Two novel non-viral gene delivery vectors: low molecular weight polyethylenimine cross-linked by (2-hydroxypropyl)-β-cyclodextrin or (2-hydroxypropyl)-γ-cyclodextrin†

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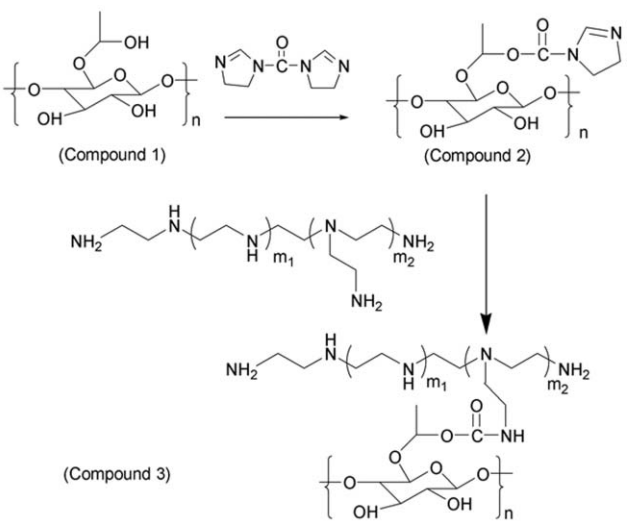
Two novel polymers of low molecular weight polyethylenimine cross-linked by (2-hydroxypropyl)-β-cyclodextrin or (2-hydroxypropyl)-γ-cyclodextrin showed lower cytotoxicity and higher transfection efficiency for the delivery of plasmid DNA compared with those of polyethylenimine (PEI, 25 kDa).

The delivery of DNA in gene therapy needs efficient and safe vectors. Viral vectors are used in most laboratory and clinical trials because of their high efficiency, associated with some safety issues such as insertional mutagenesis,[1] immunogenic and inflammatory responses.[2] Non-viral vectors, including cationic liposomes[3] and other polycations,[4] are now hot spots. Polyethylenimine (PEI) has shown a high transfection efficiency depending on its molecular weight. High molecular weight (HMW) PEI has shown high transgene expression but significant cytotoxicity. Low molecular weight (LMW) PEI has shown non-toxic and poor transfection activity.[5,6] Petersen *et al.* significantly enhanced the plasmid DNA condensation of LMW PEI by cross-linking it with star-shaped polyethylene.[7] Thomas *et al.* carried out the cross-linking of branched 2 kDa PEI with a linear 423 Da PEI *via* ester and/or amide-bearing linkages used in *in vitro* and *in vivo* gene delivery.[8] In this study, two novel biodegradable polymers of LMW PEI cross-linked by (2-hydroxypropyl)-β-cyclodextrin (2-hy-β-CD) or (2-hydroxypropyl)-γ-cyclodextrin (2-hy-γ-CD) have been developed. Both showed lower cytotoxicity and higher transfection efficiency for the delivery of plasmid DNA compared with those of polyethylenimine (PEI 25 kDa).

In this communication, LMW PEI (600 Da) was cross-linked to 2-hy-β-CD and 2-hy-γ-CD *via* a facile synthetic route (data shown in ESI†). The hydroxy groups on the outside of 2-hy-β-CD and 2-hy-γ-CD (compound **1**) were firstly activated by 1,1-carbonyldiimidazole (in DMSO) and stirred in the dark for 1.5 h. PEI (600 Da, in DMSO) was added dropwise and stirred in the dark for more than 5 h. The hydroxy groups on the outside of 2-hy-β-CD and 2-hy-γ-CD were cross-linked with amino groups of the PEI (compound **3**). When $n = 7$ in Scheme 1, **3** was named as 2-hy-β-CD-PEI600. When $n = 8$ in Scheme 1, **3** was named as 2-hy-γ-CD-PEI600. The mixture was dialyzed with a dialysis tube (MW 2 000) in running water for 2 d and the aqueous solutions lyophilized for 3 d. Two end products were obtained. The ratio of CD and PEI in the new polymers was calculated based on the proton integral values of ^{1}H NMR spectrum (Varian 400 MHz, D_2O): 1.038 ppm (CH_3 of hydroxypropyl) and 2.4–3.0 ppm (CH_2 of PEI). It was found to be 1 : 3.3 for CD and PEI600 Da (mole to mole) (Fig. 1).

The polymers could condense plasmid DNA efficiently. Agarose electrophoresis (1% agarose) was used to determine polymer and DNA binding. Various amounts of the polymers were mixed with 0.5 μg pGL3 plasmid DNA. DNA bands in the gel were visualized by a UV illuminator. As showed in Fig. 2, the migration of DNA was completely retarded when the weight ratio of polymer/DNA was at 2 : 1 (w/w, weight to weight). The migration of DNA was completely retarded when the *N*/*P* ratio (the number of nitrogen residues of PEI per DNA phosphate) of PEI 25 kDa/DNA and PEI 600 Da/DNA was 4 : 1 (data shown in ESI†).

Polymer/DNA complexes were prepared at a DNA concentration of 25 μg ml^{-1} in 150 mmol L^{-1} NaCl. The particle sizes of different weight ratios of polymer/DNA were measured with a 90Plus/BI-MAS (Brookhaven Instruments Corporation) at room temperature. Scattered light was detected at a 90° angle. Each sample was run for 200 s and analyzed in Unimodal Analysis mode. Results showed that the size of polymer/DNA complexes was less than 300 nm (data shown in ESI†).



Scheme 1 Synthesis of **3** ($n = 7$, **3** is 2-hy-β-CD-PEI600 or $n = 8$, **3** is 2-hy-γ-CD-PEI600).

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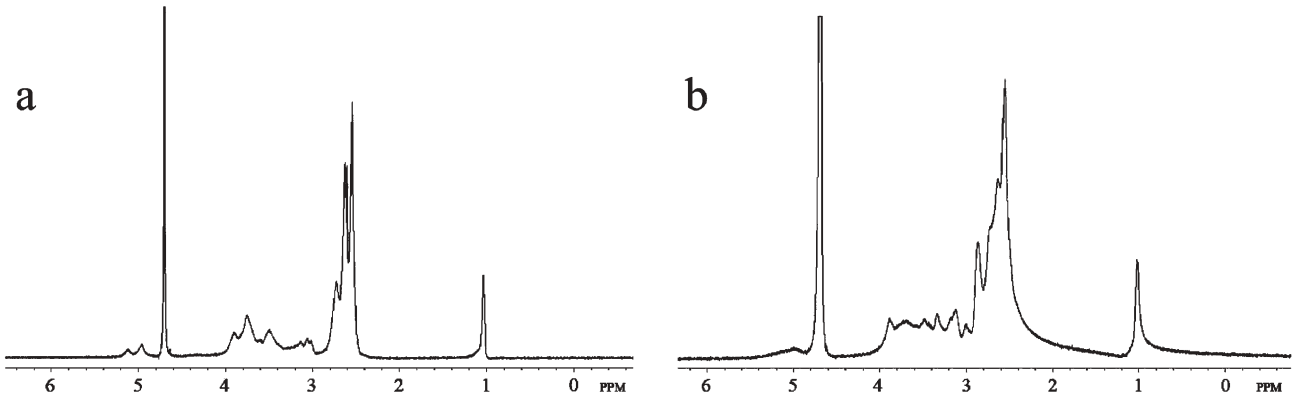

Fig. 1 ^{1}H NMR spectra of (a) 2-hy-β-CD-PEI600, (b) 2-hy-γ-CD-PEI600.

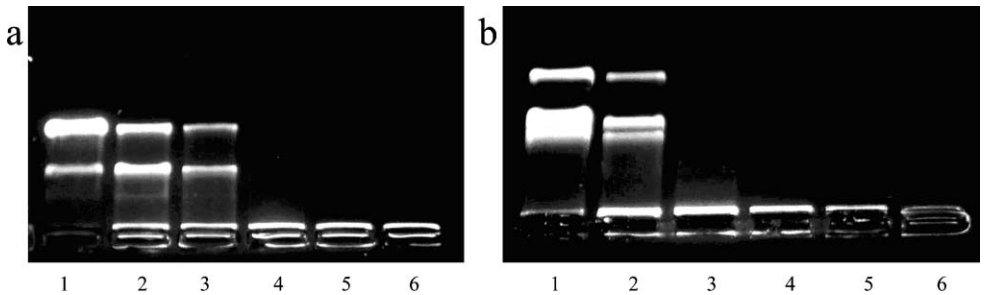

Fig. 2 Agarose gel electrophoresis retardation of pGL3 plasmid DNA by (a) 2-hy-β-CD-PEI600 and (b) 2-hy-γ-CD-PEI600. Lane numbers correspond to different polymer/DNA weight ratios: (1) 0 : 1 (DNA only), (2) 0.5 : 1, (3) 1 : 1, (4) 2 : 1, (5) 3 : 1 and (6) 5 : 1.

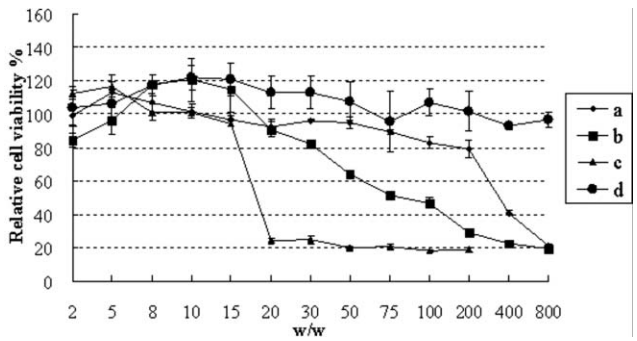

Fig. 3 Comparison of the cytotoxities induced by (a) 2-hy-β-CD-PEI600, (b) 2-hy-γ-CD-PEI600, (c) PEI 25 kDa and (d) PEI 600 Da in SKOV-3 cells measured by a MTT assay.

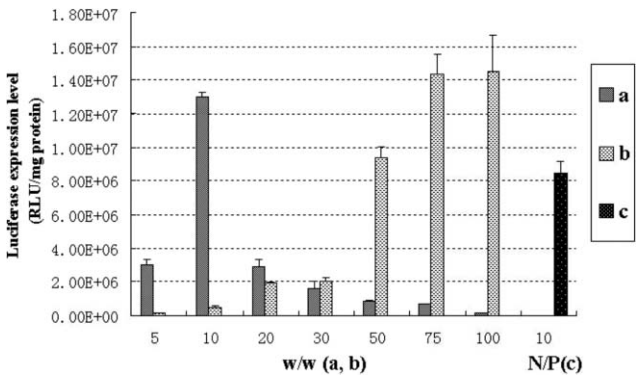

Fig. 4 Transfection efficiency of (a) 2-hy-γ-CD-PEI600, (b) 2-hy-β-CD-PEI600 and (c) PEI 25 kDa (*N*/*P* = 10) in SKOV-3 cells.

The cytotoxicity of the new polymers were compared with PEI 25 kDa and PEI 600 Da using a MTT assay in SKOV-3 cells (shown in Fig. 3). PEI 600 Da showed no cytotoxicity in Fig. 3. The new polymers showed significantly lower cytotoxicity than that of PEI 25 kDa.

The transfection efficiency of the delivering of plasmid DNA was evaluated for the two polymers in SKOV-3 cells. The assay was performed with a firefly luciferase reporter gene pGL3 plasmid. PEI 25 kDa, 2-hy-β-CD-PEI600 and 2-hy-γ-CD-PEI600/DNA complexes were incubated with SKOV-3 cells in a serum free medium for 4 h at 37 °C. After transfection, the luciferase activity in cell extracts was measured by a luciferase assay kit on a single well lucimeter (Berthold Lumat LB9507, Germany) for 10 s. The results showed the optimal ratio of transfection efficiency *in vitro*. The optimal *N*/*P* ratio for transfection efficiency of the PEI 25 kDa/DNA complex was 10 : 1, the optimal w/w ratio of the 2-hy-β-CD-PEI600/DNA complex was 100 : 1 and that of the 2-hy-γ-CD-PEI600/DNA complex was 10 : 1 (shown in Fig. 4). PEI 600 Da showed a low transfection efficiency (data shown in ESI†). The Relative Light Unit (RLU) of the two new polymer/DNA complexes was 1.5–1.7 fold higher than that of PEI 25 kDa/DNA complexes and over 20-fold higher than that of PEI 600 Da/DNA complexes.

The two novel polymers could condense plasmid DNA efficiently. They showed low cytotoxicity and high transfection efficiency. Their properties can be further optimized, and they are promising candidates for *in vitro* and *in vivo* gene therapy applications.

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