In Vitro Gene Delivery Using Polyethylene Glycol Based Vehicles Coupled to Endosomal Escape Peptides

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Statement of Purpose: The purpose of this project is to design a novel synthetic gene delivery vehicle using a polyethylene glycol (PEG) backbone functionalized with peptides to target barriers to gene delivery. Current non-viral vehicles often incorporate cationic polymers, such as polyethylenimine (PEI), for compacting DNA along with peptides for cellular targeting. However, the high density of positive charge on the cationic polymers limits their stability under physiological conditions and results in rapid clearance from the blood stream [1]. Incorporating PEG onto the vehicle surface increases vehicle stability, but the effect of the targeting peptides is often diminished [1]. We have designed vehicles containing a combination of DNA binding peptide (DBP) and endosomal escape peptide (INF7) coupled to a PEG backbone. We hypothesize that by using a PEG backbone, we can maintain the targeting effect of the peptides and reduce the overall charge density of the vehicle. In this study, particle size, zeta potential, hemolytic activity, and transfection efficiency of PEG-based vehicles complexed with plasmid DNA were determined.

Methods: PEI (25,000 MW, Sigma) was used as 10 mM stock solution. PEG (10,000 MW-4 arm, Shearwater Polymers) was dried and acrylated as previously described [2]. DBP and INF7 were coupled to PEG tetraacrylate (PEG-TA) using a Michael-type addition [2]. Plasmid DNA (95% pSV-β-Galactosidase and 5% pGFPemd-c[R]*)* was purified from overnight bacterial culture using a Qiagen Maxiprep kit. Polymer and plasmid DNA (80 μg/mL) were mixed in 150 mM NaCl and HEPES buffer (pH 7.4) to achieve desired charge ratios and incubated for 1 hr at 20°C. Particle size and zeta potential were measured with 90S Particle Size Analyzer with a Zeta PALS detector (Brookhaven Instruments). Chinese hamster ovary (CHO) cells were plated 24 hrs prior to transfection in F-12K Media (Gibco) supplemented with 10% FBS and 1% ABAM. Media was replaced with 200 μL Optimem media with 1% ABAM 2 hrs prior to transfection. 50 μL of particles was added to each well and incubated for 4 hrs at 37°C. Media was then replaced with Optimem media with 4% FBS. After 48 hr, cell lysates were analyzed by Bradford Assay (Biorad) and β-galactosidase (β-gal) assay (Promega) to determine transfection efficiency (the ratio of β-gal expression to total cell protein). The hemolytic activity of the INF7 peptide alone and PEG-DBP-INF conjugates were determined using a hemolysis assay. Human red blood cells (RBCs) were collected, washed with 150 mM NaCl, and centrifuged for 5 min at 200 x g. RBCs were diluted to $2x10^8$ cells/mL in 0.1 M phosphate buffer (pH 5.0, 5.5, 6.0, 6.5, 7.0, or 7.4) and incubated with INF or INF-PEG (20, 40,80, or 120 μg/mL INF) for 2.5 hr at 37ºC. Solutions were centrifuged, and the absorbance of the supernatant was measured at 541 nm . 100% lysis was determined by using 0.1% TritionX-100.

Results/Discussion: The PEG-DBP_2-INF_2 vehicles demonstrated similar hemolytic activity (~60 % lysis) to the INF peptide alone at pH 5.0 at 80 μg/mL (Fig. 1). PEG-DBP_2-INF_2 particle diameters were typically in the range of 250-300 nm similar to that of PEI particles formed 150 mM NaCl. The zeta potentials of the PEG/DNA particles with and without INF7 peptide were approximately -5 to +10 mV for all charge ratios, which was lower than that of the PEI particles. The highest transfection efficiency for the PEG-DBP_2 $INF7_2$ particle was seen at a charge ratio of 8:1 (+/-) (Fig. 2). The transfection efficiency of the PEG-DBP_2 $INF7_2$ particles was higher than the transfection efficiency of PEG-DBP_2 particles for charge ratios 7:1 and 8:1 and up to 60% of that observed for PEI particles.

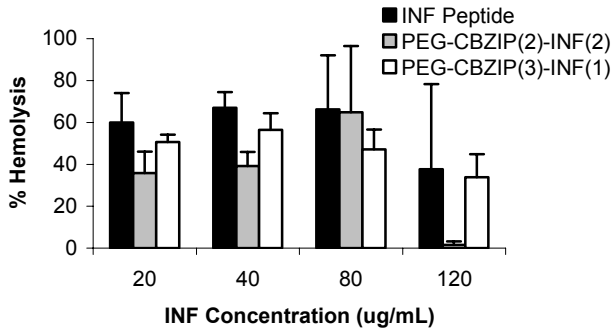

Figure 1. Percent hemolysis of the endosomal peptide (INF) and PEG-DBP-INF conjugates at pH 5 and at various concentrations of INF.

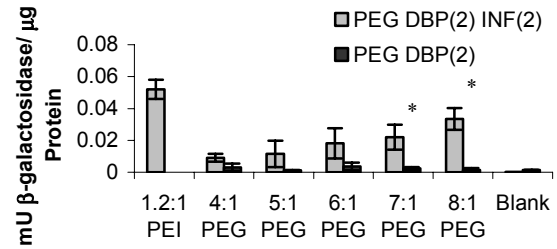

Figure 2. CHO cells transfected with PEG–TA coupled with two DBPs and two INF7 peptides compared to PEG-TA coupled with two DBPs at charge ratios ranging from 4:1 to 8:1. The error bars represent standard deviation. The * indicates difference in particles at that charge ratio ($p < 0.05$).

Conclusions: These results suggest that PEG based gene delivery vehicles coupled to endosomal escape peptides (INF7) have transfection efficiency similar to that of PEI. These vehicles maintain a near neutral zeta potential while packaging DNA and maintaining the pH dependent membrane lytic ability of the endosomal escape peptide. The increase in transfection efficiency with the INF7 peptide, suggests that endosomal escape is a key step in the gene delivery pathway of these vehicles.

References:

1. Plank et al. Hum Gene Ther. 1996; 7: 1437-1446
2. Elbert et al. Biomacromolecules. 2 (2001), 430-441.