Cell Binding and Cytotoxicity of Palmitic-Acid Modified Polyethylenimine (PEI) and Poly-L-lysine (PLL) on Bone Marrow Stromal Cells (BMSC)

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Statement of Purpose:

As gene delivery system, cationic polymers such as polyethylenimine (PEI) and poly-L-lysine (PLL) have been extensively tested *in vitro* and *in vivo*. They have been found to be one of the most efficacious non-viral transfection agents; however, despite their general efficacy, their use has been hampered by their toxicity and low transfection efficiency in some cases (especially with primary cells). To explore the effect of increasing hydrophobic character of these polymers on DNA delivery, this study attempted to conjugate naturally-occurring palmitic acid (PA) to the polymers. The PA is utilized by mammalian cells to control the intracellular trafficking of proteins, especially enabling protein crossing through lipid membranes. We therefore conjugated PA to PEI and PLL and investigated several properties of the conjugates critical for DNA delivery, namely, binding to plasmid DNA and cells, and toxicity on cells. Bone Marrow Stromal Cells (BMSC) derived from rats were used as the cell model, since BMSC are actively pursued for gene therapy protocols in clinics.

Methods:

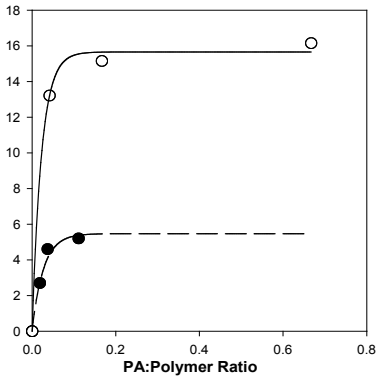
PEI (MW: 25 KDa) was reacted with palmitic acid chloride at a PA/amine ratio of 1:10, 1:64 and 1:85 for 2 hours. PLL (MW: 28 KDa) was reacted succinimide ester of palmitic acid with PA/amine ratios of 1:1.5, 1:3 and 1:6 for ~24 hours. Polymers were precipitated with ether, dried and characterized by ^{1}H-NMR to determine the extent of PA substitution. TNBS assay (Picrylsufonic acid solution 5% (w/v)) was performed to determine the concentration of free amines in polymer solutions. DNA binding of the polymers was determined by incubating the polymers with a plasmid DNA (pEGFP-N2) for 30 minutes in HEPES buffer, and analyzing the complexes formed on agarose gel (0.5%) electrophoresis. To determine binding of polymers to BMSC, the polymers were labeled with FITC (1 mM) and dialyzed to remove unreacted FITC. The cell binding was determined by incubating FITC-labeled polymers with BMSC grown in 38-well plates, and determining the amount of fluorescence bound to the cell monolayer by using a fluorescent plate reader (λ_{ab}: 485 nm; λ_{em}: 527 nm). The cytotoxocity of polymers were determined by the MTT assay, after the incubation with the cells for 24 hours.

Results and Discussion:

A series of PEI and PLL derivatives were successful synthesized with N-alkylation by using palmitoyl chloride and palmitic acid-succinimide ester, respectively. Syntheses were confirmed by the ^{1}H-NMR based on the unique chemical shifts of cationic polymers and PA, and the obtained substitution ratios were summarized in **Figure 1**. The extent of PA substitution was higher on PLL (13-16 PA/PLL) as compared to PEI, which gave up to ~5 PA/PEI. Polymers with higher substitution were not soluble in aqueous buffers.

Cell binding study of PEI-PA conjugates with low PA substitution showed no apparent difference in cell binding as compared to the unmodified PEI. With a higher substitution (~5 PA/PEI), PEI-PA exhibited a significantly higher binding to BMSC. Despite the improved binding, the toxicity of this PEI-PA was lower than the toxicity of native PEI. Similarly, PLL-PA showed higher binding to BMSC than the native PLL, but no apparent effect of PA substitution on the cytotoxicity of PLL was evident.

The binding of synthesized polymers to plasmid DNA was demonstrated as a function of polymer concentration. Based on IC_{50} values, (i.e., concentration required for 50% binding to 1 μg plasmid DNA), the binding of PEI-PA samples were lower than the native PEI (as much as 3-fold lower; i.e., 3-fold higher IC_{50}). This was the same for PLL-PA samples, which gave 2-fold lower binding to plasmid DNA as compared to PLL.



Conclusions:

Modification of the cationic polymers PEI and PLL with hydrophobic side groups increases the binding of the polymers to the cells. Toxicity, in some cases is reduced as a result of PA substitution. Although DNA complexation ability of reduced, the hydrophobic polymers did retain the ability of condensing DNA at higher polymer:DNA ratios. Further studies are warranted to investigate the ability of these polymers to carry DNA intracellularly, and allow expression of genes delivered with a plasmid DNA.

References:

(**1**) Han et al., "Water-soluble lipopolymer for gene delivery". *Bioconjugate Chem*. 2001, 12, 337-345. (**2**) Reddy et al., "Optimization of Folate-Conjugated Liposomal Vectors for Folate Receptor-Mediated Gene Therapy". *J. Pharm. Sci.* Vol. 88, No. 11, 1999.