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Review

# Polymer coatings for delivery of nucleic acid therapeutics

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ABSTRACT

Gene delivery remains the greatest challenge in applying nucleic acid therapeutic for a broad range of diseases. Combining stability during the delivery phase with activation and transgene expression following arrival at the target site requires sophisticated vectors that can discriminate between cell types and respond to target-associated conditions to trigger expression. Efficient intravenous delivery is the greatest single hurdle, with synthetic vectors frequently found to be unstable in the harsh conditions of the bloodstream, and viral vectors often recognized avidly by both the innate and the adaptive immune system. Both types of vectors benefit from coating with hydrophilic polymers. Self-assembling polyelectrolyte non-viral vectors can achieve both steric and lateral stabilization following surface coating, endowing them with much improved systemic circulation properties and better access to disseminated targets; similarly viral vectors can be 'stealthed' and their physical properties modulated by surface coating. Both types of vectors may also have their tropism changed following chemical linkage of novel ligands to the polymer coating. These families of vectors go some way towards realizing the goal of efficient systemic delivery of genes and should find a range of important uses in bringing this still-emerging field to fruition.



**Contents**



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## 1. The challenges of gene delivery

Encoding therapeutic proteins within genes provides an exceptionally powerful pharmaceutical approach, allowing production of hundreds or thousands of copies of the therapeutic agent at sites where the gene is transcribed and expressed [1,2]. The approach also allows tailored control of protein expression using promoter sequences that are only active in certain disease-related environments [3]. Together, gene therapies allow great potency to be combined with highly selective activity, promising important new treatments for a broad range of diseases.

Unfortunately the potential of gene therapy approaches is currently restricted by poor delivery, with successful application being limited to settings where delivery presents no obstacle. These include the use of isolated autologous haematopoietic stem cells, transduced with viruses ex vivo before being reintroduced to the patient [4], or where gene vectors can be injected directly into targets sites, such as the retina [5]. However these approaches enable only a very limited number of applications, and the great potential of gene therapy remains untapped until a system is developed allowing efficient delivery and expression of therapeutic nucleic acids within target cells following intravenous injection.

Nucleic acid pharmaceuticals are generally particulate, whether based on viruses or self-assembling complexes, and when they are administered intravenously they are rapidly eliminated by components of the innate immune system. Probably the most versatile approach to addressing this problem is to provide the agent with a hydrophilic polymer coating, effectively stealthing the agent during its delivery phase and allowing it to reach target cells in order to express its nucleic acid payload [6,7]. This approach requires a close interdisciplinary collaboration between polymer chemists, biologists and virologists, and the key technical challenges and important developments are discussed in detail below.

Polyelectrolyte complexes (PECs) containing DNA, also called polyplexes, are often used as the basis of non-viral gene delivery systems. [8–10] PECs are usually prepared by mixing of aqueous solutions of two oppositely charged macromolecules — positively charged hydrophilic polymers (polycations) and negatively charged polynucleotides that self-assemble into compact nano-sized particles (Fig. 1). Synthetic homopolymers based on poly(ethyleneimine) (PEI) or poly(L-lysine), statistical copolymers of amino or ammonio groups containing acrylates or methacrylates or radially branched poly(amidoamine) (PAMAM) dendrimers are the most frequently used polycations. A–B type diblock copolymers formed by polycation A block and B block of hydrophilic polymer (most frequently PEG) hold a specific position among the synthetic polycations (see Section 3). The condensed particles are formed primarily due to the attractive coulombic forces acting between ionizable phosphate groups of polynucleotides and amino or ammonio groups on the cationic polymer, with hydrogen bonds, hydrophobic, van der Waals and other interactions also playing significant roles in complex self-assembly. [11,12] PEC formation is a very fast process accompanied by changes in thermodynamic parameters, where the decrease in overall entropy (sum of entropy changes caused by the mutual fixation of the polymer chains, by the release of low-molecular weight electrolyte during the PEC formation and by the hydrophobic or other interactions in formed complex) is the driving force of the whole process. [11,13,14] The physicochemical properties and the morphology of the PECs can be influenced by many factors like cationic polymer architecture and nucleic acid form, degree of protonization (pH), distance between charged groups along the cationic polymer chain as well as ionic strength of the environment and overall charge ratio between positively and negatively charged polyelectrolytes. Most frequently, DNA is condensed with a high charge excess of cationic polymer in aqueous buffer solutions forming physiologically stable particles of submicron size with positive ζ-potential [15].

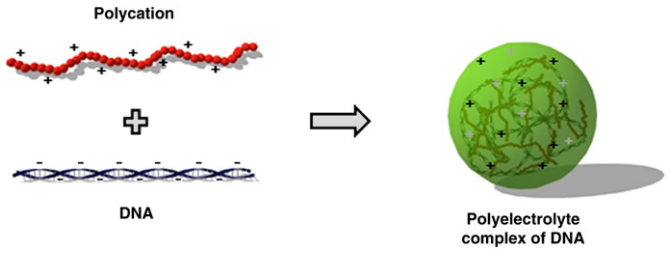


**Fig. 1.** Schematic diagram of the polyelectrolyte complexes of DNA prepared by mixing oppositely charged polyelectrolytes (usually with a moderate excess of positive charges on the cationic polymer to negative charges on the DNA) under physiological conditions. The (+) signs on the polycation chain represent protonated amino or ammonio groups, the (−) signs on the DNA helix represent deprotonated phosphate groups.

On the one hand, the positive surface charge prevents particle aggregation because of electrostatic repulsion, but on the other hand it allows non-specific interactions with negatively charged groups of plasma proteins, vessel endothelium and blood cells following intravenous injection. Adsorption of blood components to the PECs surface leads to the formation of large aggregates, which are primarily accumulated in the lung. [16,17] It is due to the physical retention of large aggregates in very tiny capilars in pulmonary alveoli. Because of low aggregates stability, most of the complexes are again released to the bloodstream, where they are recognized by immune system and rapidly eliminated through reticuloendothelial system (RES) followed by accumulation in the liver, kidney and spleen. [18,19] This fact significantly limits the utility of simple polycation/DNA PECs, and steric stabilization of the complexes is crucial to protect them during any delivery phase involving exposure to biological fluids such as the blood.

Viral vectors are also widely used to deliver therapeutic genes, but they too have poor circulatory properties and show rapid binding to antibodies and other plasma proteins, coupled with clearance into the liver and spleen [20–22]. Encapsidated viruses (such as adenovirus and adeno-associated virus) are relatively stable nanoparticulate structures. Accordingly, while they do not spontaneously disintegrate within the bloodstream, they require protection from components of the immune system and 'stealthing' during the delivery phase.

Hydrophilic polymer coatings provide an interesting way to improve the properties of both viral and non-viral vector systems, and this review will describe some of the chemistry being explored and its application in enabling systemic delivery of therapeutic genes.

## 2. Synthesis of reactive polymers

### 2.1. Semitelechelic reactive polymers

The term telechelic polymer is used to describe linear macromolecules with identical functional groups capping both ends. These polymers are characterized by the chemical composition of the main polymer chain, by the type and the content of functional end-groups (polymer functionality). Telechelic polymers containing two different end-groups