Development of a novel pH-responsive diblock copolymer for plasmid DNA delivery

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Statement of Purpose: Development of synthetic polymeric vectors for plasmid DNA (pDNA) delivery has been met with limited clinical success, namely due to low transfection activities, high cytotoxicities, and/or poorly controlled polymerization techniques (1). Based upon previous synthetic strategies (2), a diblock copolymer was developed that is capable of electrostatically condensing pDNA into serum-stable nanoparticles while facilitating endosomal release and decomplexation following cellular internalization. The free polymer was shown to assemble into a micelle consisting of a predominantly hydrophobic terpolymer core and a hydrophilic cationic corona. Upon exposure to the low pH environment of the endosomal-lysosomal pathway, it was hypothesized that protonation of tertiary amines within the core would promote micelle destabilization, exposing membrane-interactive hydrophobic groups leading to enhanced cytosolic delivery.

Methods: The diblock copolymer was synthesized using the controlled reversible addition fragmentation chain transfer polymerization (RAFT) method and is composed of a cationic block to mediate pDNA condensation and a second endosomal releasing terpolymer block. The polymer was characterized by gel permeation chromatography (GPC) to determine block lengths and polydispersity indices and ^{1}H-NMR to calculate the ratio of incorporated monomers. Dynamic light scattering (DLS) measurements were used to determine free polymer and polymer/pDNA nanoparticle mean diameters. Gel retardation assays were performed to confirm polymer-mediated pDNA condensation. pH-responsive membrane disruptive activity was assessed by hemolysis assays. Lactase dehydrogenase (LDH) measurements of treated cell populations were used as an indicator of polymer cytotoxicity. Fluorescence microscopy was employed to determine intracellular trafficking properties of complexed pDNA as well as to visualize expression of a GFP-encoding vector. Flow cytometry of RAW cells treated with this condensed vector provided two metrics of transfection activity: total gene expression (raw GFP fluorescence) and transfection efficiency (percentage of GFP-positive cells).

Results: DLS measurements found that the free polymer assembles into 29 ± 2 nm particles at physiological pH and decreases in size to 6 ± 1 nm at a pH of 5.8. An inverse size-to-pH relationship was determined for the polymer/pDNA complexes: at a pH of 7.4, a mean diameter of 144 ± 38 nm was determined compared to 362 ± 67 nm at a pH of 5.8. The polymer additionally possessed the ability to lyse biological membranes in the endosomal pH regime yet remained inert at physiological pH based on a hemolysis assay. Fluorescence microscopy of labeled pDNA demonstrated that the polymer carrier was able to release its pDNA cargo into the cytosol. An assay for cytotoxicity in RAW cells found that the polymer exhibited similar toxicity when compared to the commercial standard transfection reagent, Lipofectamine 2000 (L2K, Invitrogen, Carlsbad, CA). A preliminary flow cytometry study demonstrated that the polymer transfected 45 ± 4% of cells compared to 14 ± 1% for L2K, a finding validated by fluorescence microscopy imaging.

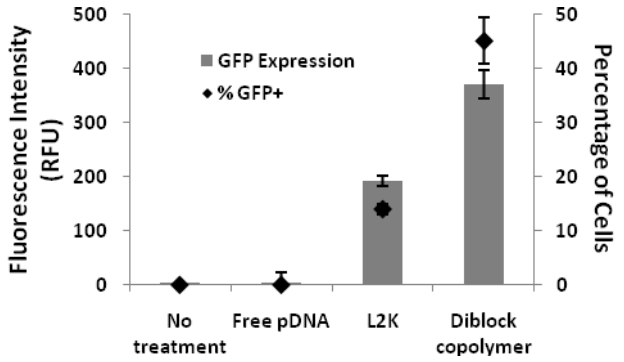

Figure 1. Flow cytometry analysis of RAW cells transfected with a GFP expression vector.

Conclusions: DLS in conjunction with a gel retardation assay confirmed that the polymer was able to condense pDNA into nanoparticles with sub-200 nm mean diameters. DLS additionally validated the hypothesis that free polymer undergoes a conformational change from micelle to unimer as a function of pH. The dependence of polymer/pDNA particle size on pH can likely be attributed to the polymer being unable to condense pDNA as effectively as a unimer when compared to the micelle structure, possibly due to a lower charge density and greater hydrophobicity of the polymer in this form. The unimer conformation was expected to be more membrane interactive, an activity that was demonstrated by a hemolysis assay that showed greater membrane destabilization at a lower pH. Through a mechanistic study of the intracellular trafficking properties of condensed pDNA, we found that the polymer mediates a greater extent of pDNA cytosolic release than L2K. When comparing the biological activities of the new diblock carrier to L2K, it was found that the diblock copolymer exhibited enhanced transfection efficiency with similar toxicity. New designs to further decrease toxicity while maintaining transfection efficiency are being conducted.

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References: 1. Schaffert D. Gene Ther. 2008;15:1131-1138. **2.** Convertine AJ. J Control Release. 2009;133:221-229.