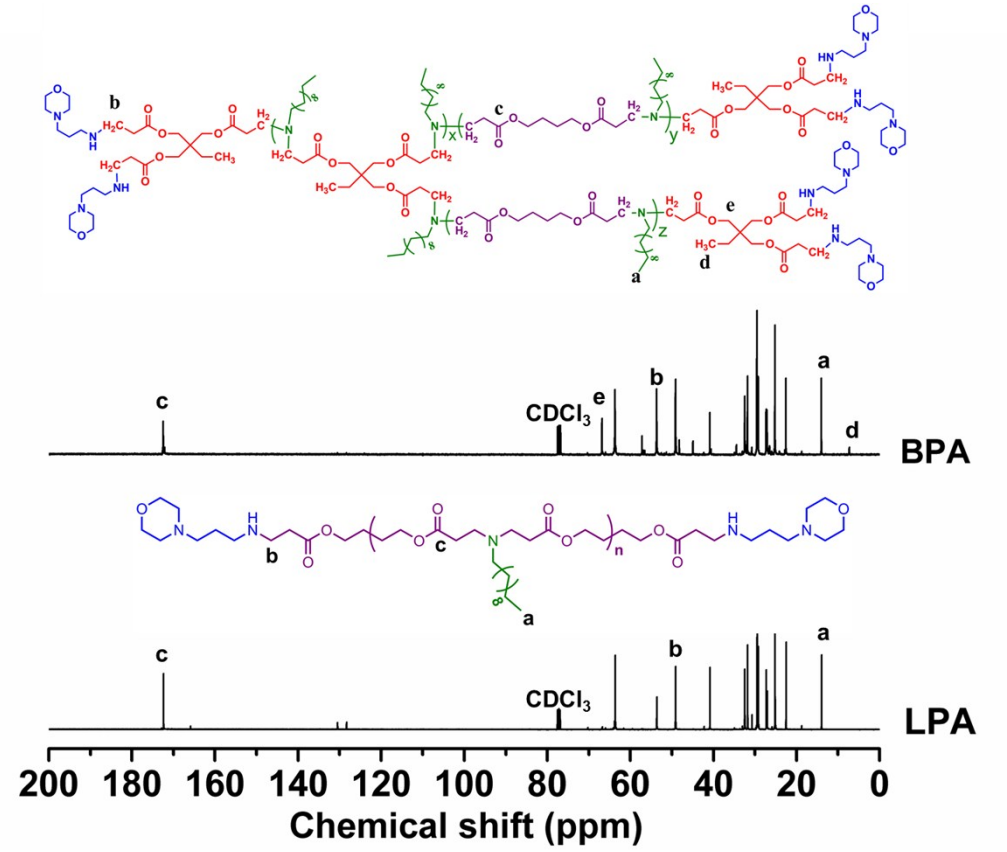

Fig. S2 ^{13}C NMR spectra of BPA and LPA.

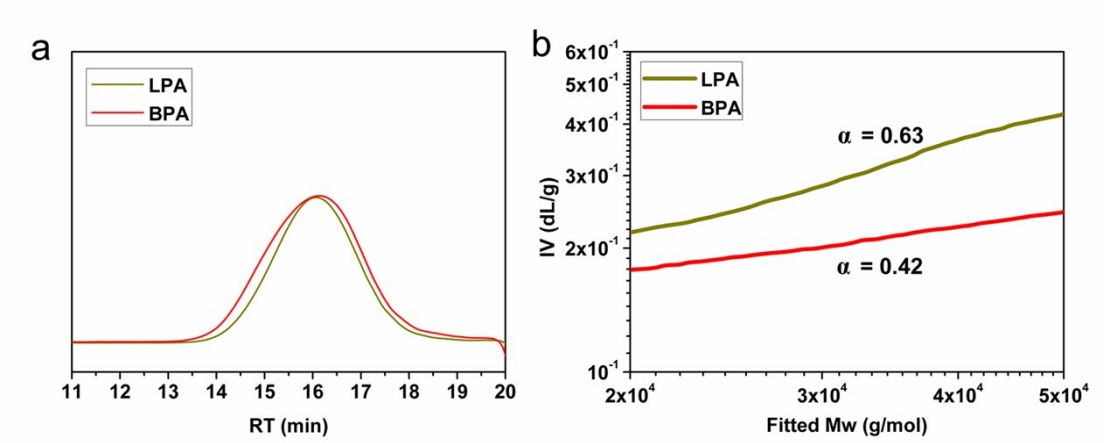

Fig. S3 (a) GPC traces show that LPA and BPA have similar molecular weights; (b) Mark-Houwink (MH) plots of LPA and BPA.

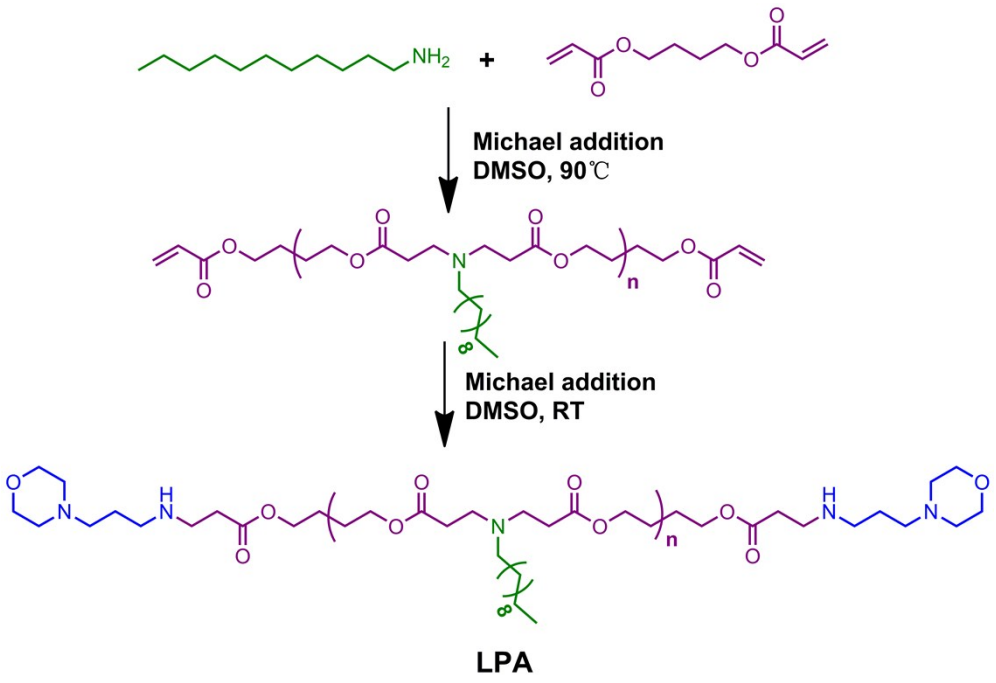

Fig. S4 Synthesis of linear poly(β-amino esters) with alkyl chains (LPA)

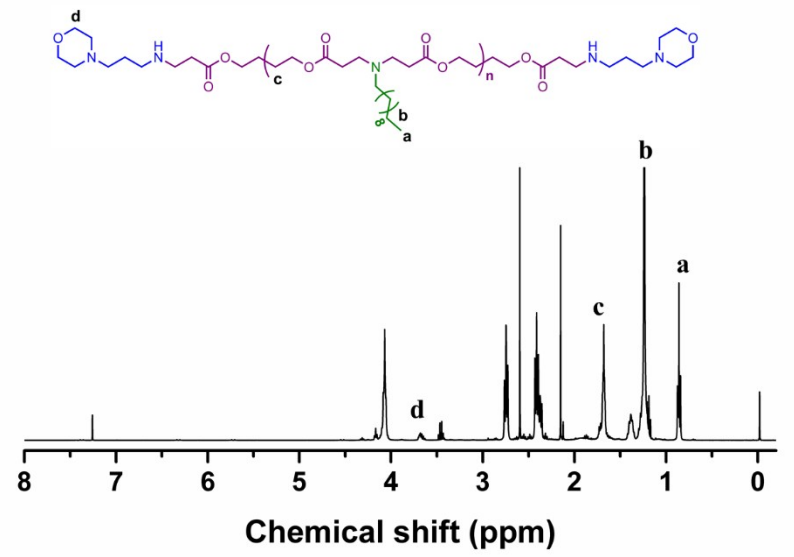

Fig. S5 ^{1}H NMR spectra of LPA. The peaks at 1.68 ppm are assigned to methylene of B4, and the peaks at 1.25 are attributed to methylene of UDA. The characteristic peaks of MPA can be identified at 3.69 ppm.

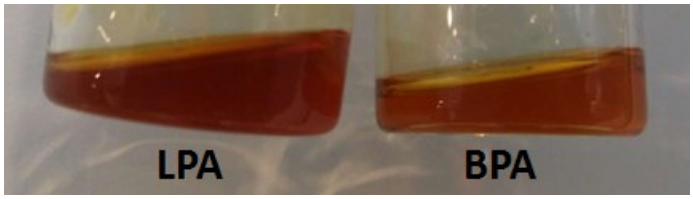

Fig. S6 The physical appearance of LPA and BPA.

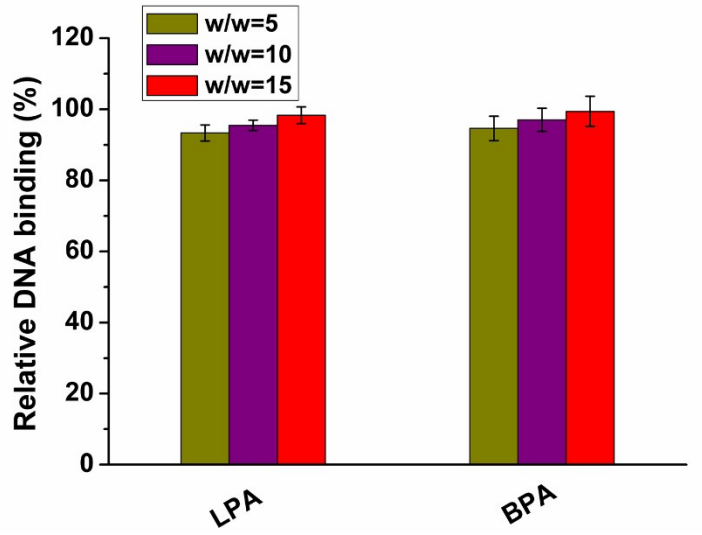

Fig. S7 Picogreen assays of relative DNA binding by LPA and BPA at w/w from 5:1 to 15:1. Data represent mean ± SD (n=3).

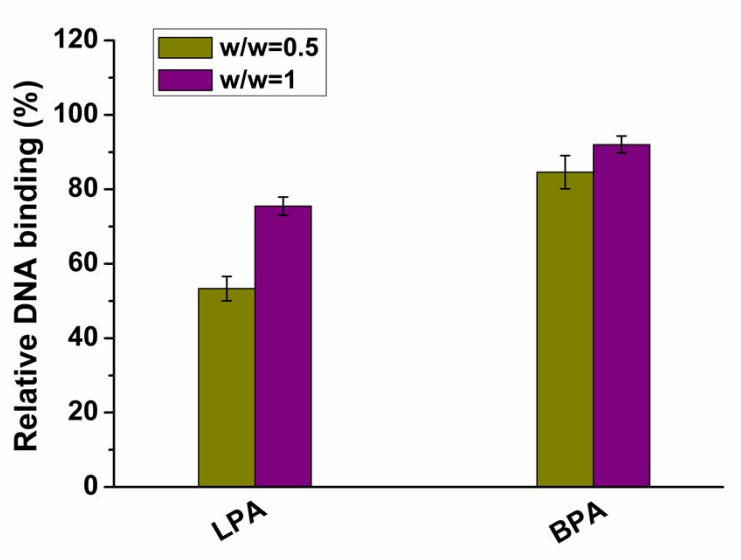

Fig. S8 Picogreen assays of relative DNA binding by LPA and BPA at low weight ratios. Data represent mean ± SD (n=3).

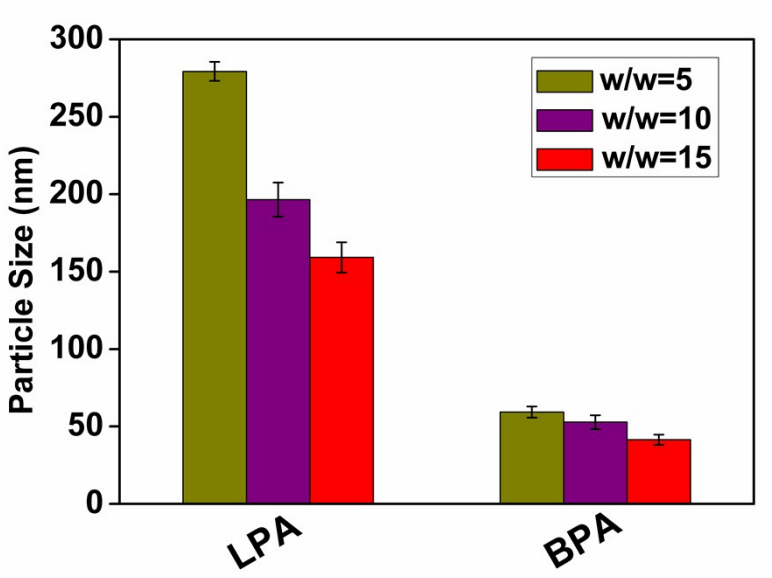

Fig. S9 Particle sizes of LPA/DNA and BPA/DNA polyplexes in DMEM media containing 10% serum for 8 h (n=3).

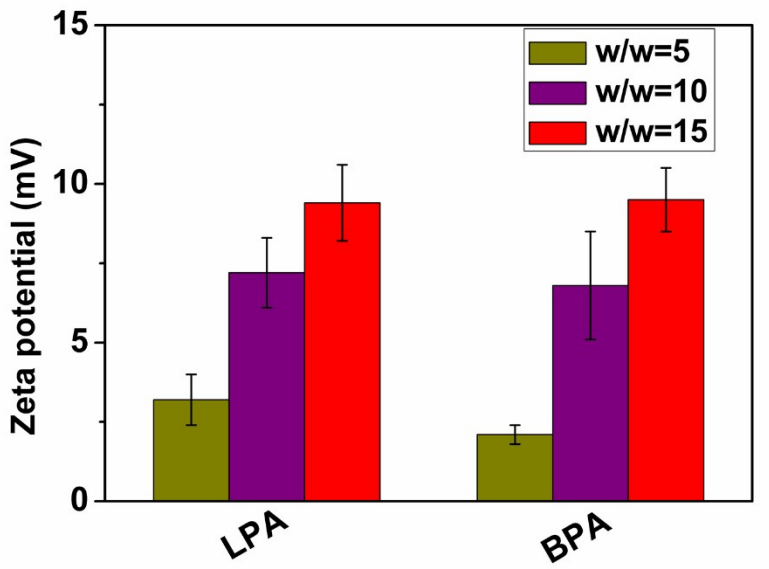

Fig. S10 Zeta potentials of LPA/DNA and BPA/DNA polyplexes. Data represent mean ± SD (n=4).

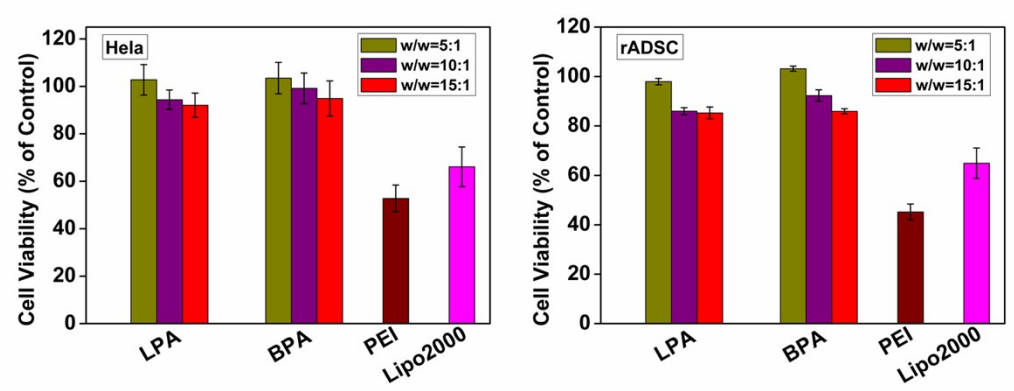

Fig. S11 Cell viability of LPA/DNA and BPA/DNA polyplexes in Hela and rADSC cells.

Table S1 Monomer feed ratio and structural information of BPA and LPA.

Polymer	Feed ratio [TMPTA]:[B4]:[UDA]	M_w (Da)	M_n (Da)	PDI	α
BPA	0.13:1:1	9075	3640	2.49	0.42
LPA	0:1.2:1	8130	4168	1.95	0.63