Development and Evaluation of "Smart" Polymer-Nucleic Acid Complexes

Mohamed E.H. El-Sayed[1], Eileen M. Bulger[2], Allan S. Hoffman[1], and Patrick S. Stayton[1]
University of Washington, Department of Bioengineering[1], and Department of Surgery[2], Seattle, Washington 98195, USA

Statement of Purpose: The objective of this research is to develop "smart", pH-sensitive, membrane-destabilizing polymer-nucleic acid complexes that can escape the endosomal membrane and reach the cytoplasm of targeted cells. This report summarizes the formulation, characterization, *in vitro* evaluation, and *in vivo* toxicity and biodistribution of "smart" polymer- nucleic acid complexes with special emphasis on therapeutic antisense oligodeoxynucleotides (ASODN) and siRNA molecules.

Methods: The selected "smart" polymer composition is poly(PAA-*co*-BA-*co*-PDSA) terpolymer with a weight average molecular weight (M_W) of 14 KDa, which exhibits a pH-dependent, membrane-destabilizing activity in response to endosomal pH gradients (1). Cationic poly-L-lysine (PLL) chains with M_W 2.5, 10, or 48 KDa were grafted to this polymer backbone through serum-stable disulfide linkages to form "smart" polymer-PLL conjugates, which were used to complex a phosphorothioate ASODN (18 bases, 5699 Da) designed to block the pro-inflammatory IRAK-1 gene pathway in alveolar macrophages. The formulation of "smart" polymer-ASODN complexes was confirmed by the gel shift assay followed by measuring the size/zeta potential of the formed complexes using dynamic light scattering. The change in cellular uptake and sub-cellular distribution of free Alexa Fluor-labeled ASODN and "smart" polymer-ASODN complexes in THP-1 macrophage-like cells was examined using fluorescence microscopy. The compatibility of promising "smart" polymer-PLL conjugates was investigated *in vivo* as a function of the administered polymer dose (20, 40, 60 mg/Kg body weight). *In vivo* biodistribution of free ^{3}H-labeled ASODN and its complexes with ^{14}C-labeled "smart" polymer-PLL conjugates was investigated as a function of time using the corresponding plasma profile, net accumulation in vital organs (heart, lungs, liver, kidneys, spleen), and excretion in urine and feces as key markers.

Results / Discussion: "Smart" polymer-PLL conjugates were synthesized and retained their pH-dependent, membrane-destabilizing activity after their complexation with therapeutic ASODN. However, the optimum NH_2/PO_4 (+/-) ratio required to form stable complexes and the size/zeta potential of the formed complexes varied based on the length of the PLL graft. "Smart" polymer-PLL conjugates with PLL grafts of M_W 2.5, 10, and 48 KDa formed stable complexes with ASODN at N/P ratios of 8/1, 3/1, and 1/1, respectively. Similarly, the corresponding size/zeta potential of the formed complexes was 188 ± 16 nm/-6.2 ± 2.9 mV, 602 ± 119 nm/11.1 ± 3.6 mV, and 892 ± 112 nm/16.7 ± 3.6 mV, respectively. The polymer-ASODN complexes incorporating PLL grafts of M_W 2.5 and 10 KDa were serum stable (> 75%) and caused no toxicity when incubated with THP-1 cells for 24 hours. Nano-sized polymer-ASODN complexes (< 200 nm) significantly increased cellular uptake and cytoplasmic distribution of the incorporated ASODN (Figure 1).

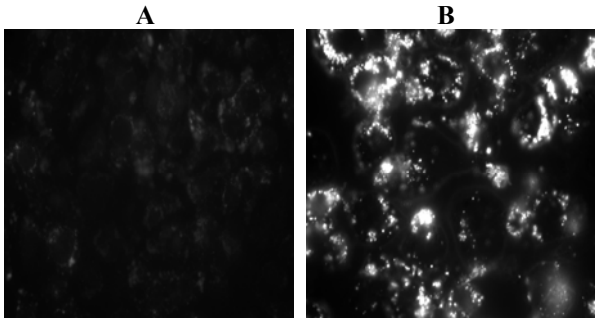

Figure 1. Fluorescent microscopy images of THP-1 cells after incubation with: A) Alexa Fluor-labeled ASODN, and B) their ionic complexes with poly(PAA-*co*-BA-*co*-PDSA)-PLL 2.5 KDa conjugate at NH_2/PO_4 ratio of 8/1.

Additionally, nano-sized polymer-ASODN complexes were highly biocompatible up to a conc. 60 mg/Kg body weight as shown in the histological examination of different organs. Free ^{3}H-labeled ASODN was rapidly cleared (< 0.5 hour) from the systemic circulation when administered intravenously, whereas "smart" polymer-ASODN complexes showed slower elimination from the systemic circulation and higher accumulation in target organ, the lungs. Cationic, polymer-ASODN complexes with 10 KDa PLL grafts, achieved significant accumulation in the lungs (26 ± 14 % of the administered dose) within 2 hours of their injection into the jugular vein, which declined to 8 ± 2% of the administered dose over a period of 24 hours. For anionic, polymer-ASODN complexes with 2.5 KDa PLL grafts, an average of 15 ± 5 % of the administered dose was retained in the lungs 24 hours after complex administration into the jugular vein. It is important to note that both ^{3}H-labeled ASODN and ^{14}C-labeled polymer-PLL conjugates showed similar distribution profiles when administered as pre-formulated complexes, which further confirms the *in vivo* stability of these complexes.

Conclusions: Results show that "smart" polymers can form stable complexes with therapeutic ASODN, achieve high serum stability both *in vitro* and *in vivo*, and accumulate in target organs by varying their properties such as size/net charge.

References:

1. M. E.H. El-Sayed, A. S. Hoffman, and P. S. Stayton, *J. Cont. Rel.*, 101 (1-3): 47-58, 2005.

Acknowledgment: This research is supported by NIH Grant R01 EB2991-02 (Hoffman & Stayton), and the U.S. Congressionally Directed Medical Research-Multidisciplinary Postdoctoral Award (El-Sayed).