Supporting Information

Polyaspartamide derivative nanoparticles with tunable surface charge achieve highly efficient cellular uptake and low cytotoxicity

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General procedures

a) Structure characterization of PASP-pg-TEPA

The chemical structure of PASP-pg-TEPA was characterized by ^{1}H-NMR and FT-IR. ^{1}H-NMR spectra were recorded on an Avance AV 400MHz Instrument (Bruker Co., Switzerland). The chemical shifts were reported in ppm on the d scale with tetramethylsilane as the internal reference and D_2O as the solvent. FT-IR spectra of PASP-pg-TEPA were measured on a TENSOR37 Fourier Transform Infrared

Instrument (Bruker Co., Germany). The samples were prepared in form of KBr pellets, and were scanned from 4000 to 400 cm^{-1}. The chemical shift of poly succimide (PSI), poly (aspartic acid) (PASPA), tetraethylenepentamine (TEPA) and PASP-pg-TEPA were shown in Figure S1.

In the ^{1}H-NMR spectra of PASP-pg-TEPA, the chemical shifts of proton appeared in 2.65~2.79 ppm were correlated to the methylene proton of TEPA (-NH-CH_2-CH_2-NH-). And the chemical shifts of proton appreared in 2.99~3.07 ppm were correlated to the methylene protons of -CH_2-COOH in PASPA. However, the signal of each peak is very small because the PASP-pg-TEPA is poorly soluble in NMR solvents (DMSO-d_6, $CDCl_3$ and D_2O).

The FT-IR spectra showed that the TEPA group was grafted to PASP backbone (Figure S2). C=O stretches of carboxyl group at 1659 cm^{-1} and N-H stretches of grafted TEPA group at 1538 cm^{-1} exist together compared with PASPA.

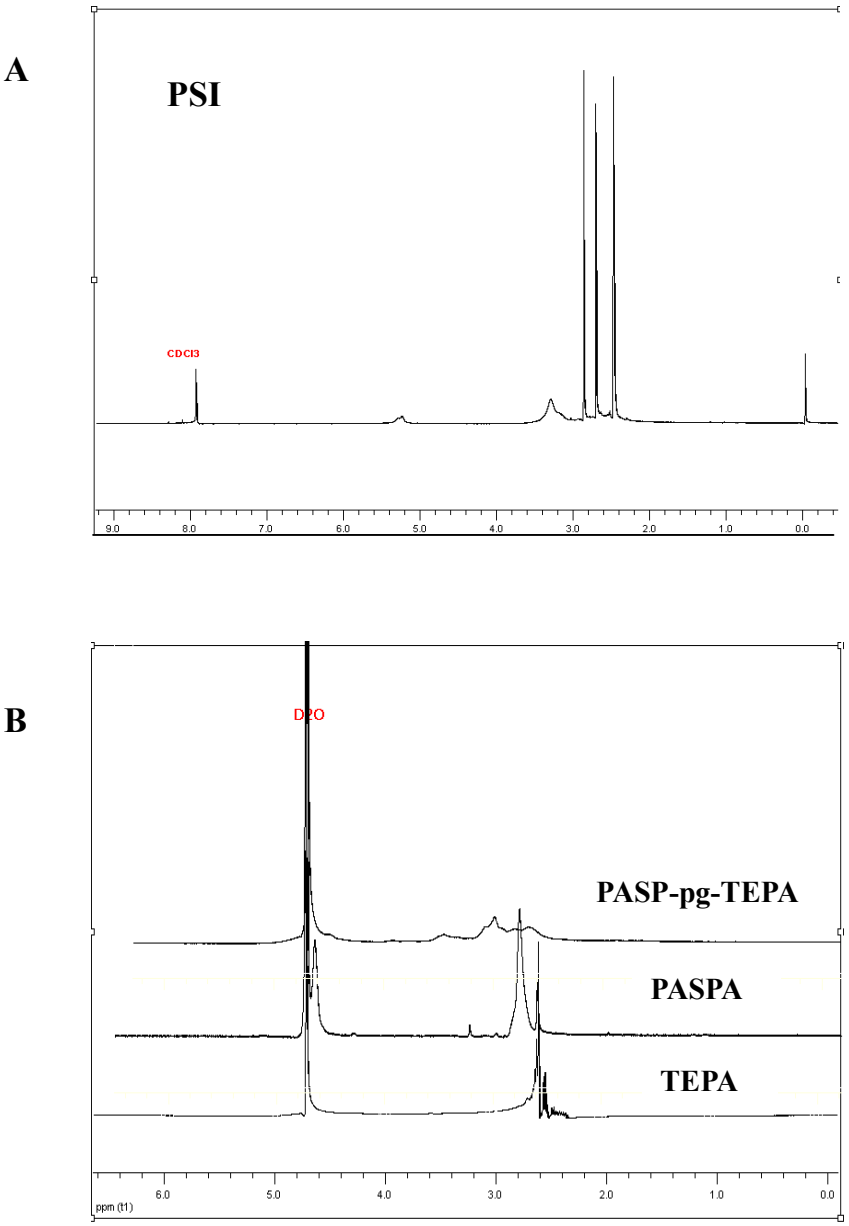
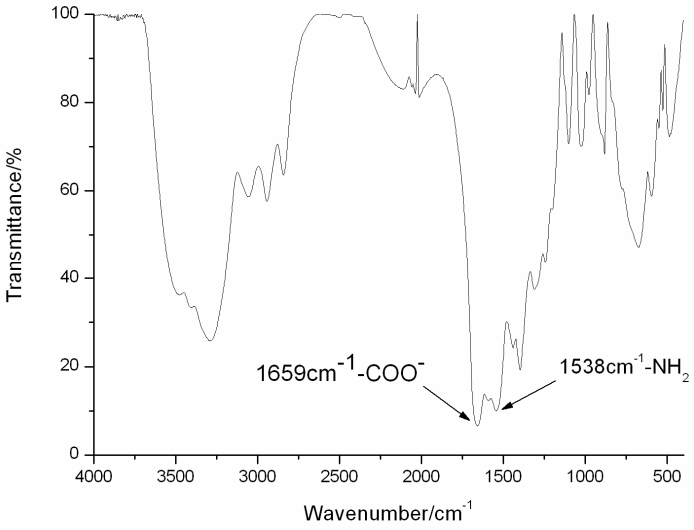


Figure S1 ^{1}H-NMR Spectra

A



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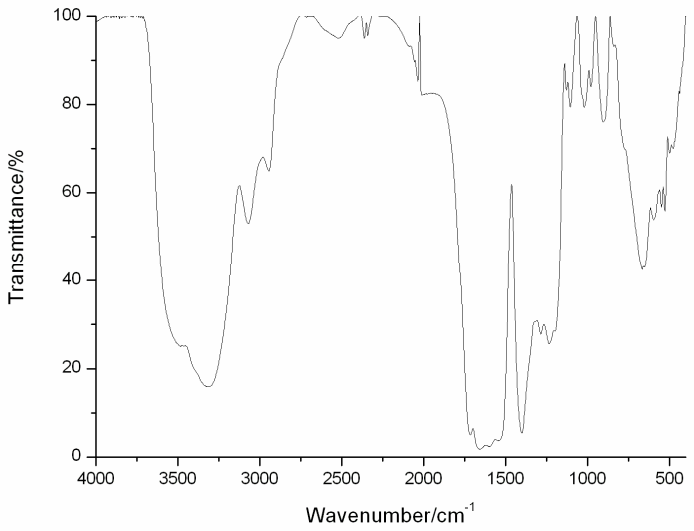

Figure S2 FT-IR spectra of (A) PASP-pg-TEPA and (B) PASPA

b) Elemental analysis of PASP-pg-TEPA

Carbon, hydrogen and nitrogen content of PASP-pg-TEPA by elemental analysis were determined using a Vario EL (Elementar, Germany) elemental analyzer. Elemental analysis was used to confirm the reaction of TEPA and PSI and to calculate the degree of substitution (DS) of the obtained PASP-pg-TEPA. The DS of the ligand TEPA on PASPA was calculated by the following equation and listed in Table S1.

$$DS = \frac{N(\%)m - N(\%)o}{5 \times N(\%)o} \times 100\%$$

Where N(%)m is the weight percentage of N element of PASP-pg-TEPA with different IEPs, $N(\%)_o$ is the weight percentage of N element of poly(aspartic acid), and 5 is the atom number of N element in TEPA.

Table S1 Elemental Analysis of PASPA and PASP-pg-TEPA with different IEPs

	C（%）	N（%）	Degree of substitution(%)
Poly (aspartice acid)	30.38	8.850	
PASP-pg-TEPA (IEP=8.5)	35.92	13.86	11.32
PASP-pg-TEPA (IEP=8.8)	37.00	14.75	13.33
PASP-pg-TEPA (IEP=9.1)	35.88	15.22	14.40

c) Measurement of light transmittance of zwitterionic PASP-pg-TEPA nanoparticle dispersion

The light transmittance of nanoparticle dispersion at different pHs was measured with a UV-Vis spectrophotometer at 500nm. Adjustment the pH of the solution was by careful addition of 0.1 N HCl with stirring.

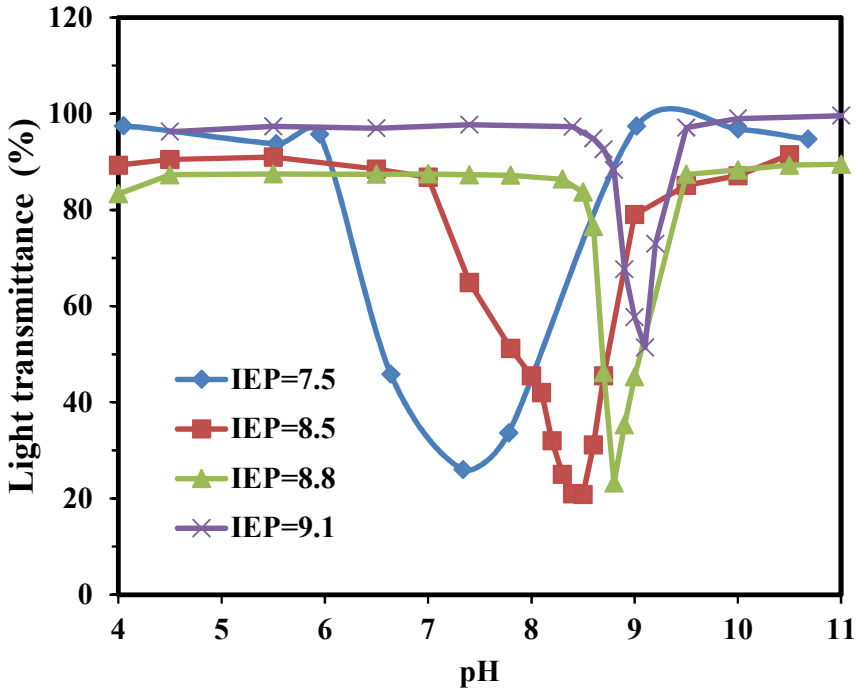

Figure S3 Light transmittance of zwitterionic PASP-pg-TEPA nanoparticle samples at different pHs

d) The effects of salt concentration and pH on the formation of PASP-pg-TEPA nanoparticles

To study how the assembly conditions affect the particle size, the hydrated diameter of PASP-pg-TEPA nanoparticles in solution at different pHs and NaCl concentrations were measured using a Malvern Zetasizer NS90 (Malvern Instruments, UK). The synthesized PASP-pg-TEPA in DMF was dialyzed against 0.1mol/L HCl, ddH_2O and 0.1mol/L NaOH. The pHs of the three kinds of solutions are, respectively, 1, 7 and 13. The particle size of IEP 9.1 nanoparticles in different pH medium was shown in Figure S4. The PASP-pg-TEPA precipitates in acidic dialysis medium, but it formed into nanoparticles with diameters around 55 nm in basic medium and around 100nm in neutral medium. This may be explained by the higher graft ratio of carboxyl group than amine group in the polymer according to the result of elemental analysis. When the medium is acidic, a large amount of carboxyl group exits in the form of –COOH and associates tightly because of hydrophobic forces, which causes precipitation. When the medium is basic or neutral, only a small fraction of amine exits in the form of $–NH_2$ and associates into the hydrophobic core of nanoparticles while a large amount of $–COO^-$ exposes on the surface of nanoparticles.

Furthermore, the effects of salt concentrations on the particle size were also investigated. NaCl solutions with serially diluted concentrations were used as the dialysis medium. The particle size of PASP-pg-TEPA was measured immediately after dialysis. According to the result shown in Figure S5, varying ionic strength of dialysis medium (in the range of 50 - 250mM) does not affect self-assembly formation of

PASP-pg-TEPA nanoparticles.

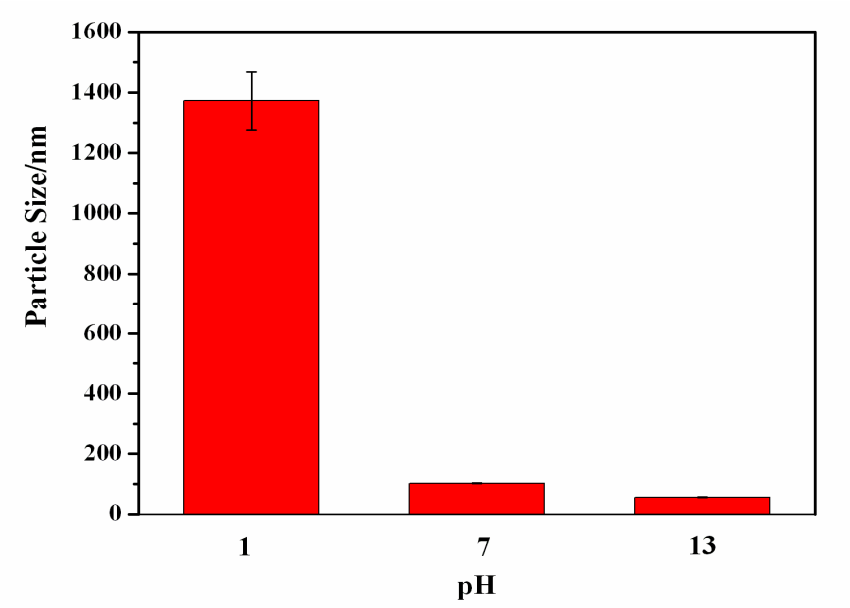

Figure S4 Particle size of PASP-pg-TEPA (IEP=9.1) nanoparticles which were dialyzed against medium with different pHs

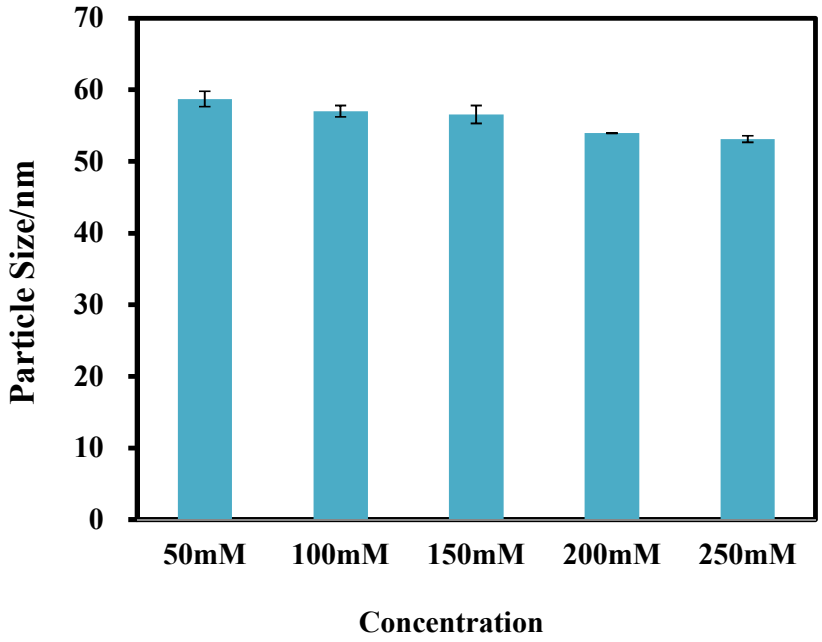

Figure S5 Particle size of PASP-pg-TEPA (IEP=9.1) nanoparticles which were dialyzed against NaCl solution with different concentrations