GDNF gene delivery via a 2-(dimethylamino)ethyl methacrylate based cyclized knot polymer for neuronal cell applications

B. Newland,† M. Abu-Rub,† M. Naughton, Y. Zheng, A. V. Pinoncely, E. Collin, E. Dowd, W. Wang* and A. Pandit*

Supporting Information

Materials

The three monomers 2-(Dimethylamino)ethyl methacrylate (DMAEMA), poly(ethylene glycol) methyl ethyl methacrylate (PEGMEMA) and ethylene glycol dimethacrylate (EGDMA), were purchased from Sigma Aldrich. *N,N,N′,N′,N″*-pentamethyldiethylenetriamine (PMDETA, 99%, Aldrich), ethyl 2-bromoisobutyrate (EBriB, 98%, Aldrich), copper(II) chloride ($CuCl_2$, 97%, Aldrich), *d*-Chloroform (99.8%, Aldrich), L-ascorbic acid (AA, 99%, Aldrich), 2-Butanone (HPLC grade, LabScan), tetrahydrofuran (THF, HPLC grade, Fisher), hydrochloric acid (HCl, 37%, Aldrich), *n*-hexane (ACS reagent grade, Fisher), dichloromethane (ACS reagent grade, Fisher) and dimethylflormamide (DMF, HPLC grade, Fisher) were used as received. Agarose (for electrophoresis, Aldrich), SYBR® Safe gel stain (Invitrogen), BioLux™ Gaussia Luciferase Assay Kit (New England Biolabs), alamarBlue® (Invitrogen) were used as received according to protocols.

Polymer Synthesis

The ratios of the monomers DMAEMA, EGDMA and poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, M_n = 475) were 820:100:80 molar equivalents respectively. The initiator Ethyl 2-bromoisobutyrate (1 mole equiv.), the catalyst $Copper^{II}$chloride (0.6 mole equiv.) and the ligand Pentamethyldiethylenetriamine (0.6 mole equiv.), along with the monomers, were added to a two necked flask containing 50mls of Tetrahydrofuran (HPLC grade, Fisher). Argon was bubbled through the reactant solution for 30 minutes to remove oxygen before adding ascorbic acid (0.09 molar equiv.) made up in 20µl of distilled water. This reduces the copper to the $copper^{I}$ state allowing the reaction to proceed (held at 50^{o}C with vigorous stirring). The reaction was monitored by gel permeation chromatography (GPC) at one hour intervals, and terminated by allowing oxygen to enter the flask.

Characterisation of Polyplexes

Polyplexes were made according to the procedure outlined in the manuscript. A 0.1% agarose (for electrophoresis, Sigma Aldrich) gel was made up in Tris-borate-EDTA (TBE) buffer and SYBR® Safe gel stain (Invitrogen) was added upon cooling. 5µl of polyplex or naked DNA controls were mixed with 5µl of loading dye then added to each well of the gel. Using a voltage of 80mV, electrophoresis was performed for between 15 and 20 minutes. Gels were then visualised using a G:Box (Syngene) and associated GeneSnap software. 1µl of polyplex or control samples for UV spectroscopy analysis were added onto the lower pedestal of a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Dublin, Ireland) for analysis. 3µl samples for transition electron microscopy (TEM) analysis were added directly onto copper

TEM grids and allowed to dry before subsequent imaging using a Hitachi H7500 microscope at 80kV. Analysis of the ability of the cyclized knot polymer to protect DNA from enzymatic degradation was carried out using the DNase-1 enzyme (Sigma) as reported previously[1]. Cyclized knot polyplexes were formed with 2µg of GDNF plasmid DNA at ratios of 3:1 and 5:1 (w/w) and compared with PEI polyplexes and naked DNA. A total sample volume of 15µl was then subject either to no treatment (1µl of distilled water), or enzyme treatment (1µl of DNase-1 (0.5 units)) for 15 minutes. Then 5µl of 100mM ethylenediaminetetraacetic acid (EDTA) was added to both sample groups for 10 minutes to inactivate the enzyme. Lastly the polyplexes were dissociated for 2 hours using 10µl of heparin (5mg/ml (Sigma)) to allow agarose gel electrophoresis visualisation (as described above) of the non-complexed DNA.

Cell Culture

Media used for various cell types are as follows:

Primary astrocytes, B104 and SHSY-5Y cells – 50% Dulbecco's Modified Eagles Medium (DMEM)(Sigma Aldrich, Dublin, Ireland), 50% F12 Ham (Sigma Aldrich, Dublin, Ireland), 10% filtered fetal bovine serum (FBS) (Sigma Aldrich, Dublin, Ireland) and 1% Penicillin/Streptomycin (P/S) (Sigma Aldrich, Dublin, Ireland).

Neu7 Astrocytes – Low glucose DMEM (Sigma Aldrich, Dublin, Ireland), 10% filtered FBS and 1% P/S

PC12 cells – DMEM, 10% Horse Serum (Sigma Aldrich, Dublin, Ireland), 5% filtered FBS and 1% P/S which were differentiated using 100ng/ml of nerve growth factor (NGF) (Alamone, Jerusalem, Israel) for two days prior to analysis.

Plasmids and Preparation

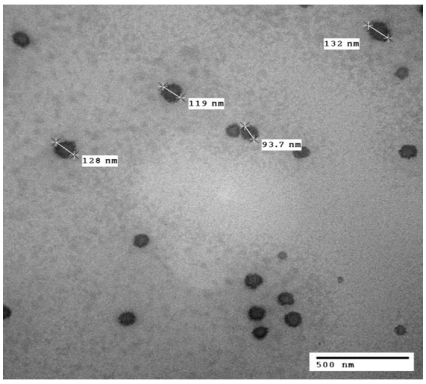
Gaussia princeps luciferase plasmid (pCMV-GLuc) was obtained from New England BioLabs®. The human GDNF isoform 1 (accession number: NM_000514) open reading frame plasmid (pBLAST49-hGDNFa) was obtained from InvivoGen (San Diego, CA, USA). Both are cell secreted forms of the transgene product. Expansion and purification was performed as per protocol using a Giga kit (Qiagen, West Sussex, UK).

Results

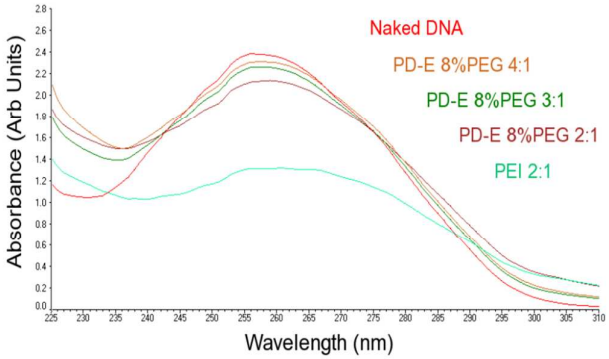
PEI Polyplex Size/Zeta Potential

Size (diameter): 88nm (+/- 21nm)

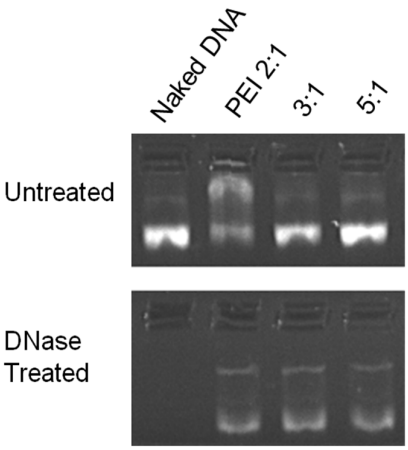
Zeta Potential: 42.5mV (+/- 5.5mV)



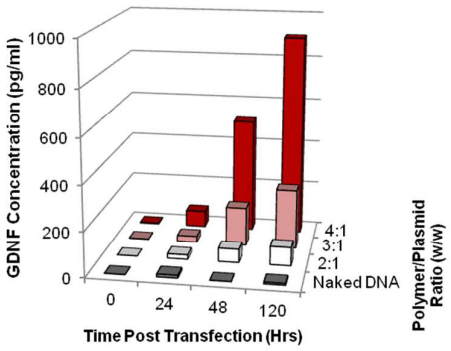
SI Figure 1 – Transition Electron Microscopy image of PD-E 8%PEG polyplexes, showing that the dehydrated diameter is approximately 100nm (50,000x magnification, scale bar represents 500nm).



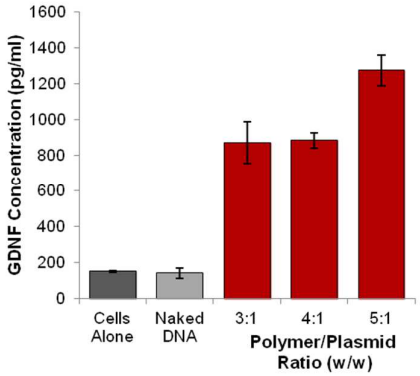
SI Figure 2 – UV spectroscopy analysis of polyplexes formed with PD-E 8%PEG at 2:1, 3:1 and 4:1 w/w ratios showing a slight decrease in absorbance due to distortion of the aromatic bases compared to those in the largely affected PEI polyplexes (2:1 ratio (w/w)).



SI Figure 3 – DNase-1 protection assay showing that PEI and PD-E 8%PEG at the indicated polymer to plasmid ratios (w/w) protect plasmid DNA from enzymatic degradation, whereas naked DNA is completely digested (n=3, representative images shown).



SI Figure 4 – Effect of polymer/plasmid ratio and time post transfection on the cumulative GDNF production from transfected Neu7 cells, showing a 4:1 polymer/plasmid ratio (w/w) gives the highest transgene expression after 120 hours (n=4).



SI Figure 5 – shows the higher levels of GDNF production 24 hours post transfection, when 10,000 cells are seeded in 8-well glass chamber slides supplemented with 300µl of serum containing media. Higher cell density and reduced media content allows GDNF levels of ~1ng/ml after 24 hours (n=4, error bars indicate +/- standard deviation).

Reference

1) Gebhart, C. L.; Sriadibhatla, S.; Vinogradov, S.; Lemieux, P.; Alakhov, V.; Kabanov, A. V. Design and formulation of polyplexes based on pluronic-polyethyleneimine conjugates for gene transfer, *Bioconjugate Chemistry* **2002**, *13*, 937-944.