Polyethylenimine (PEI), Polyethylene glycol (PEG) & Mannose Tri-Component Vehicles for siRNA Delivery

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Statement of Purpose:
Gene therapy using small interfering RNA (siRNA) has potential as a treatment for genetic defects. However, due to its inherent instability, large molecular weight and polyanionic nature, naked siRNA does not easily cross the cell membrane. To overcome this challenge, we synthesized PEI-tricomponent polymers. Each polymer was linked to polyethylene glycol (PEG) and/or mannose to prolong serum half-life *in vivo* and achieve target specific delivery, respectively. Biophysical characteristics and delivery efficacy of these polymer constructs are being evaluated.

Methods:
Branched PEI of 25,000 kDa (Sigma-Aldrich, St. Louis, MO), PEG of 2,000 kDa (Creative PEGWorks, Winston Salem, NC) and mannose (Sigma-Aldrich, St. Louis, MO) were used to construct two different polymers. Mannose and PEG chains were directly conjugated to the backbone of the cationic PEI in the first architecture (Mannose-PEI-PEG), whereas the mannose was conjugated to the PEG and the PEG was conjugated to the cationic PEI polymer backbone (PEI-PEG-Mannose) in the second. All the experiments were performed after forming polymer/siRNA complexes at desired N/P (nitrogen in cationic polymer per phosphate in nucleic acid) ratios. siRNAs were kindly provided by Integrated DNA Technologies (Coralville, IA). Size distribution and surface morphology were observed using Scanning Electron Microscopy (SEM, Hitachi S-4800). The complexation ability of polymers with siRNA was evaluated with gel retardation assay (images taken by Panasonic DMC-FX30 digital camera). Oregon Green 488 labeled polymer/Cy3 labeled siRNA complexes with DAPI stained nuclei were used to track cellular uptake of the nanoparticles using Multiphoton/Confocal microscope images (Bio-Rad Radience 2100MP). The HEK293 cell line was used as *in vitro* transfection host.

Results:
The polymer/siRNA complexes were spherically shaped with porous surfaces in SEM images. Sizes ranged from 100 to 600 nm diameter. (Figure 1).

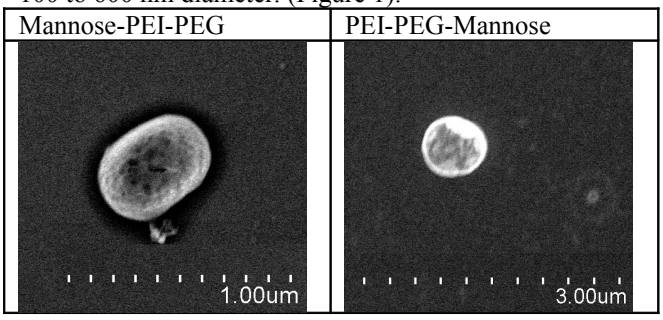

Figure 1. Representative SEM images of polymer/siRNA complexes

Both polymers showed effective complexation with siRNA throughout the wide range of N/P ratios (Figure 2). From left: polymers only, N/P=1, 3, 5, 7, 10, 12, 15, siRNA only.

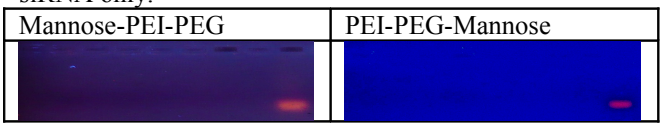

Figure 2. Gel retardation assay

Cellular uptake was observed using Oregon Green 488 labeled PEI (shown in green) / Cy3 labeled siRNA (shown in red) complexes with DAPI stained nuclei (shown in blue). The polymer/siRNA complexes (arrow heads) had been internalized and were beginning to dissociate at 2 hours post-transfection in the HEK293 cells (Figure 3).

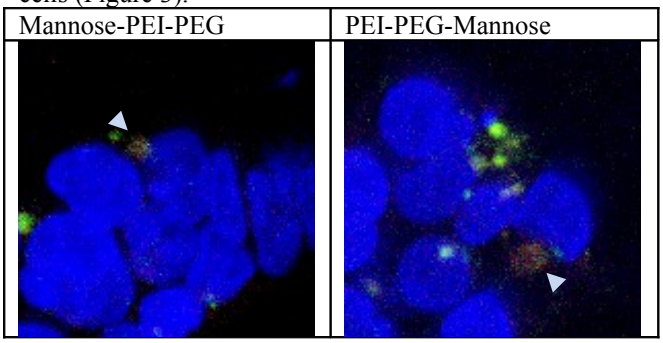

Figure 3. Cellular uptake of polymer/siRNA complexes

Conclusions:
Both Mannose-PEI-PEG and PEI-PEG-Mannose can efficiently form nanoparticle complexes with siRNA. These nanoparticles were efficiently internalized by cells and dissociate in the cytoplasm. siRNA can be found in the nucleus and cytoplasm. Future and current studies will evaluate the gene knockdown efficacy of these constructs, cell binding efficiency and toxicity.