Potential of Palmitic-Acid Modified Polyethylenimine (PEI) and Poly-L-Lysine (PLL) to Transfer Plasmid DNA into Bone Marrow Stromal Cells (BMSC)

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Statement of Purpose:

Cationic polymers such as polyethylenimine (PEI) and poly-L-lysine (PLL) have been extensively tested *in vitro* and *in vivo* to deliver anionic plasmid DNA into mammalian cells. The cationic polymers condense the long, string-like DNA molecules into compact structures, which are readily taken up by the mammalian cells. It is believed that neutralization of the charge of DNA molecules is the primary reason for the beneficial effect of cationic polymers. This process allows the passage of the DNA molecules through the primary barrier of the cells, i.e., anionic plasma membrane. To determine the influence of hydrophobic groups on DNA transfer, cationic polymers with hydrophobic residues were synthesized for this study. The polymer internalization and DNA delivery into the cells, as well as the expression of a model gene were investigated by using bone marrow stromal cells (BMSC), which is an attractive cell phenotype for clinical gene therapy.

Methods:

The BMSC used for this study was obtained from Sprague-Dawley rats according to previously published procedures [1]. The hydrophobic derivatives of PEI (MW: 25 KDa) and PLL (MW: 22 kDa) were prepared by reacting the cationic polymers with palmitoyl chloride and succinimide ester of palmitic acid (CH_3-$(CH_2)_{14}$-COOH), respectively. The extent of PA substitution was assessed by the ^{1}H-NMR. The polymers were labeled with fluorescent isothiocyanide (FITC; 1 mM) to investigate cell uptake by the flow cytometry. To investigate DNA delivery into the cells, a plasmid DNA (pEGFP-N2) was labeled with a succinimide ester of Cy5.5 dye (Amersham). The polymers were complexed with the labeled DNA for 30 minutes in HEPES buffer, and then added to the cells in 25 cm^2 flasks. After 4 or 24 hours, the cells were trypsinized and assessed with flow cytometry to determine the DNA uptake. To determine expression of a model gene (Enhanced Green Fluorescent Protein; EGFP), pEGFP-N2 plasmid was complexed with the synthesized polymers, and exposed to the cells for 24 hours. At indicated time points, the cells were trypsinized and analyzed by flow cytometry for EGFP expression.

Results and Discussion:

Both PEI and PLL were readily internalized by the BMSC after 2 hours of incubation, as assessed by flow cytometry. The polymers were localized to the cell nucleus. Conjugating palmitic acid to the PEI did not increase the internalization of the polymer to a significant degree. The PA substituted PLL (~15 palmitic acids per PLL), on the other hand, exhibited an increased internalization by the BMSC. At the highest polymer concentrations used (~100 μg/mL corresponding to 100 μL of PLL addition to cells in **Figure 1**), the cells became leaky to propidium iodine (PI), indicating a compromise in the integrity of the plasma membrane. At lower concentrations, though, no adverse effect of polymers on membrane integrity was observed. Consistent with better uptake by the cells, palmitic acid-grafted PLL gave ~3 and ~7-fold higher delivery of pEGFP-N2 to the BMSC (**Figure 2**). Consistent with increased delivery, pEGFP-N2 delivered with palmitic acid-PLL was expressed to a higher extent than the plasmid delivered with PLL, which was similar to the background values obtained (i.e., without polymer). Under optimal conditions, we were able to transfect 10-20% of the cells in the BMSC culture by the modified PLL. This was comparable to the transfection efficiency obtained with an adenovirus containing a GFP gene [2], but the latter gave a more prolonged expression (>2 weeks) unlike polymer carriers whose efficiency was diminished after 1 week.

Conclusions:

The results of this study indicated a positive influence of the hydrophobic modification of polymers on the DNA delivery by the PLL (but not PEI). Such polymers enabled expression of a model plasmid at a comparable level to adenoviral delivery initially. Additional studies are needed to prolong the duration of expression with the cationic polymers.

References:

(**1**) Haque T, Uludag H, Zernicke RF, Winn SR, Sebald W. Tissue Eng. (2005) 11: 634-44. (**2**) Li Y, Tredget EE, Ghahary A. Hum Immunol. (2004) 65: 114-123.

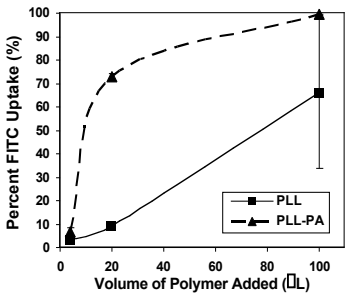

Figure 1. BMSC uptake of PLL and palmitic acid modified PLL (PLL-PA). Palmitic acid modification resulted in an increased uptake of the polymers into the cells. The vertical axis represents the % of cells that became FITC positive.

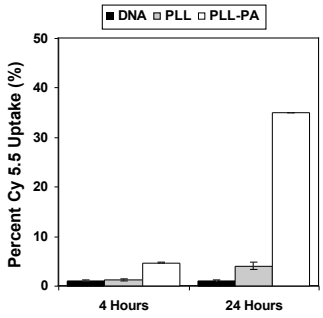

Figure 2. pEGFP-N2 delivery after 4 and 24 hrs incubation of polymer - plasmid complexes with BMSC. Note the increased delivery of pEGFP-N2 with palmitic acid-PLL. DNA incubation alone and complexed with PLL did not result in increased uptake by BMSC.