SUPPORTING INFORMATION

Surface Modification of Crosslinked Dextran Nanoparticles Influences Transfection Efficiency of Dextran-Polyethylenimine Nanocomposites

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Experimental Section

Materials

Branched polyethylenimine (PEI, m.wt. 25 kDa), (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (MTT), dextran (m.wt. ~40 kD), sodium cyanoborohydride ($NaCNBH_3$), agarose, tris, ethidium bromide (EtBr), high retention dialysis tubing (cut off = 12 kDa), xylene cyanol, bromophenol and tetramethylrodamine isothiocyanate (TRITC) were purchased from Sigma Chemical Co., USA. Bradford reagent was purchased from Bio-Rad Inc., USA. Commercial transfection agents, viz., GenePORTER 2™, Superfect™, Fugene™ and Lipofectamine™ were procured from Genlantis (USA), Qiagen (France), Roche Applied Science (USA) and Invitrogen (India), respectively. YOYO-1 iodide (491/509) was purchased from Invitrogen (India). Cell culture products and plasmid isolation kit were procured from Gibco-BRL-Life Technologies (U.K.) and Qiagen (France), respectively. FTIR spectra of nanocomposites 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. Proton nuclear magnetic resonance (^{1}H-NMR) was used to determine the degree of crosslinking in dextran nanoparticles and the spectrum was recorded on a Brucker Avance 400 MHz spectrometer in deuterated water (D_2O). The particle size and zeta potential of nanocomposites and their

DNA complexes were determined on Zetasizer Nano-ZS (Malvern Instruments, UK). The size and morphology of the nanoparticles and DNA complexes was measured by Atomic Force Microscopy (PicoSPM System, Molecular Imaging, USA). GFP reporter gene expression was observed under Nikon Eclipse TE 2000-S inverted microscope (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 imaging of labeled nanocomposites was carried out with a Zeiss LSM 510 Meta confocal microscope, USA. The analysis of the transfected cells was performed by the CellQuest software (BD Biosciences, USA).

Cell cultures

Human embryonic kidney (HEK293), Human cervical adenocarcinoma (HeLa) and Human hepatocellular liver carcinoma (HepG2) cell lines were maintained as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 50 mg/ml gentamicin.

Animals

Six to seven week old male Balb/c mice (25±3 g) were used for *in vivo* luciferase gene expression. These animals from the animal breeding colony of Indian Institute of Toxicology Research (IITR), Lucknow, were acclimatized under standard laboratory conditions and given a commercial pellet diet (Ashirwad Industries, Chandigarh, India) and water *ad libitum*. Animals were housed in plastic cages on rice husk bedding and maintained at 22±2 ºC with 12 h dark/light and 50-60 % humidity as per rules laid down by Animal Welfare Committee of IITR. Animals were cared for according to the instructions laid by the Institutional (IITR) Ethical Committee.

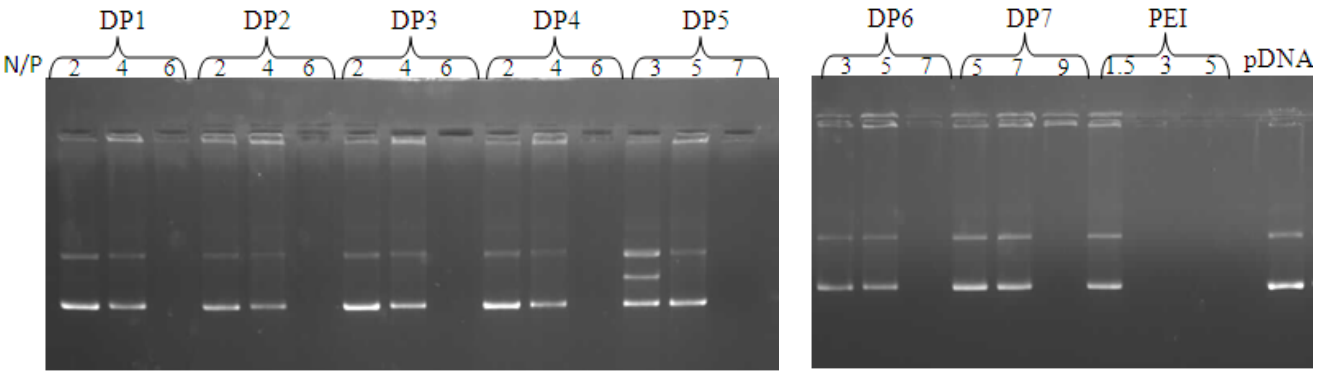

Figure S1. DNA mobility shift assay of DP nanocomposites/DNA and PEI/DNA complexes. pDNA (0.3 µg) was incubated with increasing amounts of nanocomposite in 5 % dextrose and incubated for 20 min. Samples were electrophoresed in 0.8 % agarose gel at 100 V for 45 min.

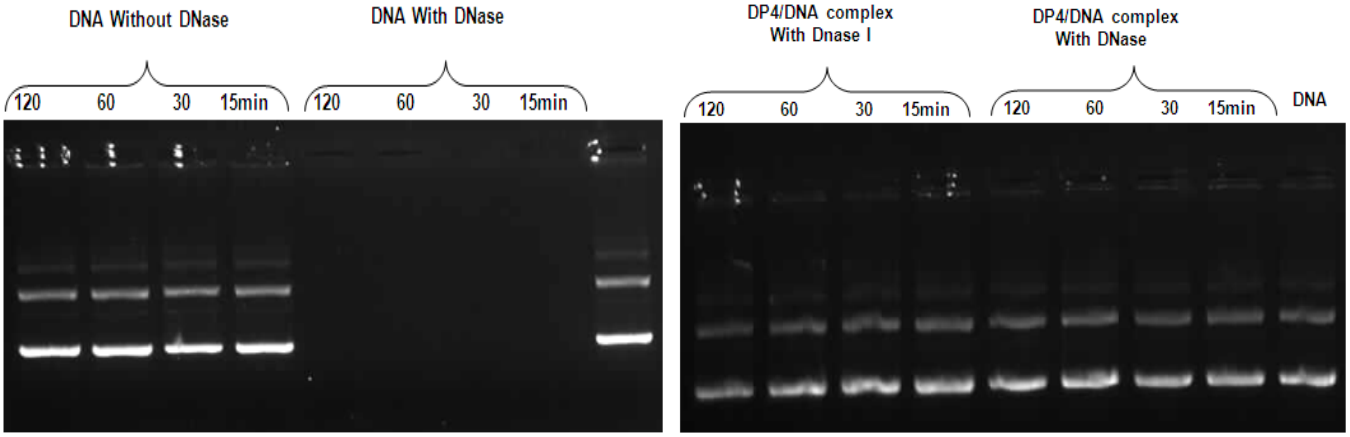

Figure S2. DNase I protection assay. DP4/DNA complex (N/P ratio 20) was treated with DNase I for different time intervals. The complexed DNA was released by treating the samples with heparin. The amount of DNA protected (%) after DNase treatment was calculated as the relative integrated densitometry values (IDV) quantified and normalized by that of pDNA values (untreated with DNase I) using Gel Documentation system.

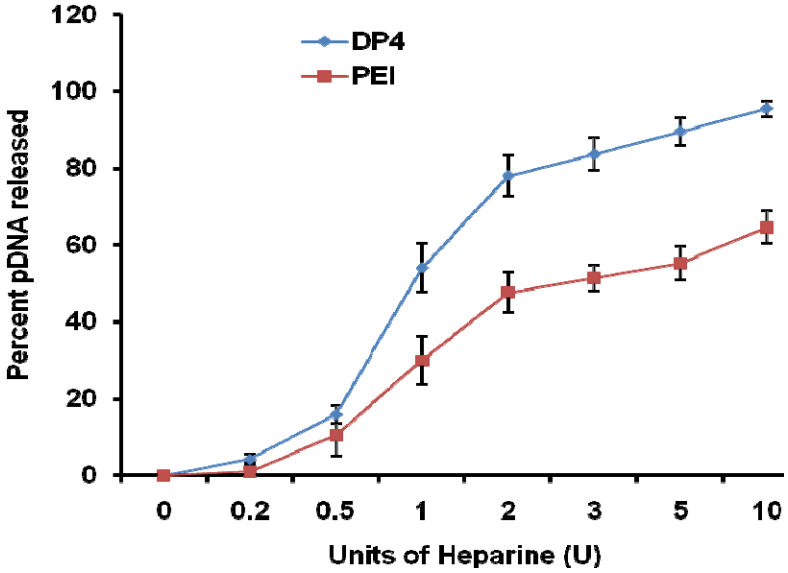

Figure S3. DNA release assay of PEI and DP4 nanocomposite. To a 20 µl solution of DP4/DNA nanoplex, heparin, in increasing concentration, was added and incubated for 20 min at 25±1°C. The samples were run on 0.8 % agarose gel at 100 V for 45 min. Error bars represent ± standard deviation from the mean.

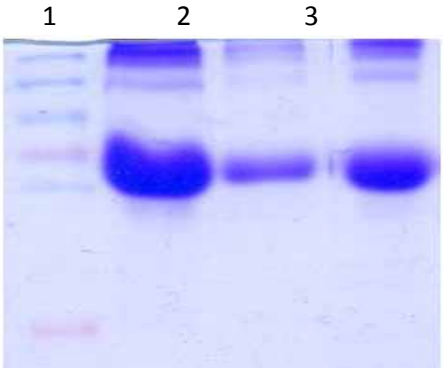

Figure S4. Protein adsorption onto surface of DP4 nanocomposite compared to adsorption onto native PEI. Lanes 1) ladder, 2) only BSA, 3) DP4 incubated with BSA and 4) PEI incubated with BSA for 3 h. Unbound BSA was removed by washing and centrifugation. Bound BSA was removed from the particles and run on a SDS-PAGE.