BIOINSPIRED NANOCARRIERS DESIGNED TO ENHANCE INTRACELLULAR DELIVERY OF BIOTHERAPEUTICS

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Abstract- **The biotechnology and pharmaceutical industries have developed a wide variety of potential therapeutics based on the molecules of biology: DNA, RNA and proteins. While these therapeutics have tremendous potential, effectively formulating and delivering them has also been a widely recognized challenge. A variety of viruses and toxins have evolved multi-functional biopolymer complexes to solve this problem by directing uptake and enhancing biomolecular transport to the cytoplasm from the low pH endosomal compartment. Here, we have designed and synthesized bio-inspired, pH-responsive polymeric carriers that mimic the multi-functional design of biology. These nanocarriers target and direct cellular uptake, as well as enhance cytosolic delivery by disrupting endosomal membranes in pH-dependent fashion. We show that the encrypted polymeric carriers significantly enhance the delivery oligonucleotides and peptides to the cytoplasm of cultured hepatocytes and macrophages, demonstrating the potential of this approach to therapeutic and vaccine development.**

Keywords **- gene therapy, vaccine, bioinspired, biotherapeutic**

I. INTRODUCTION

The efficacy of many protein and DNA therapeutics and vaccines is limited by nonproductive intracellular trafficking. We have been investigating a polymer-based approach to manipulating intracellular trafficking that is inspired by biological polymers, i.e. proteins, that are involved in controlling vesicular trafficking pathways. For example, many naturally occurring viruses and toxins have evolved pH-responsive, membrane-active proteins that enhance transport of DNA or proteins out of the endosome and into the cytosol. Because the composition of the proteins and peptides which disrupt endosomal membranes shared similarities with lipid vesicle destabilizing poly (ethyl acrylic acid) (PEAAc) [1], we were inspired to investigate whether polymers containing other related hydrophobic moieties could provide advantageous delivery properties. A second bioinspired approach has also been developed to introduce pH-responsive carrier properties. Polymeric graft copolymers were developed that contain pH-degradable linkages to pendant hydrophilic moieties that solubilized a membrane-disruptive backbone. These "encrypted" polymers can be hydrolysed at endosomal pH to enhance delivery of gene, antisense, or protein therapeutics to the cytoplasm.

II. METHODOLOGY

Ethyl and propyl acrylic acid monomers were synthesized according to the procedure outlined by Ferrito et al [2]. PEAAc, PPAAc, PBAAc and the random copolymer of ethyl acrylate and acrylic acid were synthesized by free radical polymerization, using AIBN as the initiator. For hemolysis assays, 10^8 RBCs were suspended in buffer and added to 10^8 RBCs suspended in 1ml. The absorbance of the supernatant was then measured at 541nm. The pEGFP plasmid was used to transfect NIH3T3 fibroblast cells seeded in 12-well tissue culture plates. Varying quantities of PLL were complexed to poly(propylacrylic acid) (MW 60,000 g/mol) in PBS to characterize different ratios of PLL:PPAAc. Cells were incubated with PLL:PPAAc and PLL:plasmid particles for 4 hours, and subsequently incubated for an additional 24 hours.

The encrypted polymers are designed as self-contained carriers that incorporate the primary functionalities of viruses and toxins: targeting agents that direct receptor-mediated endocytosis, a pH-responsive element that selectively disrupts the endosomal membrane, and the biomolecular component which is delivered as a free and active agent into the cytoplasm. The polymers contain a masked membrane disruptive element that is activated in the low pH environment of the endosome. Copolymers of dimethylaminoethyl methacrylate (DMAEMA) and hydrophobic alkyl acrylates were chosen for the membrane-disruptive backbone, based on previous work on endosomolytic polymers. PEG was chosen as the solubilizing hydrophilic graft because of its established ability to improve the stability, circulation lifetime, and biodistribution properties of a wide variety of delivery systems. The pH-sensitivity of the encrypted polymer is provided through the acid-degradable acetal linkers.

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III. Results and discussion

The membrane disrupting activity and pH responsiveness of the alkyl acrylic acids could be tuned by manipulating the monomer composition and molecular weight to rationally control pH-responsiveness and hemolytic concentration dependencies. Gene transfection studies with a β-galactosidase plasmid construct demonstrated that the addition of poly (propyl acrylic acid) (PPAAc) to ternary polycation condensed DNA particles resulted in a significant enhancement of transfected cells. The PPAAc also conferred serum stability to the lipoplexes. Control transfections performed with poly(acrylic acid) and poly(methylacrylic acid) confirmed that the nature of the alkyl group is critical to the mechanism of PPAAc enhancement. Finally, PPAAc was also shown to enhance the cytoplasmic delivery of a model antibody-targeted protein therapeutic, demonstrating the potential of the alkyl acrylic acids to enhance protein and peptide delivery for therapy or molecular vaccines.

The encrypted polymeric carriers were designed to release covalently grafted oligonucleotides, peptides, and proteins when the pH-degradable linkages were hydrolyzed in the endosome. The encrypted polymers showed very low background hydrolysis rates at physiological pH, but were rapidly hydrolyzed at lower pH values typical of the endosome. The Acetal-PEG copolymer was shown to enhance cytoplasmic delivery of a PEG-FITC conjugate and an oligonucleotide conjuage to hepatocytes. Lysine grafted encrypted polymers efficiently complexed AS-ODNs as determined in a gel shift assay. The encrypted polymers were then complexed with AS-ODNs at various charge ratios and their ability to inhibit iNOS synthesis in the RAW cells was measured. As a control, the encrypted polymers were complexed with a scrambled ODN and incubated under the same conditions. The encrypted polymer was able to significantly enhance the delivery of AS-ODNs at a 1/1 +/- charge ratio into macrophages. At this charge ratio, the Polymer E2 + AS-ODN complex causes approximately 80% inhibition of iNOS, whereas the iNOS AS-ODN causes only 25% inhibition by itself.

IV. Conclusion

The striking enhancement of transfection efficiency with cationic lipid/DNA/PPAA mixtures, along with antibody conjugates, suggests that PPAA may provide significant improvements for the *in vivo* intracellular delivery of drugs such as DNA, oligonucleotides, proteins and peptides. The Encrypted Polymers efficiently deliver antisense oligonucleotides into the cytoplasm of hepatocytes and macrophages, as well as peptides into macrophages. No toxicity was observed and the delivery systems were effective in serum. These new families of polymers could therefore find numerous applications in the development of delivery systems for protein and DNA therapeutics and vaccines [3].

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