157a Functionalizable Polymer Precursors for Non-Viral Gene Delivery Vectors

Sharon Y. Wong and David Putnam

Advances in genetic research and molecular biology continue to improve the clinical potential of gene therapy. However, researchers in the field are hindered by a mélange of obstacles that prevents the potential from becoming a reality. Such difficulties include *in vivo* cell targeting specificity, gene vector immunogenicity, gene expression regulation, and gene delivery inefficiency. Furthermore, the mechanisms underlying transfection are poorly understood, making rational design of efficient gene delivery vectors a challenge. Early research in gene therapy employed modified viral vectors to deliver therapeutic genes. Their high efficiency and evolutionary capabilities make viral vectors attractive candidates for therapeutic gene delivery applications. However, in some instances, they can elicit immunogenic responses or activate oncogenes following chromosomal insertion. Thus, the strategy we employ to develop safe and efficient gene delivery vehicles involves iteratively constructing libraries of non-viral biomaterials that are evaluated via cell-based cytotoxicity and cell transfection efficiency bioassays. Constructing *libraries* of non-viral gene vectors, as opposed to linearly generating different compounds, in an iterative fashion affords us the ability to synthetically evolve and evaluate a large number of compounds concurrently, thereby mimicking nature's evolutionary process in a short amount of time. The libraries comprise of polymethacrylate-based polymers constructed of various combinations of cations, carbohydrates, and hydrophobic residues. We have chosen to use polymethacrylates, namely poly(N-methacryloxysuccinimide), to serve as the precursor to our library components, because they can be 1) functionalized with a variety of ligands through reaction with their active esters; and 2) synthesized to have narrowly distributed molecular weights (M_W). These two characteristics enable us to synthesize libraries of distinct and well-characterized polymers, thus allowing a correlation between polymer structure and DNA delivery efficiency to be made. Furthermore, with the aid of automated fluidics, we can feasibly synthesize libraries of functionalized polymers with low, medium, and high M_W with all permutations of the desired ligands attached. Finally, we quantify and assess the clinical efficacy of each compound in the library by performing biologically-relevant cell-based assays, such as cytotoxicity and cell transfection efficiency experiments. The information gleaned from these assays enables us to 1) determine the structure-function significance, if any, of polydispersity by carrying out identical processing and testing protocols to a library of polymers with wider polydispersities (e.g., synthesized from free radical polymerization); and 2) establish structure-function relationships and hypothesize cellular mechanisms of gene delivery in order to iteratively synthesize more efficient libraries in an effort to continue our search for safe and efficient gene delivery vehicles.