Electronic Supplementary Material (ESI) for Nanoscale.

SUPPLEMENTAL INFORMATION

Hydroxyl PAMAM dendrimer-based gene vectors for transgene delivery to human retinal pigment epithelial cells

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Synthesis of Fmoc-functionalized bifunctional dendrimer (BiD-Fmoc, 1):

Fmoc-GABA-OH (287 mg, 0.882 mmol) was dissolved in DMF (5 mL) in a 50 mL round bottom flask under nitrogen environment. PyBOP (574 mg, 1.1035 mmol) dissolved in DMF (5 mL) and DIEA (200 µL) were added, and the reaction mixture was allowed to stir for 1 hour in an ice bath. Finally, PAMAM G4-OH (309 mg, 0.022 mmol) dissolved in anhydrous DMF (10 mL) was added to the reaction mixture and was stirred for 48 hours. The mixture of solvents was evaporated at 25 °C under vacuum. The crude product was redissolved in DMF (20 mL) and subjected to dialysis in DMF (membrane MW cutoff = 1 kDa) for 26 hours by changing the solvent at least 3 times. The obtained solution was evaporated under reduced pressure at room temperature, and the final product was subjected to high vacuum overnight, to produce an off-white semi-solid Fmoc-functionalized dendrimer conjugate (BiD-Fmoc, **1,** 460 mg). ^{1}H NMR (DMSO-d_6) δ 1.63 (m, CH_2 protons, linker), 2.27-3.39 (m, CH_2 protons of G4-OH and COCH_2 protons of linker), 3.99 (bs, CH_2OC=O protons, G4-OH), 4.18 (bs, C*H* proton of Fmoc group), 4.28 (bs, OCH_2 protons of Fmoc group), 4.71 (bs, OH protons of G4-OH), 7.30-7.38 (d, aromatic protons of Fmoc), 7.65 (s, aromatic protons of Fmoc), 7.86-8.13 (m, aromatic protons of Fmoc, carbamate protons, and internal amide protons of G4-OH).

Synthesis of bifunctional dendrimer (BiD, 2):

The whole batch of above BiD-Fmoc dendrimer was dissolved in anhydrous DMF (10 mL) and 10 mL of piperidine/DMF (2:8) was added to it under nitrogen. The reaction mixture was stirred for 1 hour and the mixture of solvents was evaporated under vacuum. The crude product was co-evaporated with 10 mL of DMF under high vacuum and subjected to dialysis against DMF (membrane MWCO = 1000 Da) for 24 h. The solvent was evaporated and the product was triturated with diethylether to take out traces of DMF. Finally, the semi-solid product was re-dialyzed with pure DI water for 2-3 hours and subjected to freeze dryer to get bifunctional dendrimer (BiD, 280 mg). ^{1}H NMR (DMSO-d_6) δ 1.24-1.53 (m, CH_2 protons, linker), 2.21 (bs,

CH_2 protons of G4-OH), 2.29 (m, COCH_2 protons of linker), 2.43 (bs, CH_2 protons, G4-OH), 2.65 (bs, CH_2 protons, G4-OH), 3.09-3.12 (t, CH_2 protons, G4-OH), 3.27-3.28 (d, CH_2NH protons, linker), 3.38-3.41 (t, CH_2 protons, G4-OH), 3.99-4.01 (t, CH_2OC=O protons, G4-OH), 7.84-8.11 (m, N*H*CO protons of G4-OH).

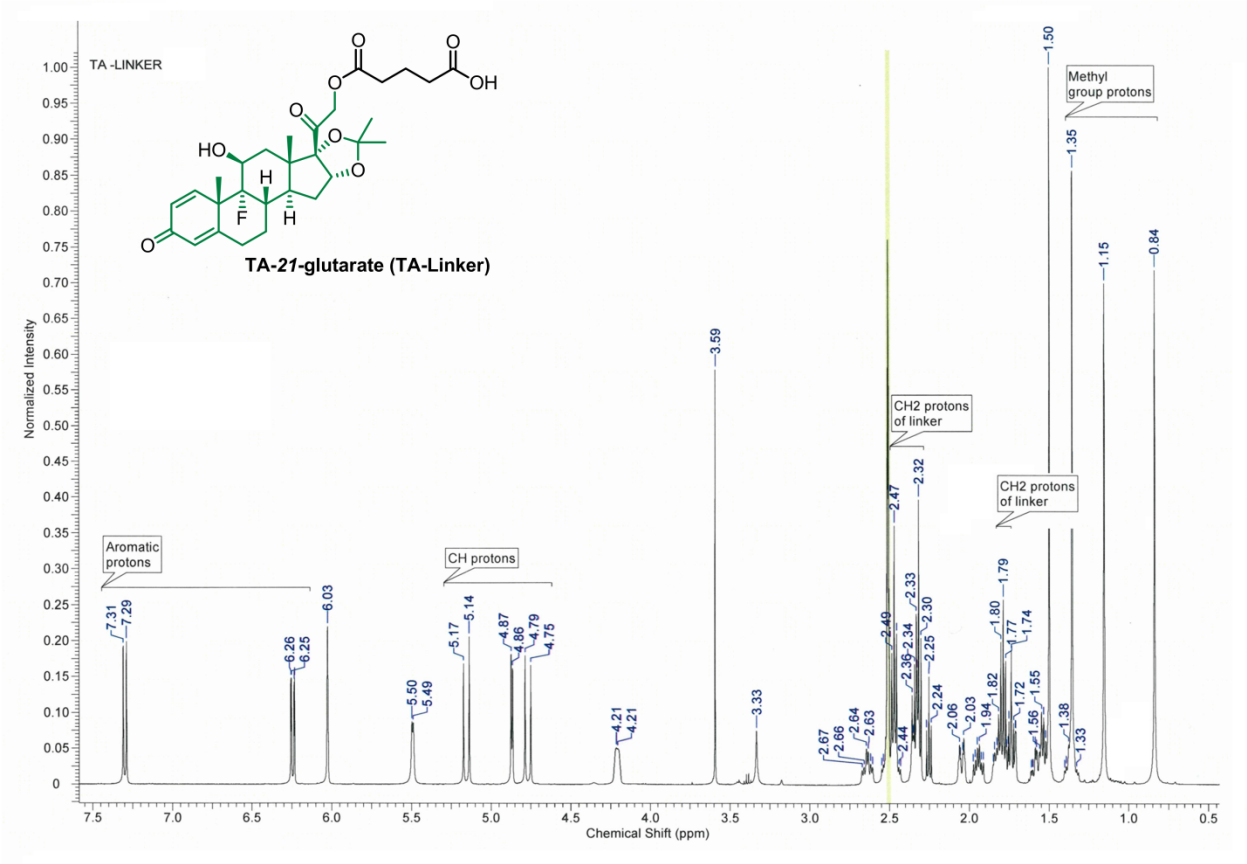

Fig.S1: ^{1}H NMR spectrum of triamcinolone acetonide-21-glutarate (TA-linker) in DMSO-d_6.

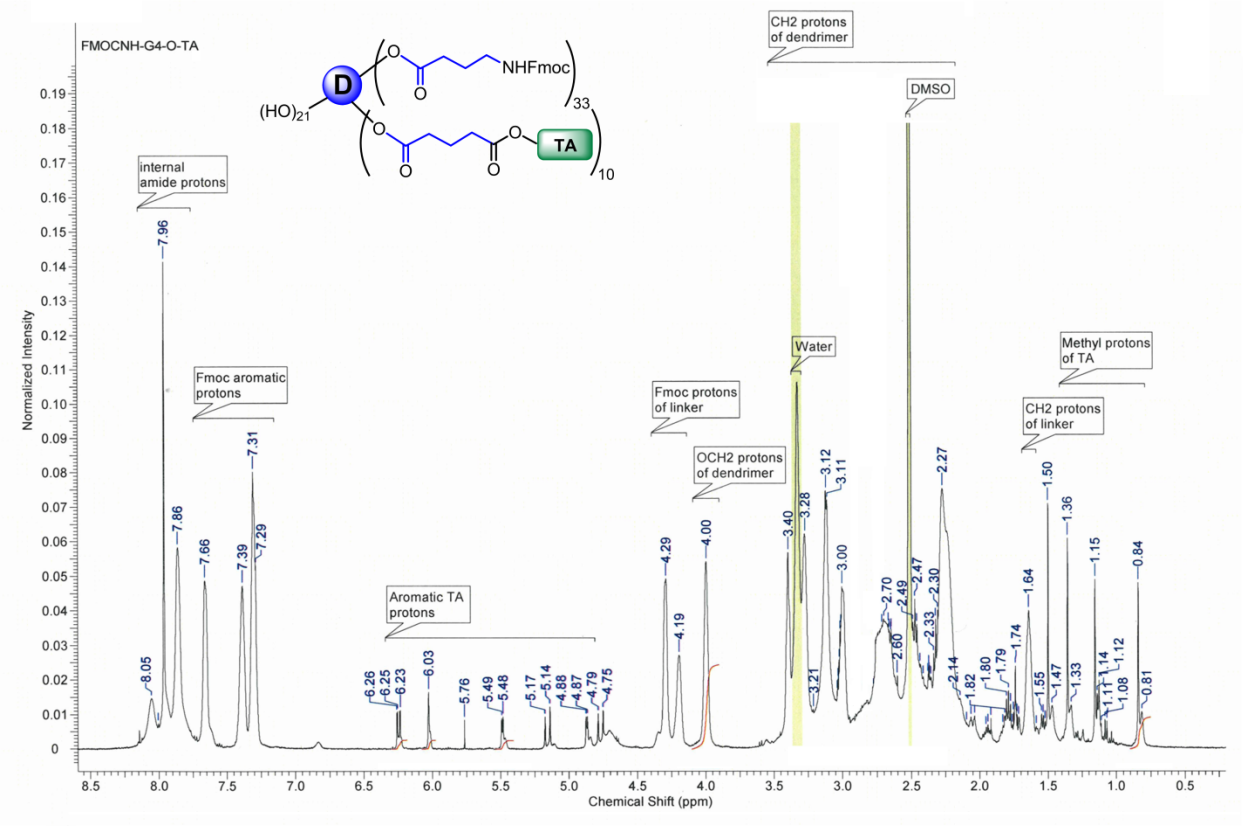

Fig.S2: ^{1}H NMR spectrum of Fmoc-functionalized bifunctional-triamcinolone acetonide (BiD-Fmoc-TA, ***3***) in DMSO-d_6.

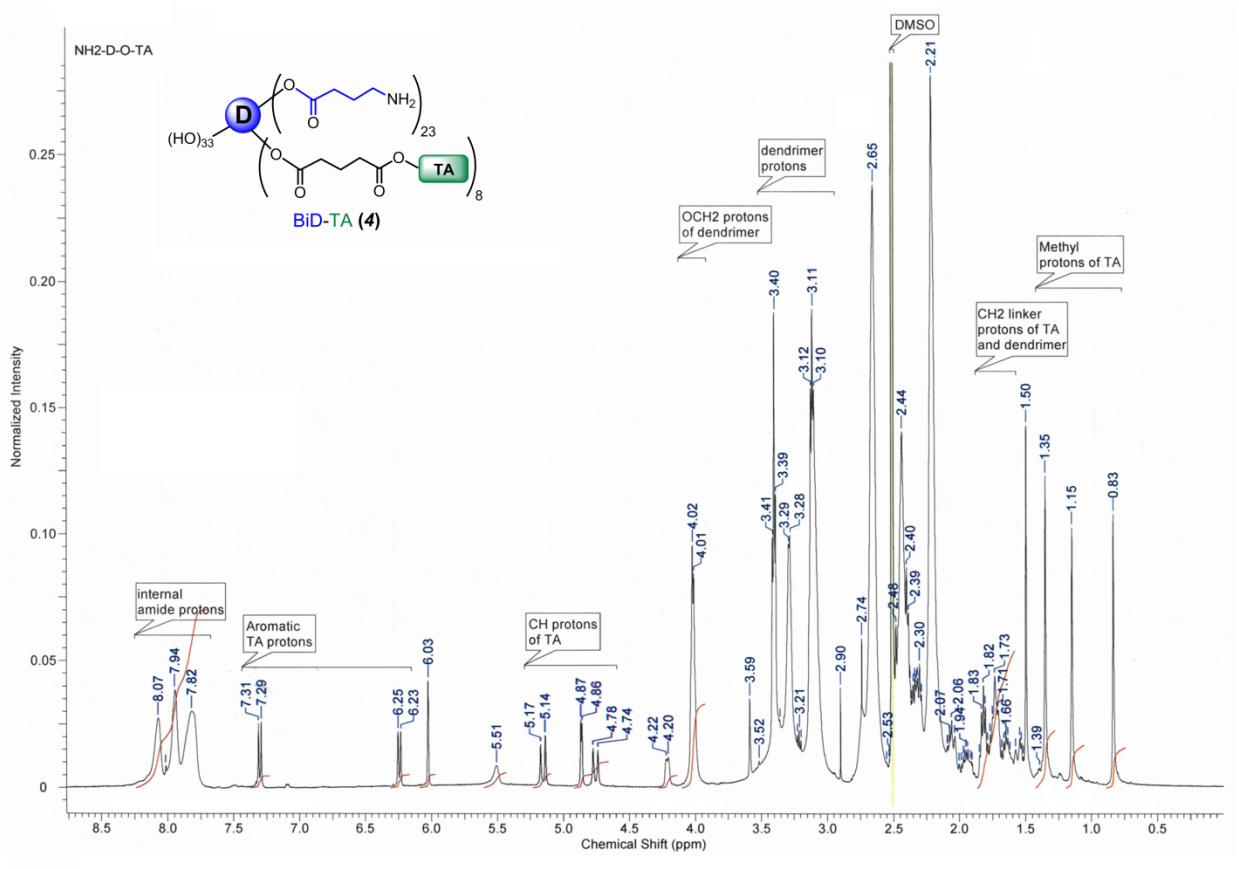

Fig.S3: ^{1}H NMR spectrum of bifunctional-triamcinolone acetonide (BiD-TA, ***4***) in DMSO-d_6.

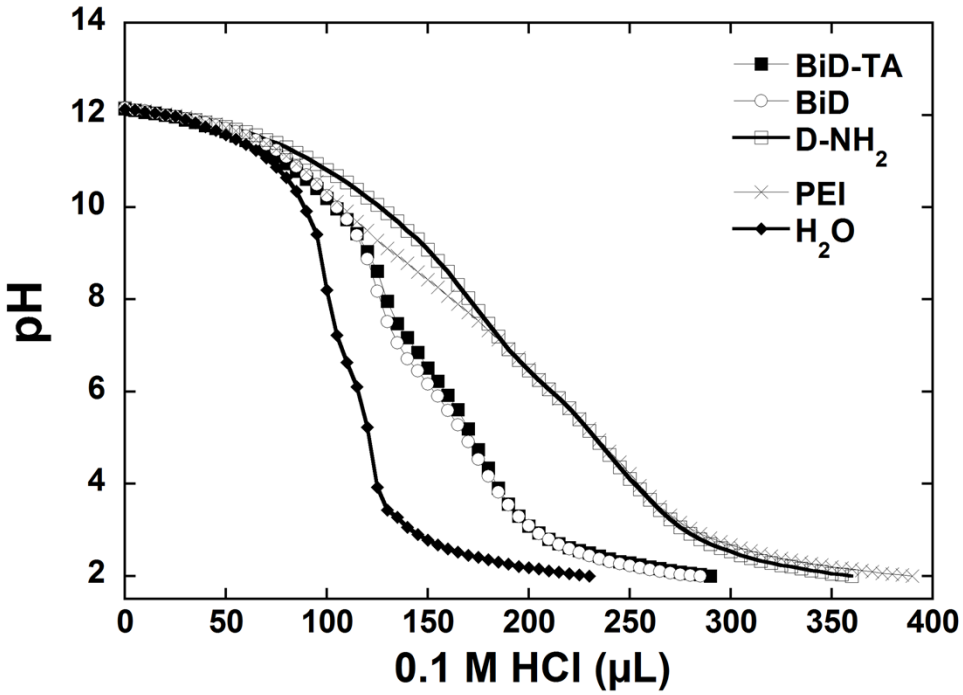

Figure S4: Buffering capacity. Measurement of buffering capacity of dendrimer base polymers by acid titration; PEI and ultrapure water were used as a control and solutions were titrated from pH 12 to pH 2 using 0.1 M HCL.

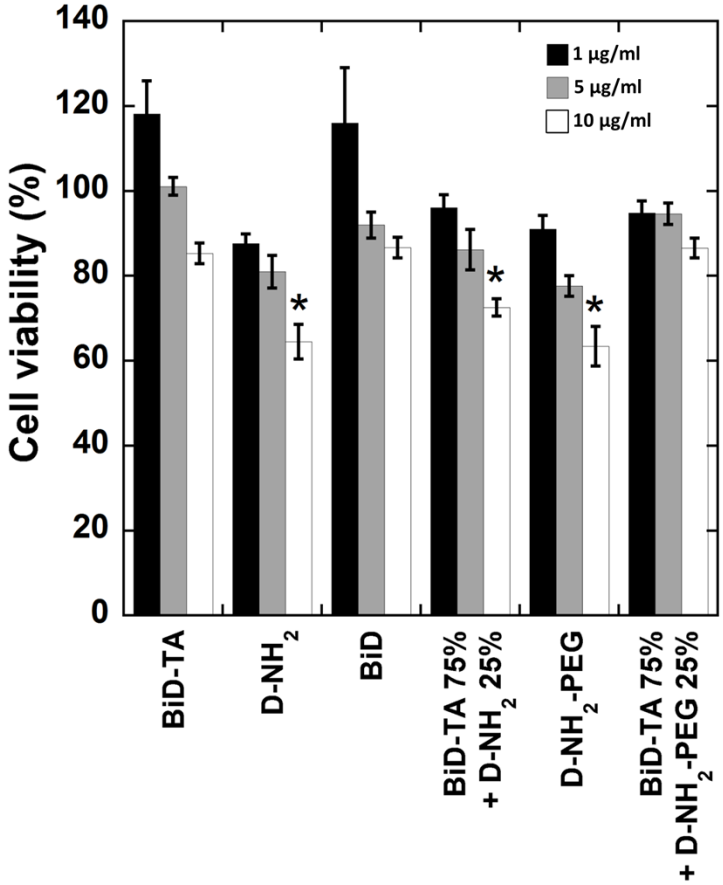

Figure S5: Cytotoxicity of dendrimer-based gene vectors to human aRPE cells. Cells were treated with varying concentrations of respective gene vectors. Cell viability was measured after 24 h of treatment and compared to non-treated controls. Data represents the mean ± SEM. * Denotes statistically significant ($p < 0.05$) difference from 80%.

Gene vectors	Hydrodynamic diameter (nm) ± SEM	PDI	Zeta Potential (mV) ± SEM
BiD-TA 5% + BiD 95%	45.8 ± 1.5	0.23	16.9 ± 1.4
BiD-TA 10% + BiD 90%	46.2 ± 1.4	0.25	15.3 ± 0.9
BiD-TA 25% + BiD 75%	38.8 ± 2.3	0.23	13.6 ± 1.0
BiD-TA 50% + BiD 50%	34.5 ± 0.8	0.20	15.8 ± 0.9
BiD-TA 75% + BiD 25%	34.0 ± 2.0	0.25	9.9 ± 0.8
BiD-TA	42.9 ± 2.8	0.28	16.5 ± 3.3
BiD-TA 75% + D-NH_2 25%	43.2 ± 1.5	0.15	12.1 ± 1.5

Table S1: Physicochemical characteristics of additional gene vectors studied. Size, ζ-potential and polydispersity (PDI) were measured by dynamic light scattering (DLS) in 10 mM NaCl at pH 7.0 and are presented as average of at least 3 measurements ± standard error (SEM).