# Gene Delivery Using Polymeric Vectors: Review Article

**Anshika Kushwaha**
Assistant Professor, Department of Food and Biotechnology, Jayoti Vidyapeeth Women's University, Jaipur, India.

Received: July 21, 2018 Accepted: August 31, 2018

**ABSTRACT** *Peptides, proteins, nucleic acids and their analogs with high therapeutic value have shown great potential as biopharmaceuticals to treat various diseases such as cancer, infectious diseases, genetic disorders etc. However, successful delivery of such biomolecules is a major challenge as their molecular properties lead to poor physical and chemical stability under in vivo conditions with limited cellular uptake. Through the past researches, a wide range of lead molecules have been identified which have shown high binding affinity and great potential in In-vitro non-cellular assays via direct interaction with the molecular targets, however, physicochemical properties, viz., size, poor aqueous solubility, hydrophobic nature and negative charge, of several of these compounds have limited their permeability across cell membrane. Charged polymeric materials possess significant potential and are extensively investigated for various therapeutic applications. Compared to anionic materials, cationic materials are more extensively explored. These form electrostatic complexes with anionic biomolecules (nucleic acids and proteins) and also exhibit bioactive properties such as antimicrobial, antioxidant and antitumor making cationic materials more promising as therapeutics.*

***Keywords:*** *Gene Delivery, Polymeric Vector, Biopharmaceuticals, Antitumor, Anionic materials, Cationic materials*

## Introduction

The gene therapy, involves the treatment of genetically caused diseases by transferring exogenous nucleic acid into specific cells of patients or by delivering therapeutic genes to diseased cells. Gene therapy has attached great interests over the past few decades. It has been gradually realized that the development of safe, efficient and comfortable gene-delivery vectors has become a bottleneck in clinical applications (Jin *et al*., 2014).

Naked DNA molecules do not enter cells efficiently because of its negative charge, large size and hydrophilic nature. In addition, these molecules are very susceptible to nuclease-mediated degradation. Therefore, the primary challenge for gene therapy is to develop carriers (commonly called vectors) that can facilitate gene transfer to targeted cells without degradation of the delivered genes. The gene transfection vectors can be generally divided as viral and non-viral ones.

Initial research concentrated on using viral vectors, including retroviruses, adeno-associated viruses, adenoviruses etc exhibited high efficiencies for delivery and expression but associated immunogenicity, pathogenicity and difficulties in large scale production have limited their use for the proposed application. Recently, non-viral delivery system have attracted the attention of researchers and are being considered to be more favorable in therapy because of their reduced safety concerns, ability to deliver high-molecular weight DNA, and ease of preparation, purification and modification (Midoux *et al*., 2009). For these reasons, polymeric materials have been explored as potential gene delivery vectors, a viable alternative to viral gene vectors.

Methods of non-viral gene delivery have been explored using physical (carrier-free gene delivery) and chemical approach (Natural or Synthetic vectors based-gene delivery)

Physical approaches, including needle injection, electroporation, gene gun, ultrasound mediated gene delivery etc. employ a physical force that permeates the cell membrane and facilitate intracellular gene transfer.

The chemical approaches including cationic lipid, dendrimers (PAMAM, PPI), peptides and cationic polymers (Poly L-Lysine, Polyethylenimine, chitosan) use synthetic or naturally occurring compounds as carriers to deliver the transgene into cells. PEI is available in two forms: linear and branched. Although, linear PEI is preferred for *in vivo* applications because of its advantageous toxicity profile. Branched PEI (bPEI) contains a higher percentage of primary amines and is thus susceptible to modification. bPEI contains $1^0$ , $2^0$ and $3^0$ amines in the ratio of 1:2:1 with pKa values spanning around physiological pH, providing remarkable buffering capacity. The $1^0$ amines are responsible for high degree of DNA binding while $2^0$ and $3^0$ amino groups provide good buffering capacity to the system (Gioia *et al*., 2008). Among the

various commonly used cationic polymers, branched polyethylenenimine has shown to be most efficient gene carrier (gold standard) *in vitro* because of its inherent proton sponge property (Midoux *et al*., 2009) namely, partially proponated PEI absorbs more protons inside endocytic vesicles embedded with some ATPase proton pumps, accompanied by an influx of chloride counter-ions, ultimately rupturing endocytic vesicles due to higher osmotic pressure (Barar *et al*., 2013).

In this study we propose to idea about modified cationic polymer for in-vitro transfection studies. and evaluation for their in vitro transfection efficiency and cell viability.

**What is Gene Delivery?**

Over the past two decades, tremendous progress has been made in the area of biomedical sciences with the advent of an increasing number of biopharmaceuticals such as novel peptide and protein drugs as well as nucleic acid analog based drugs (Jin *et al*., 2014). Recently, drug delivery systems have become a subject of major interest in the pharmaceutical industry for the treatment of different diseases, viz., cancers, viral infections, infectious and genetic disorders (Fan *et al*., 2013). A wide range of candidate molecules have shown high binding affinity and great potential in in vitro non-cellular assays through direct interaction with molecular targets. However, successful delivery of these biomolecules poses a major challenge as their molecular properties lead to poor physical and chemical stability under in vivo conditions and limited their delivery across the cell membrane. The physicochemical properties of several compounds like size, poor water solubility, hydrophobic nature, and negative charge, limit their cellular uptake significantly (Liu *et al*., 2014). To overcome the main barriers for successful delivery of these molecules to the desired site, many approaches have been reported in the literature (Gioia et al. , 2008). However, an ideal approach to deliver a molecule of choice to a specific target is still elusive, thus there is a scope for researchers to design new delivery strategies and synthesize materials to enable these bioactive drugs to be delivered intact to the target cell.

**Various Vectors involved in Gene Delivery**

Various kind of materials are being used for nDDS development (Figure 1), which include lipids, polymers (biodegradable or non-biodegradable), antibodies, metals, magnetic substances, carbon, ceramics and viral capsids (Sun *et al*., 2012).

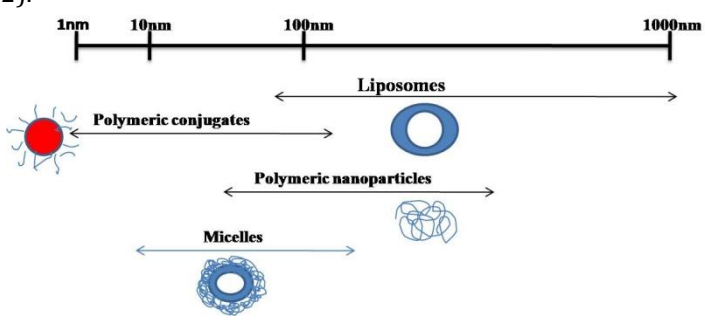


**Figure 1. Nanoengineered drug delivery systems with their sizes.**

**Different Vector Systems for Gene Delivery**

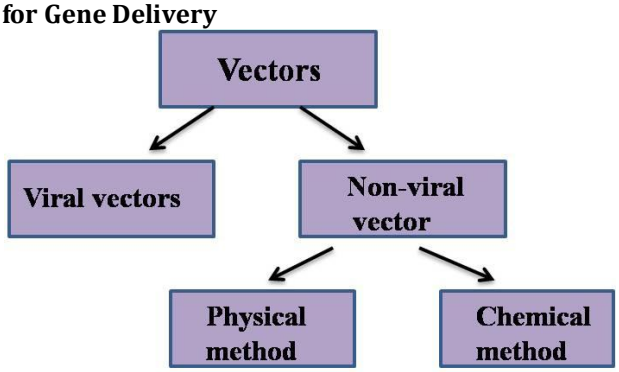


**Figure 2. Types of Gene Delivery Vectors**

**1. Viral vectors**

Viruses are considered to be one of the simpler forms of life. It represents highly evolved natural vectors for the transfer of foreign genetic information into the host cells. This characteristic property of viruses has led to extensive use of engineered recombinant viral vectors for the delivery of therapeutic genes into the diseased tissues. These genetic manipulations in viral genome are needed to prevent their replication,

inflammation, cytotoxicity and immunogenicity into the host cells (Shang *et al*., 2014). These viral vectors include both DNA and RNA viruses such as retrovirus, adenovirus (type 2 and 5), adeno-associated virus, herpes virus, pox virus, human foamy virus (HFV), and lentivirus (Kurosaki *et al*., 2009). These viral vectors can deliver gene efficiently into a broad range of cell types and are widely used in both basic research and therapeutic applications.

**2. Non-viral vectors**

2.1. Physical methods

The simplest and safest route to administer therapeutic DNA by physical/mechanical method has attracted attention as it does not require any vector (Figure 3). It usually employs a physical force to overcome the cellular membrane barrier and facilitates gene transfer inside the cell. By this method, fragment of DNA or plasmid containing transgene is directly delivered into cells without use of any vector that could be cytotoxic or immunogenic as commonly seen in viral vectors. Physical methods include gene delivery by needle injection (Bromberg *et al*., 1998), ballistic injection, electroporation, sonoporation, photoporation, hydroporation. These methods of gene transfer may find their place in specific clinical application as they overcome some of the issues linked to viral or biochemical approaches.

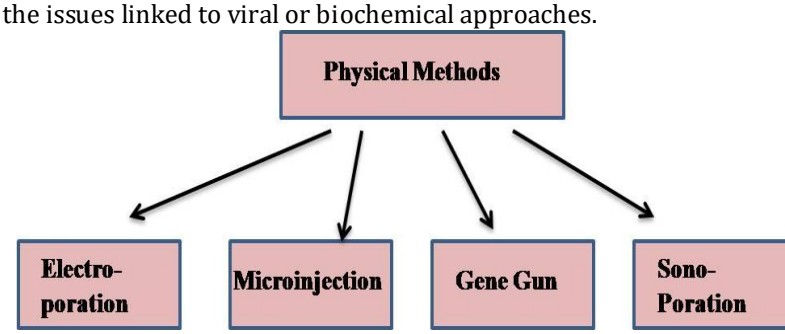


**Figure 3. Primary physical methods for gene delivery**

**2.2. Chemical methods**

These methods employ uptake-enhancing chemicals for efficient delivery of DNA. These methods are based on complex formation between positively charged chemicals (usually lipids/polymers) and negatively charged DNA molecules. These complexes lead to condensation of the genetic material into particles of a few tens to several hundred nanometres in diameter, which help in providing protection to genes and mediate cellular delivery. Such complexes of plasmid DNA with cationic lipids and polymers are known as LIPOPLEXES and POLYPLEXES, respectively. Since 1960s, N-(diethylamino)-ethyl-dextran (DEAE) (Rader *et al*., 2008) and calcium phosphate (Johnson et al., 1966) have been extensively used for in vitro gene delivery.

**Polymeric Carriers: New Emerging Vector for Efficient Gene Delivery**

Cationic polymers have shown promise as a safe, predictable, biodegradable and nontoxic alternative to viral gene therapy, relying on endocytosis of synthetic polymer-based carriers bio-conjugated to the targeted gene or other biological molecules (Schwendener *et al*., 2014). Cationic polymer-based gene carriers (polyplexes) showed good biodegradability, low toxicity, triggered nucleic acid release, structural diversity and relatively higher transfection efficiency than liposomes (Allen *et al*., 2004). Many kinds of polymers have, therefore, been investigated for gene delivery, such as chitosan, PEI, polylysine, polyamino ester and so on (Andreani *et al*., 2014). Cationic segments, organelle-escape units, and degradable fragments are essential to a polymer-based vehicle for gene delivery. The majority of these cationic segments are derived from polyamines, including polylysine, polyarginine, chitosan, polyethylenimine and polyamidoaminedendrimers.

**Table 1. Summary of commonly used polymeric carriers**

| | | |
|---|---|---|
| Chitosan | Good biocompatibility and biodegradability; low immunogenicity; low toxicity; antimicrobial activity; | Low insolubility under physiological pH condition; low transfection efficiency; |
| PEI | Strong DNA condensation capacity; intrinsic endosomal activity; unique buffering capacity; high transfection efficiency; | Bad biodegradability; the contradiction between transfection efficiency and cytotoxicity; |
| PAMAM | surface functionality; molecular weight dependent | Low transfection efficiency; |

| | | |
|---|---|---|
| | transfection efficiency and cytotoxicity; uniform size distribution | |
| PLGA | Safety; good biodegradability; | Low release rate and low encapsulation efficiency of pDNA; acidic microenvironment induced by it; |

Some recent studies have shown improved gene delivery efficiency of hydrophobically-modified chitosan, poly(amidoamine) dendrimer (Blanquer *et al*., 2014), poly (L-lysine) and PEI by enhancing DNA condensation through cooperative binding as well as promoting interactions with the lipophilic cell membranes and endocytosis, facilitating DNA release for transgene expression and alleviating serum inhibition. However, such modifications are yet to get understood completely and efforts are afoot to establish the role of hydrophobicity of cationic polymers in gene delivery.

**Polyethylenimine (PEI)**

In the arena of non-viral vectors, PEI (branched PEI, 25 kDa) is considered as gold standard for its gene transfection capability. In 1995, Behr et al. reported the first successful polyethylenimine-mediated gene transfection (Andreani *et al*., 2014). Since then, PEI has been derivatized to improve the physicochemical and biological properties of polyplexes. Polyethylenimine exists in both branched and linear structures. Branched PEI is synthesized by acid-catalysed polymerization of aziridine (Schwendener *et al*., 2014), while linear PEI is synthesized via ring opening polymerization of 2-ethyl-2oxazoline followed by hydrolysis (Allen *et al*., 2004). Various transfection agents based on PEIs have been made commercially available, viz., ExGen500 and jetPEI (Andreani *et al*., 2014). PEI nanoparticles have been prepared by two strategies that either by complexation of PEI with DNA to form nano-complexes (Thukral *et al*., 2014) or use of cross-linkers to first form PEI nanoparticles followed by DNA loading onto it.

**Conclusion**

At present, Synthetic compounds such as peptides, lipids, dendrimers and cationic polymers are being exploited widely for their use in generating non-viral delivery vehicles. Among the various chemically developed vectors, cationic polymers are the most extensively used transfection reagents for in vitro and in vivo application.

Through the present study, it can be concluded that polymeric vectors are new emerging way to transfer the gene in gene delivery methods.

**REFERENCES**


1. L. Jin, X. Zeng, M. Liu, Y. Deng, N. He, Current Progress in Gene Delivery Technology Based on Chemical Methods and Nano-carriers, Theranostics 4 (2014) 240.
2. P. Midoux, C. Pichon, J.J. Yaouanc, P.A. Jaffrès, Chemical vectors for gene delivery: a current review on polymers, peptides and lipids containing histidine or imidazole as nucleic acids carriers, Br. J. Pharmacol. 15 (2009) 166.
3. S.D. Gioia, M. Conese, Polyethylenimine-mediated gene delivery to the lung and therapeutic applications, Drug Des. Devel. Ther. 2 (2008) 163.
4. J. Barar, Y. Omidi, Intrinsic bio-signature of gene delivery nanocarriers may impair gene therapy goals, BioImpacts 3 (2013) 105.
5. M. Ahmed, R. Narain, Cell line dependent uptake and transfection efficiencies of PEI-anionicglycopolymer systems, Biomaterials 34 (2013) 4368.
6. T. Kurosaki, M. Uematsu, K. Shimoda, K. Suzuma, M. Nakai, T. Nakamura, T. Kitahara, T. Kitaoka, H. Sasaki, Ocular gene delivery systems using ternary complexes of plasmid DNA, polyethylenimine, and anionic polymers, Biol. Pharm. Bull. 36 (2013) 96.
7. T. Kurosaki, T. Kitahara, S. Fumoto, K. Nishida, J. Nakamura, T. Niidome, Y. Kodama, H. Nakagawa, H. To, H. Sasaki, Ternary complexes of pDNA, polyethylenimine, and γ-polyglutamic acid for gene delivery systems, Biomaterials 30 (2009) 2846.
8. Y. Fan, J. Yao, R. Du, L. Hou, J. Zhou, Y. Lu, Q. Meng, Q. Zhang, Ternary complexes with core-shell bilayer for double level targeted gene delivery: In vitro and in vivo evaluation, Pharm. Res. 30 (2013) 1215.
9. Y. Liu, R. You, G. Liu, X. Li, W. Sheng, J. Yang, M. Li, Antheraeapernyi silk fibroin-coated PEI/DNA complexes for targeted gene delivery in HEK 293 and HCT 116 cells, Int. J. Mol. Sci. 15 (2014) 7049.
10. S.L. Sun, Y.L. Lo, H.Y. Chen, L.F. Wang, Hybrid polyethylenimine and polyacrylic acid-bound iron oxide as a magnetoplex for gene delivery, Langmuir 28 (2012) 3542.
11. L. Shang, K. Nienhaus, G.U. Nienhaus, Engineered nanoparticles interacting with cells: size matters, J. Nanobiotechnol. (2014) 12:5.

12. T. Kurosaki, T. Kitahara, S. Kawakami, K. Nishida, J. Nakamura, M. Teshima, H. Nakagawa, Y. Kodama, H. To, H. Sasaki, The development of a gene vector electrostatically assembled with a polysaccharide capsule, Biomaterials 30 (2009) 4427.
13. L. Bromberg, polyether-modified poly (acrylic acid) : Synthesis and properties, Ind. Eng. Chem. Res. 37 (1998) 4267.
14. R. A. Rader, Nat. Biotechnol., 2008, 26, 743–751.
15. H.M. Johnson, K. Brenner, H.E. Hall, The use of a water-soluble carbodiimide as a coupling reagent in the passive hemagglutination test, J. Immunol. 97 (1966) 791.
16. R. A. Schwendener, Ther. Adv. Vaccines, 2014, 2, 159–182.
17. T. M. Allen and P. R. Cullis, Science, 2004, 303, 1818–1822.
18. T. Andreani, A. M. Silva and E. B. Souto, Biotechnol. Appl. Biochem., 2014, 1322.