Electronic Supplementary Material (ESI) for RSC Advances.

Development of novel, biocompatible, polyester amines for microglia-targeting gene delivery

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Experimental

Materials

Characterization of TGP

The composition of TGP was assessed by ^{1}H high resolution nuclear magnetic resonance (NMR) spectroscopy using a Bruker AVANCE 600 MHz spectrometer. The TG, PEI, and TGP were dissolved in deuterium oxide (D_2O) at a concentration of 5 mg/ml and measured. The molecular weight of TGP was analyzed using gel permeation chromatography performed with a Dionex ultimate 3000 instrument. Briefly, sample (50 µl) was injected and measured in triplicate. The flow rate of the column was 1.0 ml/min, and 0.1 M sodium nitrate solution was used as the mobile phase.

Transmission electron microscopy

An image of TGP/DNA nanoplexes (N/P 20) was produced using energy-filtering transmission electron microscopy (EF-TEM) (LIBRA 120, Carl Zeiss, Oberkochen, Germany). The particle solution was placed on the copper grid, stained with 2% aqueous uranyl acetate for 5 s, washed twice with distilled water, and the resulting liquid was removed with filter paper.

Measurement of zeta-potential and particle sizes

The zeta-potential and sizes of TGP/DNA nanoplexes at N/P ratio of 20 were measured using a dynamic light scattering spectrophotometer (DLS, ELS 8000, Otsuka Electronics, Osaka, Japan) with 90° and 20° scattering angles. Average and standard deviation were calculated from three measurements of each sample.

MTS assay

Mixed glial cells were grown on a 96-well plate with 3×10^4 cells per well and treated with a cytotoxic reagent for 24 h. Then, 20 µl of MTS reagent (Promega) was added directly into the cell culture media and incubated at 37°C for 1 h. Reduction of the MTS tetrazolium compound by viable cells to generate the formazan product, soluble in serum-free media, was quantified by measuring the absorbance at 492 nm using an EMax® Plus Microplate Reader (Molecular Devices, San Jose, CA, USA). Absorbance obtained from samples treated with cytotoxic reagents was compared to absorbance in control samples to obtain the percentage of cell viability.

In vitro transfection efficiency

The efficiency of transfection in primary mixed glia was evaluated using the luciferase assay. Cells were seeded at 3×10^4 cells/well in a 24-well plate and treated with TGP/pGL3 nanoplexes at 70-80% confluency. TGP and PEI were complexed with purified pGL3 plasmids (0.5 µg) at various N/P ratios (5, 10, 20, 30) and added directly to cell culture media containing 10% FBS. Luciferase assay was conducted according to the manufacturer's protocol. Relative light units (RLU) were standardized by total protein, which was measured by a bicinchoninic acid assay (BCA) and performed in triplicate with a chemiluminometer (SPARK 10M, Tecan, Männedorf, Switzerland).

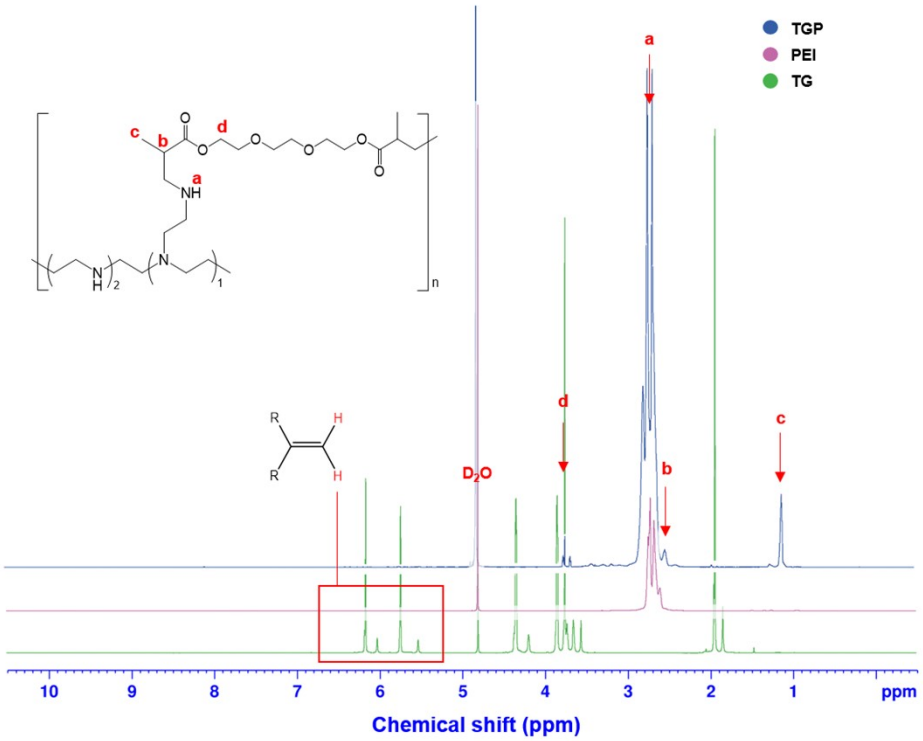

Figure S1. ^{1}H-NMR spectra of TGP in D_2O

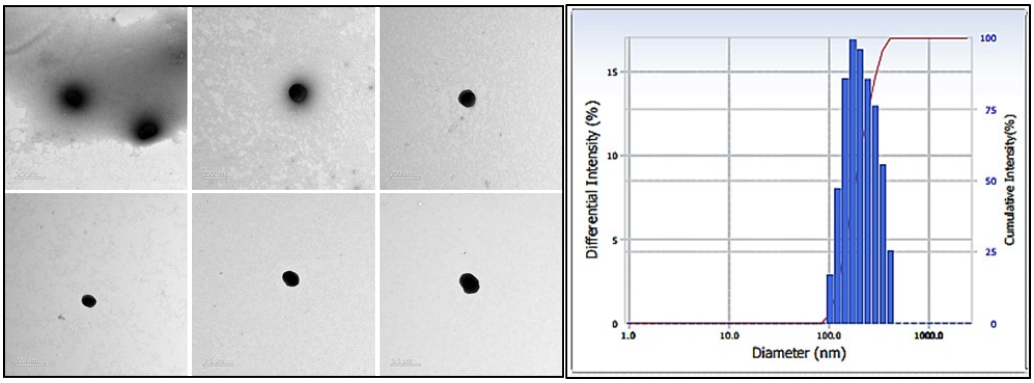

Figure S2. TEM images and particle distribution graph showing the morphology of TGP/pGL3 nanoplexes at N/P 20. (TEM image scale bar; 200 nm or 500 nm)

Table S1. Characterization of TGP measurement using gel permeation chromatography

	Mn[1]	**Mw**[2]	**Mz**[3]	**PD**[4]
1st	1587	1639	1689	1.03
2nd	1811	1852	1894	1.02
3rd	1932	1968	2004	1.02
SD	175.04	166.87	159.87	
Average	1777	1820	1862	1.02

[1]Mn (Number average molar mass); [2]Mw (Mass average molar mass); [3]Mz (Z-average molar mass); [4]PD (Polydispersity)

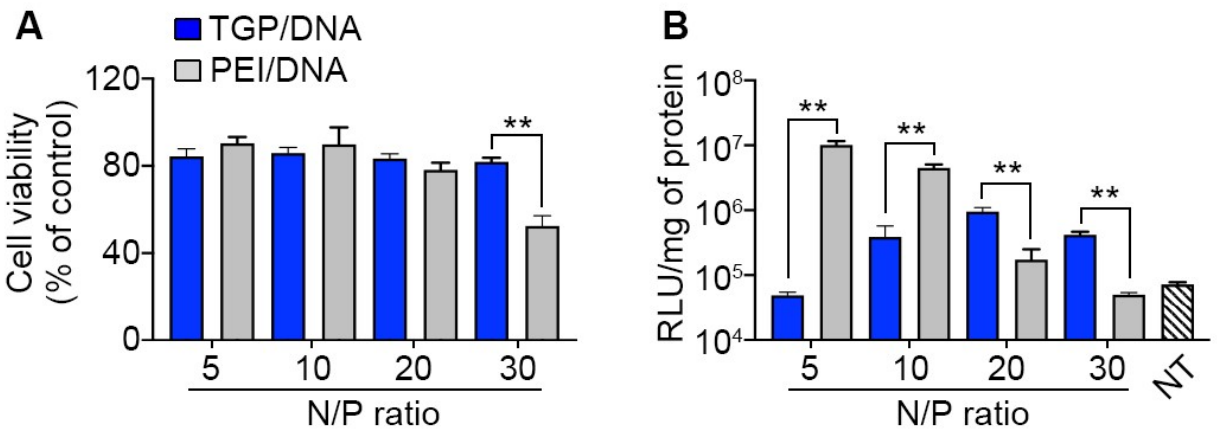

Figure S3. Cell viability (A) and transfection efficiency (B) of TGP/pGL3 in the presence of serum. Data are expressed as mean ± SEM (n = 3 per each group, $^{**}p < 0.01$).