Polymer Technology for Gene Therapy

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Overview of human gene therapy.

Gene therapy can be defined as the treatment of human disease by the transfer of genetic material into specific cells to elicit a desired therapeutic phenotype. It is not difficult to envision treatment of monogenic diseases such as hemophilia, muscular dystrophy or cystic fibrosis through replacement of errant genes within the affected cells. Gene therapies are also being developed, however, for cardiovascular, neurological and infectious diseases, wound healing and cancer, by delivering genes to augment naturally occurring proteins, to alter the expression of existing genes, or to produce cytotoxic proteins or prodrug-activating enzymes.

Because of its broad potential, gene therapy has been heavily investigated during the past 30 years. The first clinical trial of gene therapy, for the treatment of severe combined immunodeficiency (SCID), was initiated in 1990 [1]. It was not until April 2000, however, that Cavazzana-Calvo *et al.* reported the first clinical success, treating two infants with γc-SCID [2]. Also that year, Kay *et al.* reported positive data, including increased circulating levels of factor IX, in a hemophilia clinical trial [3] and Khuri *et al.* reported a successful Phase II trial using a combination of gene therapy and traditional chemotherapy to treat recurrent squamous cell carcinoma of the head and neck [4]. Despite more than 1300 clinical trials, no products have been FDA-approved.

At the same time, tragic setbacks including the deaths of patients in two trials have hindered progress. In particular, a severe inflammatory response caused by the adenovirus used in a 1999 trial for treatment of ornithine transcarbamylase deficiency was proved to be the cause of death, which resulted in a temporary halt to all gene therapy trials. Furthermore, at least two of the 11 children in the Cavazzana-Calvo γc-SCID trial developed leukemia due to retroviral insertion of the therapeutic sequence in or near a gene associated with childhood leukemias. A key limitation to development of human gene therapy remains the need for safe and efficient methods for gene delivery [5].

Current gene delivery methods comprise recombinant viruses, used in the majority of clinical trials, and synthetic materials, including peptides, polymers and liposomes. Though viruses are the most efficient vectors, they often initiate immune responses, are limited in the size of genetic material that can be carried, are difficult to produce and purify, and exhibit limited target-cell specificity (or often non-specificity). Cationic polymers [6-8] have the potential to be non-toxic and non-immunogenic, are chemically and physically stable, are relatively easy to produce in large quantities, and can be targeted to desired cell types, but in general lack the efficiency needed for clinical application. Even the most efficient polymers are orders of magnitude less efficient than viruses (micrograms of DNA are required to achieve transgene expression comparable to that resulting from a virus suspension containing ~10 picograms of genetic material).

The gene delivery problem.

To escort genes from a solution (e.g., in a vial) to the cell nucleus, gene delivery vectors must navigate a series of obstacles, both extracellular and intracellular. Viruses have evolved

functions to address each challenge. In contrast, synthetic vectors are generally unsatisfactory because they lack one or several of the necessary functions. Consideration of these barriers is important for the rational design of new materials.

Gene delivery vectors face a first set of barriers in transporting genes from the test tube to the membrane of a target cell. First, the vector must bind and condense plasmid DNA to a sufficiently small size to allow efficient cellular internalization and protect the genes from nuclease degradation. Polycations and DNA spontaneously form tight complexes (polyplexes) through entropically driven electrostatic interactions. The resulting particles typically comprise several DNA molecules and hundreds of polymer chains and range from a few tens to several hundred nanometers in diameter. Secondly, the polyplexes should form a stable solution under physiological conditions often achieved by coating with hydrophilic polymers such as polyethylene glycol. Third, for many indications it is critical that the vectors recognize specific cells by displaying cell-specific ligands (e.g., small molecules, peptides, proteins, and antibodies).

Following internalization, gene delivery vehicles are challenged with a new set of intracellular obstacles (Figure 1), and the vector must provide functionality to overcome each one. Polyplexes are generally internalized by endocytosis, and once in the endocytic pathway, polyplexes are routed through a series of vesicles. The typical endpoint of this trafficking is the lysosome, an acidic vesicle filled with degradative enzymes including nucleases. It is critical, therefore, that DNA and vector escape these compartments into the cytoplasm. Next, the vector must escort the DNA through the cytosol toward the nucleus. Particles as large as typical polyplexes cannot passively diffuse in the cytosol and, thus, require a means of active transport. The genes must ultimately enter the nucleus, with or without the vector material. Although the

nuclear envelope contains pores for transport of biomolecules into and out of the nucleus, this process is very tightly regulated so that non-desirable species, including exogenous genes, are excluded. Finally, DNA and the vector must separate from one another in order to allow transcription of the therapeutic gene. The location at which such unpackaging occurs, however, is not generally known. Although >95% of cells in culture may internalize vectors (on the order of 100,000 copies per cell) typically <50% express the transgene, suggesting that the majority are lost at one these steps.

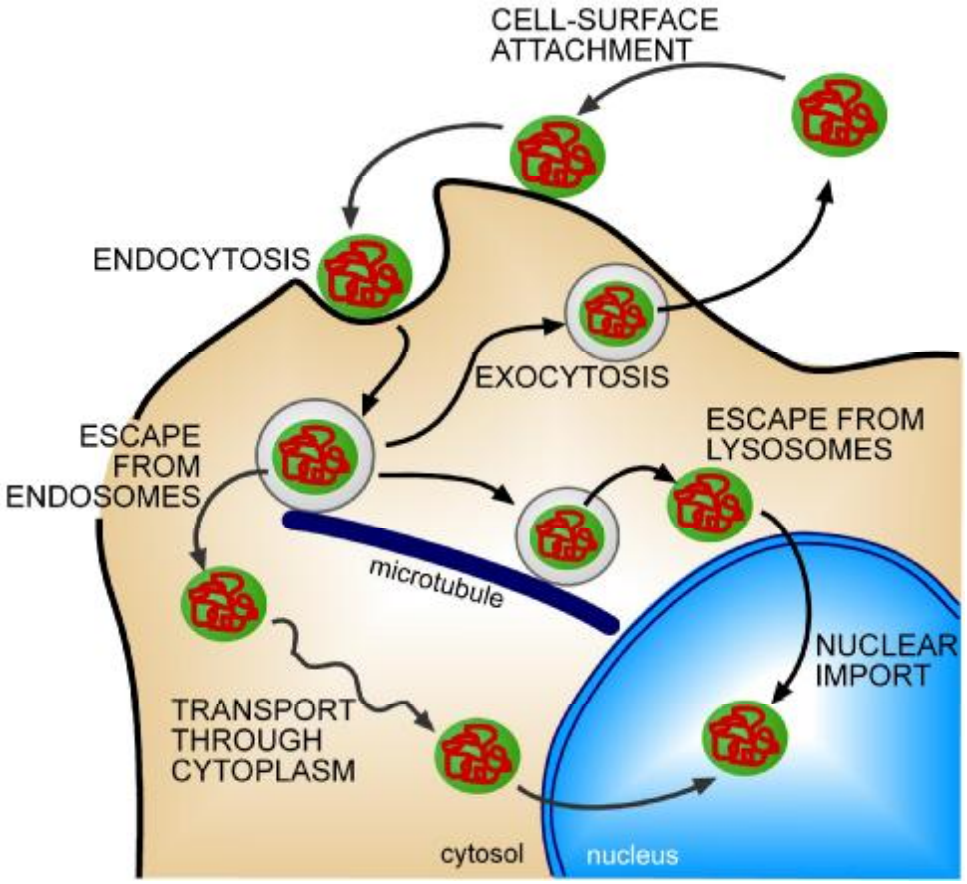

Figure 1. Schematic illustrating the important steps in intracellular processing of polymer-DNA gene delivery vectors.

Progress in design of gene delivery materials.

Many early gene delivery studies employed commercially available polymers (Figure 2). Polylysine was one of the first cationic polymers used in the modern era of gene delivery research [9, 10]. Although early studies were promising, it appears unlikely that polylysine-based polyplexes will be clinically useful due to low efficiency. Polyethylenimine (PEI), on the other hand, is one of the most effective gene delivery polymers [11]. Its effectiveness is believed to be due in large part to efficient escape from the endocytic pathway via the "proton-sponge" mechanism. Because nitrogen represents every third atom of the PEI backbone, the polymer exhibits a very high density of amines, only 15-20% of which are protonated at physiological pH. As endocytic vesicles are acidified, polyplexes containing PEI (or other proton-sponge materials) are able to buffer the vesicle lumen, leading to influx of counter ions, osmotic swelling, and vesicle rupture. PEI-mediated gene delivery has been hindered, however, by the polymer's relatively high cytotoxicity in many cell lines in culture and *in vivo*.

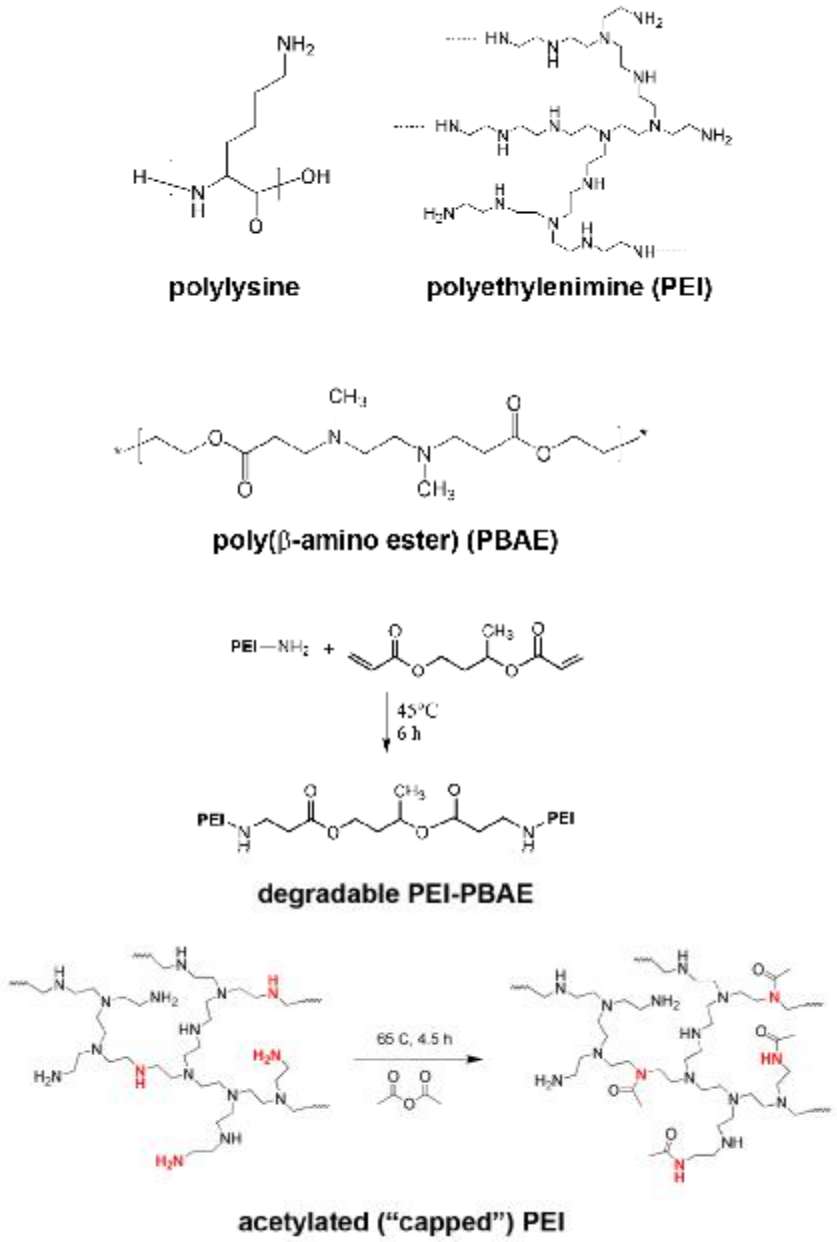

Figure 2. Structures of representative gene delivery polymers.

Many new types of polymers have been synthesized specifically as gene delivery vectors in the past two decades. Because of poor understanding of polymer-mediated intracellular trafficking, however, many of these designs are based on unproven hypotheses. Results have been mixed, with few of these materials providing highly efficient gene delivery. A current focus in the field, therefore, is developing new understanding of intracellular processing and polymer

structure-activity relationships. Because of space limitations, only a small selection of relevant studies will be described here.

One important approach has been to focus on synthesis of biocompatible, non-toxic gene delivery agents including materials such as poly[□-(4-aminobutyl)-L-glycolic acid] (PAGA), a biodegradable mimic of polylysine [12], polyurethanes, disulfide-linked polymers, or poly(□-amino esters) (PBAEs) (Figure 2). As one example of the latter, Forrest *et al*. cross-linked low molecular weight PEI – which is non-toxic, but ineffective for gene delivery – with small diacrylates [13, 14]. The resulting materials exhibited initial molecular weights sufficient to tightly bind and condense DNA, but degraded within 8-24 h to non-toxic by-products. These degradable PEI derivatives were up to 16-fold more efficient than the analogous non-degradable commercial PEI of comparable molecular weight.

PBAEs also have been exploited in combinatorial syntheses in which a panel of diacrylates and amines are cross-linked to generate more than 2000 unique polymers [14]. These materials have been screened for gene delivery activity and other important properties, including toxicity. The best polymers were more efficient than the top commercial transfection reagents and, in some situations, are comparable to adenoviruses. Perhaps most importantly, by correlating gene delivery activity with polymer/polyplex properties, the investigators may begin to extract structure-activity relationships that will guide future polymer designs.

Because PEI is an off-the-shelf material, one may also expect that its buffering capacity is not optimal. In fact, Forrest *et al.* modified the protonation profile of PEI by reaction with acetic anhydride to convert the primary and secondary amines to secondary and tertiary amides, respectively (Figure 2) [15]. Such a change should make a poorer proton sponge by decreasing the number of protonable nitrogens in the polymer. Surprisingly, gene delivery activity

dramatically increased upon acetylation, and the polymer with acetylation on ~57% of the primary amines was as much as 60-fold more efficient than unmodified PEI [16]. Subsequent investigation of the mechanisms leading to this unexpected enhancement revealed that PEI acetylation also decreases polymer-DNA binding strength, resulting in enhanced "unpackaging" of polyplexes within target cells. This report was significant in identifying polymer-DNA as a critically important design criterion for gene delivery materials.

Conclusions

A variety of polymers has been employed in gene delivery studies, but their effectiveness as gene therapy vectors remains orders of magnitude poorer than viral vectors. As a result, polymers are generally considered unacceptable for clinical applications. The important extra- and intracellular barriers to efficient gene delivery are known. The lack of efficiency of polymer gene delivery vectors, nevertheless, is due to a lack of functionality for overcoming at least one of these barriers. Based on the large number of studies of off-the-shelf gene delivery polymers, much has been learned about the structure-function relationships of polymer vectors. This knowledge has been applied to design and synthesis of new polymers, tailor-made for gene delivery, and a number of promising candidates have been reported in recent years. With a growing understanding of polymer gene delivery mechanisms, it is likely that polymer-based gene delivery systems will become an important tool for human gene therapy.

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