Electronic Supplementary Material (ESI) for Molecular BioSystems

Electronic Supplementary Information

Polyethyleneglycol crosslinked N-(2-hydroxyethyl)-polyethylenimine nanoparticles as efficient non-viral vectors for DNA and siRNA delivery in vitro and in vivo

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General

N-(2-Hydroxyethyl)-polyethylenimine (HeP, 50 kDa), branched polyethylenimine (bPEI, 25kDa), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), Tris, ethidium bromide, xylene cyanol, bromophenol blue, tetramethylrhodamine isothiocyanate (TRITC) and 4',6-diamidino-2-phenylindole dilactate (DAPI) were procured from Sigma Chemical Co. (USA). YOYO-1 for labeling plasmid DNA was purchased from Invitrogen (USA). PEG_{600} dicarboxylic acid (PEG_{600}dc) was procured from Fluka AG, Switzerland. Commercial transfection agent, Lipofectamine^{TM}, was procured from Invitrogen (USA). Cell culture products and plasmid isolation kit were procured from Invitrogen (USA) and Qiagen (France), respectively. Fourier Transform Infrared (FTIR) spectra of nanoparticles were recorded on a single beam Perkin Elmer (Spectrum BX Series), USA with the following scan parameters: scan range, 4400-400 cm^{-1}; number of scans, 16; resolution, 4.0 cm^{-1}; interval, 1.0 cm^{-1}; unit, %T. Particle size and zeta potential of nanoparticles and their DNA complexes were determined on Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). The size of the nanoparticles and DNA complexes was also determined by Atomic Force Microscopy (AFM, PicoSPM System, Molecular Imaging, Arizona, USA). GFP reporter gene expression was observed under Nikon Eclipse TE 2000-S inverted microscope (Kanagwa, Japan). Green fluorescent protein (GFP) was analyzed spectrofluorimetrically on NanoDrop ND-3300 spectrofluorometer, USA, at an excitation wavelength of 488 nm

and emission at 509 nm. Confocal laser scanning microscopy (CLSM) of labeled nanoparticles was carried out on a Zeiss LSM 510 Meta confocal microscope. Fluorescence activated cell sorting (FACS) was carried out by Flow Cytometry (Guava EasyCyte Plus System, Millipore, USA).

Animals

Six to seven weeks old male Swiss albino male Balb/c mice (25 ± 3 g) for *in vivo* experiments, were procured from the animal facility of Indian Institute of Toxicology Research (IITR), Lucknow. They were acclimatized under standard laboratory conditions with 12 h dark/light and 50-60 % humidity as per the rules laid down by Animal Welfare Committee of IITR. The animals, housed in plastic cages having rice husk as bedding, were given commercial pellet diet (Ashirwad Industries, Chandigarh, India) and water *ad libitum*. The animals were cared humanely according to the laid down guidelines of Institutional Animal Ethics Committee.

Synthesis of siRNA

The following oligonucleotide sequences were synthesized using the standard phosphoramidite chemistry and purified on RP-HPLC:

T7 primer: d (TAA TAC GAC TCA CTA TAG)

GFP sense: d (ATG AAC TTC AGG GTC AGC TTG CTA TAG TGA GTC GTA TTA)

GFP antisense: d (CGG CAA GCT GAC CCT GAA GTT CTA TAG TGA GTC GTA TTA)

After annealing T7 primer sequence to GFP sense or antisense oligonucleotides, complementary strands were synthesized by T7 RNA polymerase. The sense and antisense RNA strands were annealed and double stranded siRNA was used for transfection.

Cell culture

Chinese hamster ovary cells (CHO) were maintained in DMEM supplemented with 10% FBS, while human embryonic kidney (HEK293) and human cervical cancer cells (HeLa) were maintained in high and low glucose DMEM, respectively, supplemented with 10% FBS. Cells were grown at 37 ^{o}C in a humidified 5% CO_2 atmosphere.

Mobility shift assay

The electrophoretic mobility of DNA complexes of HePP, Hep and bPEI was assessed on a 0.8% agarose gel. Plasmid DNA (0.3µg) was complexed with HePP nanoparticles, HeP and bPEI at different weight ratios (w/w) in 5% dextrose by mixing aqueous solutions of pDNA (1µl, 0.3µg/µl) with HePP nanoparticles, HeP and bPEI (1mg/ml) and incubated for 30 min (25 ± 2 ^{o}C). DNA complexes (20µl) were mixed with 4µl xylene cyanol (1µg/4µl, in 20% glycerol), electrophoresed (100V, 1 h), stained with ethidium bromide and finally visualized on a UV transilluminator.

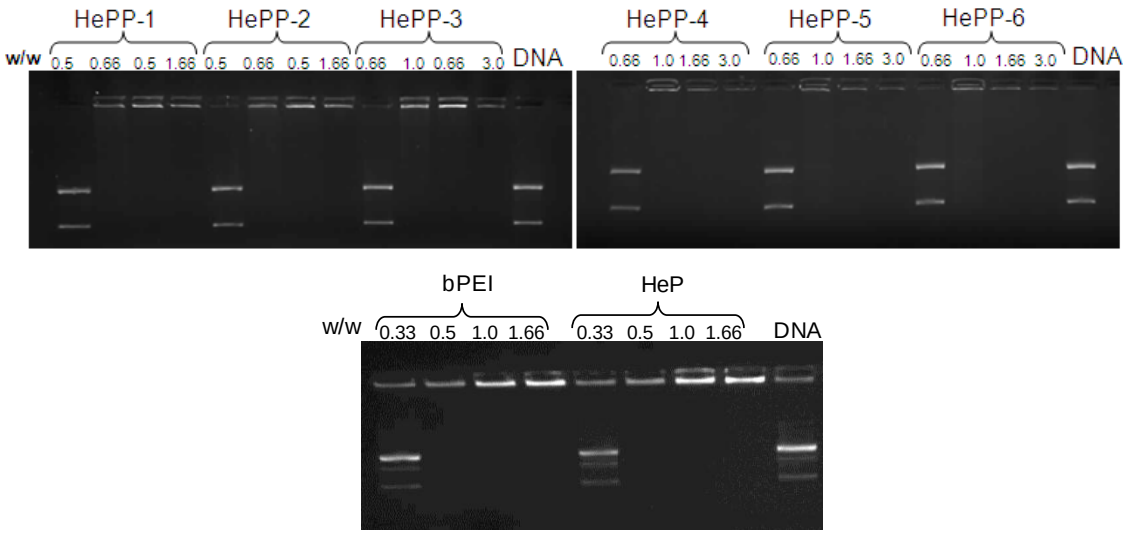


Figure S1. Gel retardation assay of HePP/pDNA, bPEI/pDNA and HeP/pDNA complexes. pDNA (0.3µg) was mixed with increasing amounts of nanoparticles / polymers and analyzed the complexes on 0.8% agarose gel.

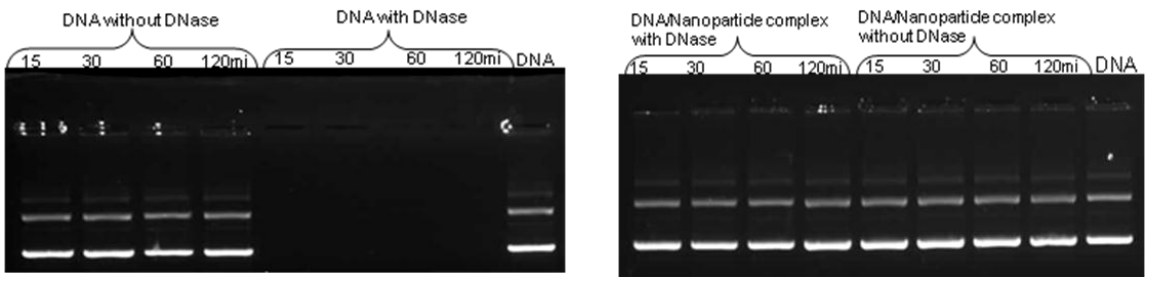
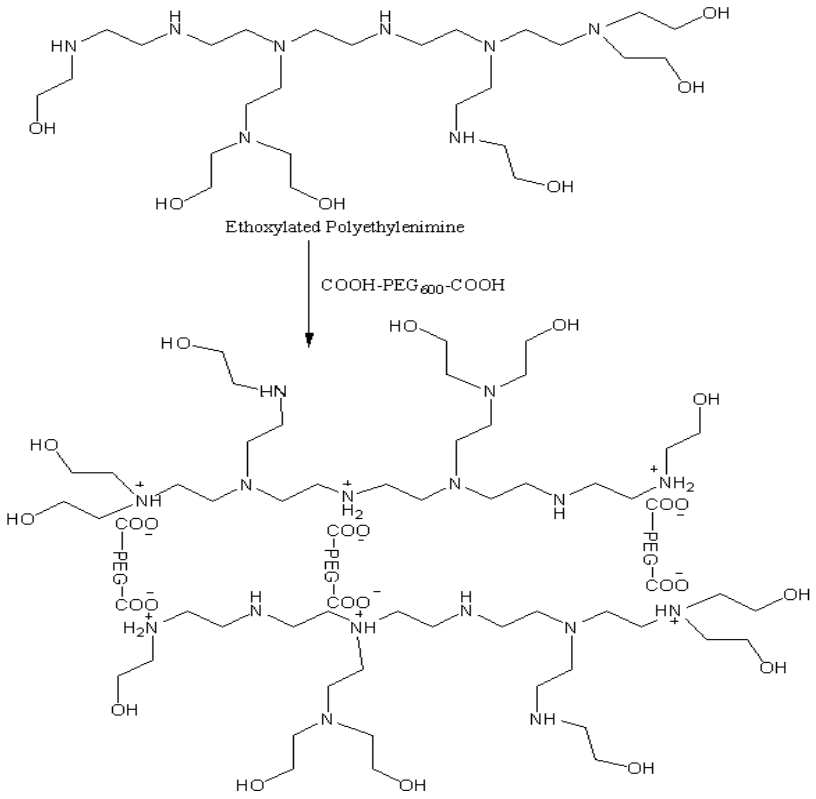


Figure S2. DNaseI protection assay. HePP-3/pDNA complex was treated with DNase I (w/w ratio 3.33) for different time intervals. The complexed DNA was released by treating the samples with heparin. The amount of DNA released (~88%) after DNase I treatment.



Scheme S1. Schematic representation of preparation of HePP nanoparticles.