Design and Characterization of a Polyethylene Glycol – Peptide Conjugate for In vitro Gene Delivery

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Introduction: The aim of this project is to design a novel synthetic gene therapy vehicle with a polyethylene glycol (PEG) backbone, which can be used to study the rate limiting step(s) of gene delivery. Current conjugated non-viral vehicles use cationic polymers, such as polyethylenimine (PEI) and poly-L-lysine (PLL), to condense DNA efficiently along with peptides for cell targeting. However, the high density of positive charge in these polymers limits their stability under physiological conditions and results in rapid clearance [1]. Conjugating PEG to these polycationic vehicles increases their stability, but decreases their cell targeting efficiency [2]. We have designed vehicles containing a combination of DNA binding peptide (DBP), endosomal escape peptide (INF7) and/or cell targeting peptide (RGD). We hypothesize that by using a PEG backbone conjugated with peptides for DNA condensation and cell targeting, we can develop a vehicle with low charge density, which does not block the peptide interactions with DNA or cells. In this study, the particle size, zeta potential, binding kinetics, and transfection efficiency of PEG-based vehicles were compared with PEI and PLL vehicles.

Materials and Methods: PEG (3400 MW-2 arm, Aldrich and 10,000 MW-4 arm, Shearwater Polymers) was dried and acrylated as previously described [3]. DBP, RGD, and INF7 peptides were synthesized using solid phase Fmoc chemistry, and purified with reverse phase HPLC. Peptides were coupled to PEG diacrylate (PEG-DA) or PEG tetra-acrylate (PEG-TA) using a Micheal-type addition [3]. Plasmid DNA (pSV-β-Galactosidase,.β-Gal*)* was purified using a Qiagen Maxiprep kit. Polymer and plasmid DNA (20 µg/mL) were mixed in 150 mM NaCl and HEPES buffer (pH 7.4) to achieve desired charge ratios (+/-) and incubated for 1 hr. Particle size and zeta potential were measured with 90S Particle Sizer (Brookhaven Instruments,). Particles were added to HEPG2 cells (ATCC) plated 24 hrs prior to transfection in a 24 well plate (25,000 cells/well). 50 µL of particles was added and incubated for 4 hrs at 37°C in serum-free Opti-mem media. After 48 hr, cells lysates were collected with 1x reporter lysis buffer (Promega) and analyzed using a Bradford Assay (Biorad) and β-gal assay (Promega). Transfection efficiency was determined by calculating the ratio of β-gal expression to total cell protein. For cell binding assays, vehicles were labeled with Oregon Green succinimidyl ester (Sigma) and added to cells at various concentrations and incubated at 37°C. At 4 hrs, media was removed and cells were washed with PBS (pH 7.4). Particles bound to the cell surface were removed with an acetic acid wash and cells were lysed with 1N NaOH, and the fluorescence of each wash was measured with a Cytofluor 4000 (Applied Biosytems).

Results and Discussion: PEG-TA was coupled with two DBP's and two RGD peptides, and used to form particles with charge ratios (+/-) of 2:1-8:1. Dynamic light scattering showed particles sizes of approximately 220-280 nm with neutral zeta potentials (-1 to +5 mV). The highest transfection efficiency for these vehicles was observed at a charge ratio of 5:1 (+/-) (Fig. 1). The transfection efficiency of PEG-TA coupled with DBP's and two IN7 peptides was also evaluated for charge ratios (+/-) of 2:1-8:1. The most efficient charge ratio for this vehicle is 6:1 (+/-) (Fig. 1). The efficiency of the PEG-TA with two IN7 peptides or two RGD peptides was equivalent to that of PEI.

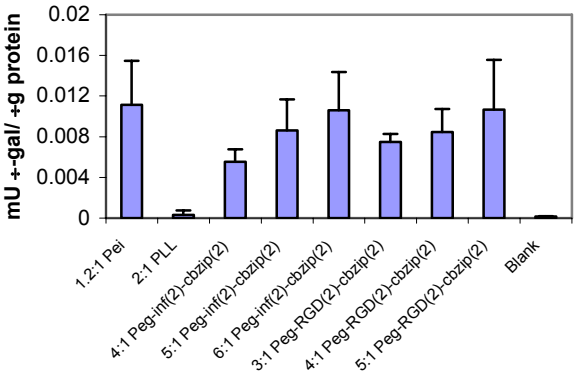

Figure 1. The transfection results of PEG-TA coupled with a DBP,RGD peptide, or IN7 peptide compared to PEI (N/P=6) and PLL (N/P=2).

Binding studies were performed to determine the effect of RGD peptide on cell surface targeting. The binding effect of the RGD-DBP-PEG-TA vehicle was compared to that of the DBP-PEG-TA vehicle.

Table 1. The binding results of PEG-TA coupled with a DBP and/or RGD peptide

Vehicle	K_d (nM)
PEG-TA DBP RGD	**38**
PEG-TA DBP	**338**

These results show an increased affinity of the RGD-DBP-PEG vehicle verses DBP-PEG vehicle toward the cell surface. The estimated K_D values are 38 and 338 nM respectively.

Conclusions: These results suggest that PEG-based vehicles for DNA delivery can be made using Michael-type addition to conjugate DBPs, cell targeting and endosomal escape peptides to multi-acrylated PEG. These vehicles have a transfection efficiency of HEPG2 cells equivalent to that of PEI. These vehicles maintain a zeta potential near neutral, while packaging and targeting the cell surface, which is promising for future studies.

References

1. Plank et al. Hum Gene Ther. 1996; 7: 1437-1446
2. Kunath et al. J Gene Med. 2003 Jul;5(7):588-99.
3. Elbert et al. Biomacromolecules. 2 (2001), 430-441.