

† Electronic Supplementary Information

Experimental

Materials

The propargyl focal point PAMAM dendron with the third generation (PFP-D₃) was synthesized according to the method reported by Lee et al. (see: J. W. Lee, H. J. Kim, S. C. Han, J. H. Kim, S. H. Jin, *J. Polym. Sci. Part A: Polym. Chem.*, 2008, **46**, 1083—1097). α -CD (Sigma, U.S.A.) was used after drying under vacuum at 65 °C for 24 h. Poly(ethylene glycol) methyl ether ($M_n=5000$, Aldrich) was dried at 50 °C in vacuo overnight. Sodium azide (NaN₃, 99%) and sodium ascorbate (99%) were purchased from Alfa Aesar. Triethylamine and p-toluenesulfonyl chloride (TsCl) were purchased from Sinopharm Chemical Reagent Corp. (Shanghai, China) and used as received. Plasmid EGFP-N1 (4.7 kb) encoding enhanced green fluorescent protein was purchased from Clontech (Palo Alto, CA). The plasmid DNA (pDNA) was isolated with PureYield (TM) Plasmid Maxiprep System Kits (USA) in accordance with the manufacturer's instructions. The pDNA was stored at -20 °C until the transfection experiments. All other reagents were of analytical grade and used without further purification.

Preparation and characterization of azide-terminated poly(ethylene glycol) (PEG-N₃)

Poly(ethylene glycol) methyl ether (10.0 g, 2.0 mmol) and toluene-4-sulfonyl chloride (3.81g, 20.0 mmol) were completely dissolved in CH₂Cl₂ (100 mL) under a N₂ atmosphere. Triethylamine (20.2 g, 20.0 mmol) was dissolved in CH₂Cl₂ (40 mL) and then added dropwise to the above solution at ice-water bath. The mixed solution was stirred for 24 h at room temperature, filtrated and precipitated into diethyl ether. After dried in vacuo at 25 °C, the monotosylated poly(ethylene

glycol) (PEG-Ts) was obtained as white powder with the yield of 93%. ^1H NMR of PEO-Ts (300MHz, CDCl_3): δ (ppm) = 2.44 (s, 3H), 3.37 (s, 3H), 3.46 (t, 2H), 3.54 (t, 2H), 3.64 (s, 450H), 3.82 (t, 2H), 4.16 (t, 2H), 7.35 (d, 2H), 7.81(d, 2H). To obtain PEG- N_3 , sodium azide (1.275 g, 19.5 mmol) was added to a solution of the obtained PEO-Ts (5.0 g, 0.975 mmol) in dry DMF (35 mL) under a N_2 atmosphere, and the reaction mixture was stirred at room temperature for 24 h. DMF solvent was removed under reduced pressure, and then the product was dissolved in dichloromethane. The mixture was extracted sequentially with NaCl (5 wt %) solution and distilled water, dried with anhydrous Na_2SO_4 , and then precipitated in diethyl ether. As a result, PEG- N_3 was obtained as white powder with the yield of 80% yield. ^1H NMR of PEG- N_3 (300 MHz, CDCl_3): δ (ppm) = 3.37 (s, 3H), 3.39 (t, 2H), 3.46 (t, 2H), 3.54 (t, 2H), 3.64 (s, 450H), 3.82 (t, 2H).

Preparation and characterization of PEGylated polyamidoamine dendron (PEG- D_3)

Typically, PEG- N_3 (500 mg, 0.1mmol) was dissolved completely in distilled water (5 mL). PFP- D_3 (500 mg, 0.3 mmol), sodium ascorbate (29.7 mg, 0.15 mmol) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (7.5 mg, 0.03 mmol) were subsequently added to the solution. After stirred for 24 h at room temperature, the mixture solution was concentrated under reduced pressure, diluted with THF and centrifuged to remove the excess PFP- D_3 . The polymer, PEG- D_3 , was recovered by the precipitation of the centrifuged product in diethyl ether, and then dried under vacuum. The yield of PEG- D_3 was 86%. ^1H NMR spectra of PEG- N_3 , PFP- D_3 and PEG- D_3 were obtained using a Bruker DPX-300 NMR spectrometer (300 MHz). CDCl_3 or D_2O was used as the solvent. FTIR spectra of PEG- N_3 , PFP- D_3 and PEG- D_3 were recorded on a Perkin-Elmer Paragon 1000 spectrometer at the frequencies ranging from 400 to 4000 cm^{-1} . The samples were thoroughly mixed with KBr and pressed into pellet form. Gel permeation chromatographic analyses of PEG- D_3 sample was performed in aqueous solution at 35 °C with an elution rate of 0.6 mL min^{-1} on a gel permeation chromatography (GPC) equipped with a Waters 1515 isocratic HPLC pump and a Waters 2414 refractive index detector.

Waters millennium module software was used to calculate the molecular weight based on a universal calibration curve generated by polyethylene glycol standards with narrow molecular weight distribution.

Formation and characterization of PEG-D₃/pDNA polyplexes

For the formation of PEG-D₃/ pDNA polyplexes, aqueous solutions of PEG-D₃ and pDNA were mixed together at room temperature, and then stirred gently for 20 min. The ultimate concentrations of PEG-D₃ and pDNA were kept to be 4.0% (w/v) and 50 µg/mL, respectively. Optical camera was used to record the appearance of the PEG-D₃/ pDNA complex solution. The zeta potentials of aqueous 4.0% PEG-D₃ solution before and after the complexation with 50 µg/mL pDNA were determined by a Zeta Potential Analyzer instrument (ZetaPALS, Brookhaven Instruments Corporation, USA). Prior to the measurements, the complexes were incubated at 37 °C for 30 min, and diluted by 0.15 mol/L NaCl solution to 1.0 mL. For the resultant PEG-D₃/ pDNA polyplexes, the mean particle size and size distribution were measured by a BI-200SM Goniometer particle size analyzer (Brookhaven, USA) at 25 °C with angle detection of 90°, and the morphology was observed by a JEM1400 transmission electron microscopy. The binding ability of PEG-D₃ to pDNA was examined by gel electrophoresis.

Formation and characterization of supramolecular hybrid hydrogel

To obtain the supramolecular hydrogel hybridized with PEG-D₃/ pDNA polyplexes, aqueous α -CD solution was added to the above complex solution consisting of 4.0% (w/v) PEG-D₃ and 50 µg/mL pDNA. A gelation occurred at room temperature due to the supramolecular self-assembly in the mixed system. To investigate the gelation process, a time-sweep rheological analysis was performed by an Advanced Rheometric Extended System (ARES, TA Co., USA) in oscillatory mode with

parallel plate geometry (50 mm diameter and 1.0 mm gap) at 25 °C. To investigate the hydrogel strength and the flow behavior under shear stress, dynamic frequency sweep and steady shear tests were also carried out. Before the tests, a dynamic strain sweep was conducted in order to ensure the rheological measurements within a linear viscoelastic region, and the corresponding strain was determined to be 0.1%. X-ray diffraction measurement was performed to investigate the supramolecular gelation mechanism by a Rigaku D/max-2200 type X-ray diffractometer. The voltage was set to be 40 kV, and the current was 30 mA. The proportional counter detector collected the data at a rate of $2\theta = 2^\circ/\text{min}$ in the range of $2\theta = 5\text{--}50^\circ$.

Gel electrophoresis retardation assays

The binding ability of PEG-D₃ to pDNA and the confirmation of PEG-D₃ / pDNA polyplexes released from the hydrogel were examined by gel electrophoresis. Agarose gel (1.0%, w/v) containing ethidium bromide (0.25 mg/mL, Sigma) was prepared in TAE buffer (40 mmol/L Trisacetate, 1 mmol/L EDTA). Ultrapure water was used as the solvent for PEG-D₃, pDNA and the released extracts in the absence and presence of heparin sodium salt used for disassembly of plasmid DNA complexes. After incubation for 30 min at room temperature, all samples were separated by electrophoresis on the agarose gel at 90 V for 1 h. Visualization and image capture was accomplished using a UV-transilluminator under a Kodak EDAS 290 digital imaging suite (Fisher Scientific, PA).

In-vitro release study

The release profile of encapsulated pDNA from the in-forming supramoleccular hydrogel was determined by measuring the amount of free pDNA after disassembly of PEG-D₃/ pDNA complexes by heparin sodium salt (see: C. H. Hu, L. Zhang, D. Q. Wu, X. S. Cheng, X. Z. Zhang, R.

X. Zhuo, *J. Mater. Chem.*, 2009, **19**, 3189–3198). After adding 500 μL PBS (0.01 mol/L, pH=7.4) as release media, the sample was transferred into a portable shaking incubator maintained at 60 rpm and 37 °C. At a given time point, 300 μL supernatant was collected from each sample, which was then replaced by the same amount of fresh pre-warmed PBS. The collected sample was recovered by incubating with 1% heparin solution for 30 min. The amount of recovered pDNA was determined using an Eppendorf Biophotometer. The cumulative release of encapsulated plasmid from the hydrogel was calculated as follows: Cumulative release (%) = (pDNA released at a given time / pDNA encapsulated) \times 100. The release study was carried out in triplicate.

In-vitro transfection test

293T cells were plated in 24-well plates at 1×10^4 cells/well. The cells were incubated for 12 h. Freshly prepared PEG-D₃/pDNA polyplexes (PEG-D₃, 4.0%; pDNA, 50 $\mu\text{g}/\text{mL}$) and the PEG-D₃/pDNA polyplexes released from the supramolecular hydrogel (PEG-D₃, 4.0%; pDNA, 50 $\mu\text{g}/\text{mL}$; α -CD, 6.0%) at various time were collected respectively and frozen to dry. The dried powders were then dissolved respectively in a required volume of DMEM. Each well of the cells was transfected with the complex solution after changing the fresh complete DMEM (containing 2.0 μg pDNA in each well). After 4 h incubation, serum-free media were changed with fresh media containing 10% serum. After 48 h incubation, the cells were directly observed with a Olympus IX71 fluorescence microscope (Melville, NY, USA). Then transfected cells were washed once with PBS and detached with 0.25% trypsin. Transfection efficiency was evaluated by scoring the percentage of cells expressing GFP, using a FACS Aria flow cytometer (Germany).

Cell viability assays

The 293T cells were seeded in the 96-well plate at a density of 1×10^4 cells/well and cultured for 24

h. The growth medium was replaced with 200 μL complete DMEM culture medium that contained the desired amount of the freshly prepared PEG-D₃/pDNA polyplexes (PEG-D₃, 4.0%; pDNA, 50 $\mu\text{g}/\text{mL}$) and the PEG-D₃/pDNA polyplexes released from the supramolecular hydrogel (PEG-D₃, 4.0%; pDNA, 50 $\mu\text{g}/\text{mL}$; α -CD, 6.0%) at various time, and five multiple holes were set for every sample. Cells treated with the same amount of PBS were used as the control group. The cells were incubated for another 24 h, and the cell viability was assayed by adding 20 μL of MTT (Sigma) PBS solution (5 mg/mL). After incubation at 37 °C for another 4 h, the formed crystals were dissolved in 150 μL of dimethyl sulfoxide (DMSO). The absorbance that correlated with the number of viable cells in each well was measured by an MRX-Microplate Reader at a test wavelength of 570 nm.