Chapter 7

Biopolymer in Gene Delivery

Sakthivel Lakshmana Prabu and
Kandasamy Ruckmani

Additional information is available at the end of the chapter

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Abstract

Nowadays, biopolymers, a class of biomaterials, represent frontier area in the drug delivery systems. Drug release from nano- and microparticles is a complex process, which involves several steps. Uptake of nanoparticle in the intracellular is affected by numerous factors. Recently, gene delivery has been considered one of the promising approaches for the treatment of various diseases acquired genetically in human being. The use of biopolymers as nanoparticles in gene delivery can potentially avoid many of the safety concerns in the gene delivery system. In gene delivery, the genetic materials such as DNA plasmids, RNA and siRNA are either encapsulated inside or conjugated to the nanoparticles, which protects the genetic materials until the drug reaches its target site. Treatment of the diseases is based on the effective delivery of the genetic materials into specific cells that are responsible for disease development. Various properties such as particle size, surface charge, morphology of the surface and release rate of the loaded molecules are the important parameters in the gene delivery system. In this chapter, various biopolymers (cationic polymers) and inorganic non-viral-delivery vectors used in gene delivery used as therapeutic agents are discussed.

Keywords: gene delivery, polymers, biopolymers, delivery system, therapeutic effect

1. Introduction

Polymers are the materials that are either prepared/produced synthetically or isolated from natural sources. Polymers can respond based on their environmental conditions such as pH, temperature, ionic strength, electric field, magnetic field, chemical and biological stimuli to deliver the desired therapeutic agents. Recently, biopolymer is a biomaterial used in various delivery systems to interact with the biological system and release the therapeutic agent. These biopolymers are utilized in the various applications due to their biocompatibility, biodegradability and low immunogenicity. Among the various biopolymers, synthetic polymers

have well-defined structure and fine-tunable degradation kinetic and mechanical properties compared to the natural polymers. Recently, biodegradable nanoparticles have a major role in the field of health sciences especially for treating various diseases through drugs, vaccines and genes [1–7]. Nanoparticle in gene-delivery system has been utilized for treating various diseases such as cancer and haemophilia. The major challenge in the gene delivery is delivering the genetic materials such as DNA, plasmids, RNA and siRNA into the target/special cells to replace the damaged genes or expression inhibition of undesired genes or expression and production of required proteins. In gene delivery, the genetic material is either encapsulated inside the nanoparticle or conjugated to the nanoparticle. The nature, source and their physico-chemical properties of the polymers play an important role in the formation of desired properties of nanoparticles and to achieve a better therapeutic effect [8–12].

2. Polymeric gene delivery vector

The important property in polymeric vector is that the polymer should be non-toxic (biocompatible), biodegradable (hence have less toxicity) and also help to release the DNA from the complex into the cytoplasm. In polymeric vector, the polymer must be condensate with the genetic material. Condensate between the cationic polymer and genetic materials can be done through electrostatic interactions. By modifying the surface of NP, NP-DNA complexes can be formed by electrostatic binding between the positive charges of the NPs and the negative charges of the DNA. Only when the medium is aqueous and hydrophilic, the polymeric vector will be mobile, because the vector needs hydrophobic and hydrophilic components and be stabilized in an aqueous solution by forming micelles [13].

2.1. Polymer properties in polymeric gene delivery

Polymers have permanent cationic charges on its surface and are not preferred due to its strong condensate property with DNA, which will not release DNA into the cell. Hence, ionizable cationic polymers with pK values between 5 and 7 are preferred in the polymeric vector delivery which is shown in **Figure 1**.

Other important factors to be considered for the polymer in the polymeric gene-delivery vector are its molecular weight, molecular structure and composition of the polymer. Increase in the polymer's molecular weight also increases its toxicity. Polymers of different molecular structures such as linear, branched, stars and dendrimers have an impact on the transfer genes into cells [14–18].

2.2. Preparation of polymeric gene vector

Polymeric vectors are prepared by mixing plasmid DNA with a cationic polymer. During condensation between plasmid DNA and polycation, plasmid DNA undergoes a conformational change from a hydrodynamic size of 200–300 nm to particles of less than 100 nm. Plasmid DNA has a highly organized chemical structure [19–22]. A condensation between plasmid DNA and polycation is shown in **Figure 2**.

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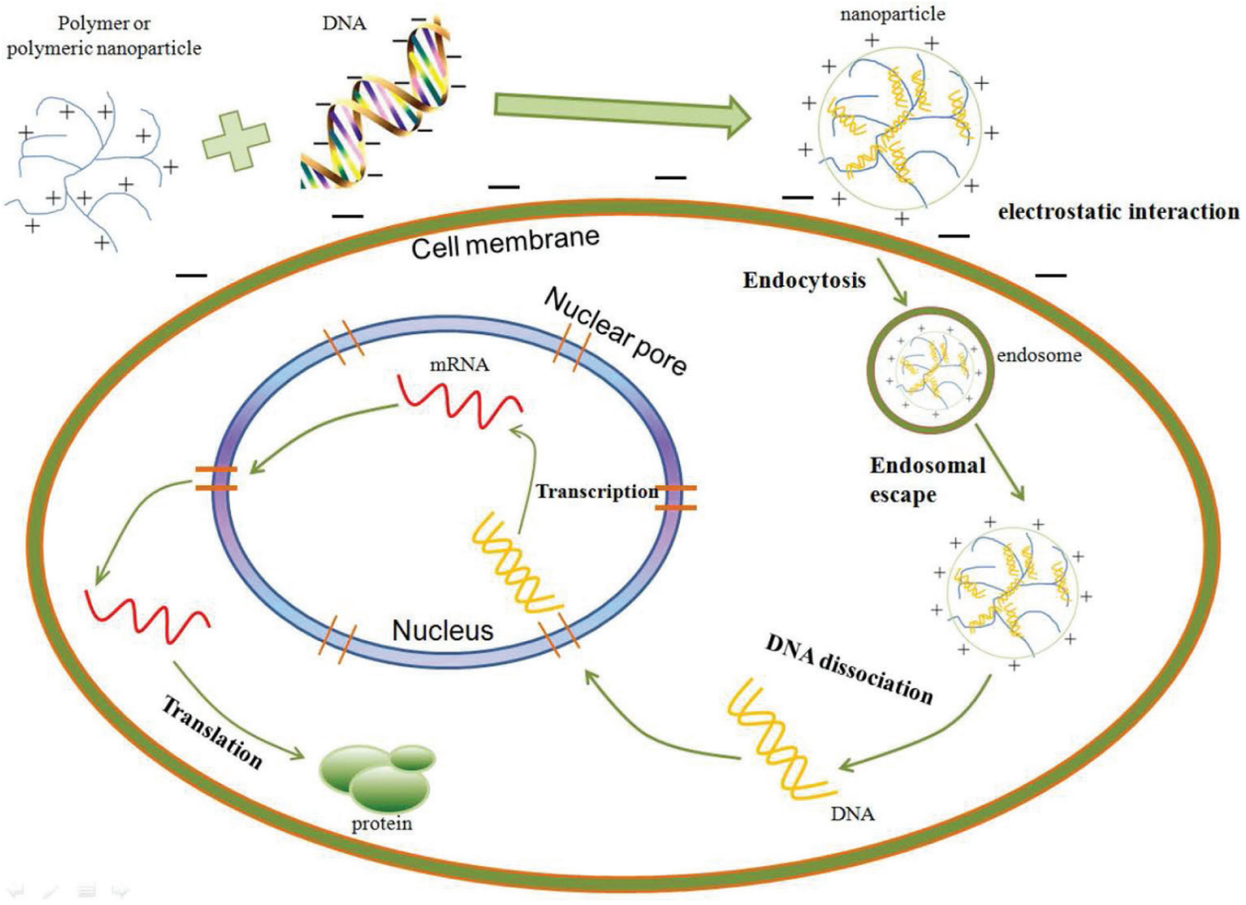

Figure 1. Gene delivery process of polymeric nanoparticle.

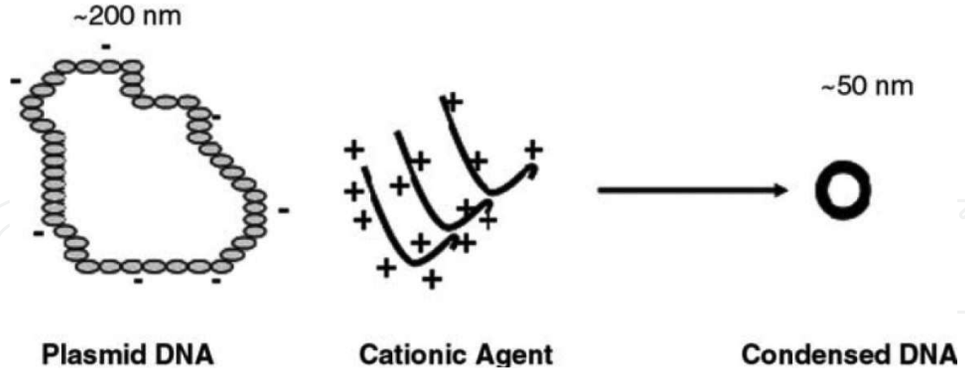

Figure 2. Condensation between plasmid DNA and polycation.

The order of mixing and vortex speed of mixing plays an important role in the size of the DNA nanoparticles. DNA can be condensate, either by evaporation under vacuum or by freeze drying. The freeze/thaw cycle can influence the particle size of DNA nanoparticles.

The charge ratio of DNA nanoparticles is the calculated ratio of amines on the polymer relative to the phosphates on DNA at a given stoichiometry of polymer to DNA. When a cationic

polymer binds to plasmid DNA, sodium ions are displaced and the electronegative charge is partially satisfied. DNA condensates are normally prepared at near-neutral pH in low ionic strength buffer [23, 24].

3. Dendrimers

Dendrimer is a monodisperse macromolecule with perfectly branched regular structure and having at least one branched junction at each repeat unit 3. These dendrimers are used in gene delivery. The dendrimer/DNA complexes are encapsulated in a water-soluble polymer, and then deposited on or sandwiched in functional polymer films with a fast degradation by dehydration to mediate gene transfection.

Biodegradable dendrimers are commonly prepared by inclusion of ester groups in the polymer backbone, which will be chemically hydrolysed and/or enzymatically cleaved by esterases in physiological solutions. These dendrimers are large molecular weights which accumulate and retain in higher amounts in the tumour tissues. Dendrimer fragments are eliminated safely through urine.

Dendrimers are prepared through either a divergent method or a convergent method.

In the **divergent methods**, as given in **Figure 3**, dendrimer grows from a multifunctional core molecule to outwards. The first-generation dendrimers are derived from the core molecule that reacts with monomer molecules containing one reactive and two dormant groups. This periphery molecule is then activated to react with more monomers. This step is subsequently repetitive to produce layer-by-layer dendrimers for several generations.

In the` convergent approach, stepwise dendrimer is constructed, starting from the end groups and progressing inwards. The growing branched polymeric arms are called dendrons, which can attach to a multifunctional core molecule (**Figure 4**).

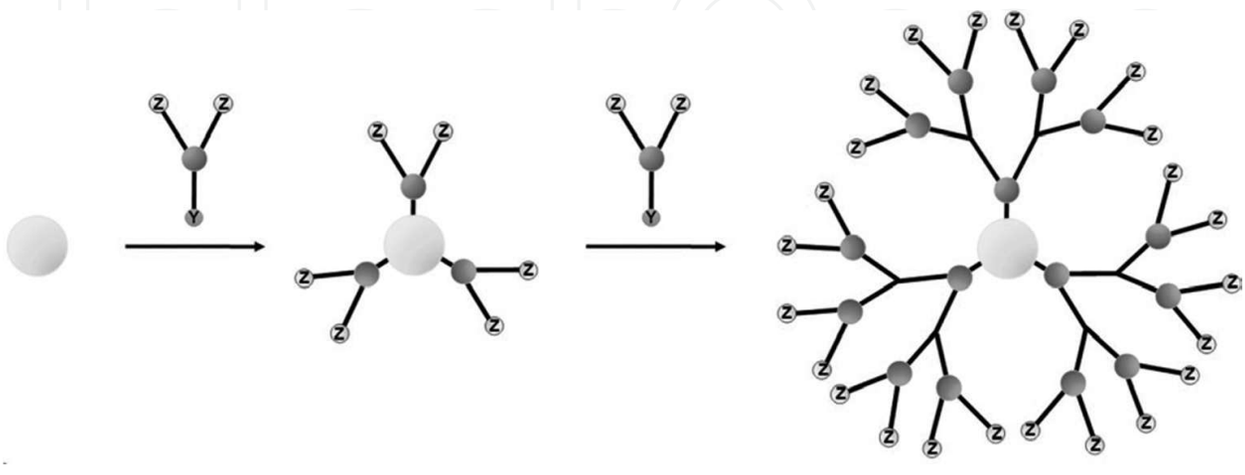

Figure 3. Formation of dendrimer by divergent methods.

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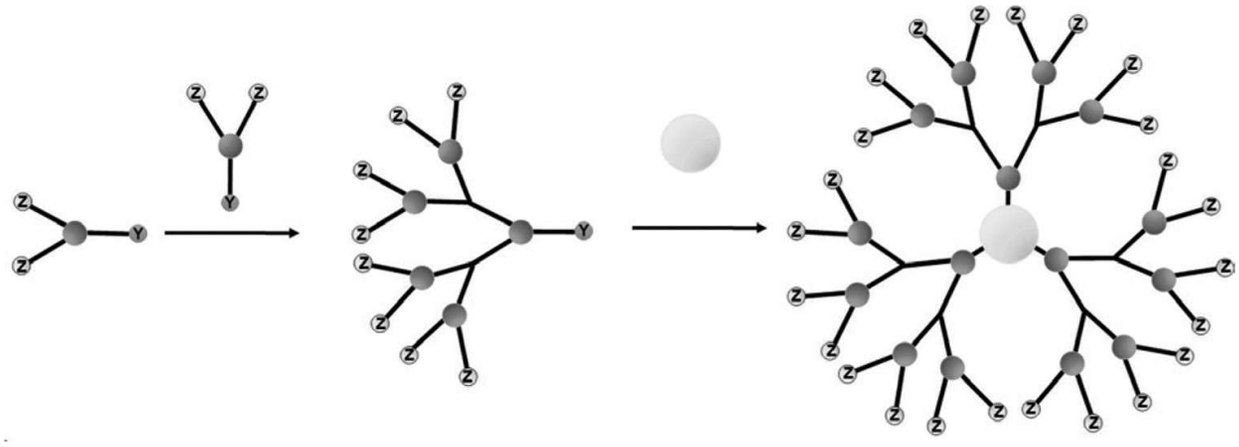

Figure 4. Formation of dendrimer by convergent method.

Whereas the structure Y in dendrimer is chemically active focal point and Z is the functional chemical group of another monomer.

3.1. Other types of dendrimers

3.1.1. Amino acid-based dendrimers

Amino acid-based dendrimers were developed to capitalize on the unique properties of the amino acid-building blocks, including chirality, hydrophilicity/hydrophobicity, biorecognition and optical properties. Optically active protein-mimetic dendrimers have been synthesized using various amino acids, such as tryptophan, phenylalanine, glutamic acid, aspartic acid, leucine, valine, glycine and alanine.

Amino acid-based dendrimers can be synthesized by

1. amino acid or peptide grafting and display on the surface of a conventional dendrimer

2. attachment of amino acids or peptides to an organic or a peptide core.

3.1.2. Glycodendrimers

Carbohydrate interactions with different receptors displayed at the cell surface control a number of normal (e.g., lymphocyte activation and cell-cell adhesion) and abnormal (e.g., cell-pathogen adhesion and cancer cell metastasis) biological processes. Glycodendrimers have been synthesized by coupling isothiocyanate-functionalized glycosyl and mannopyranoside ligands as well as an *N*-hydroxysuccinimide (NHS)-activated galactopyranosyl derivative to amine-terminated dendrimers.

3.1.3. Hydrophobic dendrimers

Dendrimers with hydrophobic interiors and a hydrophilic surface are called hydrophobic dendrimers. Hydrophobic dendrimer gives better encapsulation and efficient solubilization of hydrophobic drug molecules. Specifically, dendrimers with hydrophobic cores were proved

to effectively retain hydrophobic drug molecules in the voids of their branching architecture, mimicking amphiphilic polymer micelles.

3.1.4. Asymmetric dendrimers

Asymmetric dendrimers are synthesized by coupling dendrons of different generations to a linear core, which yields a branched dendrimer with a nonuniform orthogonal architecture.

There are two different types of dendrimeric copolymers:

1. **Segment-block dendrimers**—segmented with segments of different constitution.
2. **Layer-block dendrimers**—concentric spheres of differing chemistry [25–42].

4. Cationic polymers

DNA, when combined with sufficient amounts of cationic polymers, will condense into discrete entities which are called as polyplexes [43]. The polyplexes are compact nanoparticles formed through electrostatic interactions between the positive charges of amines and the negative charges of DNA phosphates. The strength of DNA binding to the polymers is related to the N:P ratio.

The most common cationic polymers used as nonviral gene-delivery vectors include chitosan, PLL, polyethylenimine (PEI), poly(amido amine) (PAMAM) dendrimers and select polypeptides [24, 44, 45].

4.1. Chitosan

Chitosan is a polysaccharide copolymer composed of randomly distributed β-(1-4)-linked d-glucosamines and *N*-acetyl-d-glucosamines, obtained by partial alkaline deacetylation of chitin [46], with different molecular weights (50–200 kDa), degrees of deacetylation (40–98%) and viscosities [47]. Chitosan is a natural polymer, **Figure 5**, with linear polyamine, having reactive amino and hydroxyl groups, biodegradable to normal body constituent, safe and non-toxic, and binds to mammalian and microbial cells. The main commercial sources of chitosan are the crustacean shell wastes of crabs, shrimps and

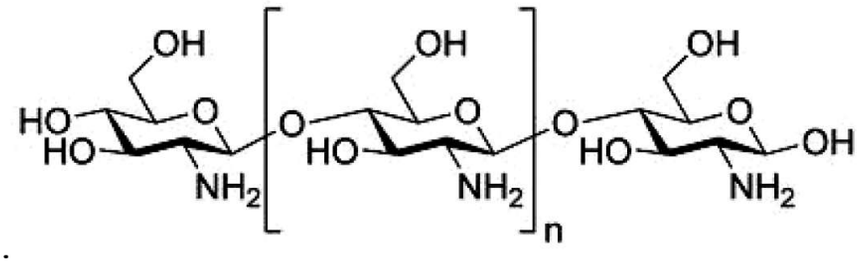

Figure 5. Structure of chitosan.

lobsters [48]. Chitosan is soluble in aqueous solutions of some acids and some selective N-alkylidination. Its solubility, biodegradability, reactivity and adsorptivity of many substrates depend on the amount of protonation of the $–NH_2$ function on the C-2 position of the D-glucosamine unit, whereby the polysaccharide is converted to a polyelectrolyte in acidic media. Chitosan is considered one of the most valuable polymers for biomedical and pharmaceutical applications due to its biodegradability, biocompatibility, antimicrobial, non-toxicity and anti-tumour properties.

Chitosan effectively condenses DNA and protects it from nuclease degradation. Various conjugates such as thiolation, glycolation and folate chitosan are available. Chitosan is biodegradable, biocompatible, low immunogenicity and non-toxic at low molecular weights (10–50 kDa). It has been suggested that the toxicity of chitosan is perhaps due to impurities in the chitosan polymers [49–60].

4.2. Poly-L-lysine

Poly-L-lysine (ε-poly-L-lysine), as given in **Figure 6**, is a small natural homopolymer of the essential amino acid L-lysine that is produced by bacterial fermentation. Poly-L-lysine is a positively charged amino acid polymer with approximately one HBr per lysine residue. The hydrobromide allows the poly-L-lysine to be in a crystalline form soluble in water. Adhesion

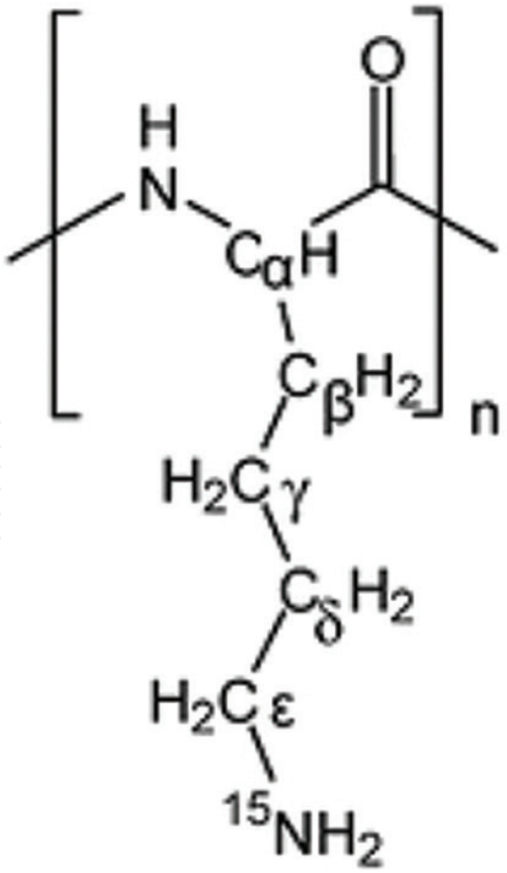

Figure 6. Structure of poly-L-lysine.

into the cell wall is based on the interaction between the negatively charged ions of the cell membrane and positive charge of poly-L-lysine. Simple electrostatic mixing of DNA and poly-L-lysine produces DNA particles with various structures. The mode of binding between the poly-L-lysine and DNA is cooperative and non-cooperative binding. Condensation between the DNA with the PLA depends upon the PLL chain length. Increase in the length of the PLL chain increases the condensation [61–68].

4.3. Polyethylenimine

Polyethylenimine (PEI), as given in **Figures 8** and **9**, is water-soluble, linear or branched polymers composed of the amine group and two carbon aliphatic CH_2CH_2 spacer. It is a weakly basic aliphatic polymer and polycationic one due to primary, secondary and tertiary amino groups. PEIs are available in different molecular masses and forms. Various forms of PEIs are shown in **Figure 7**–9. Linear polyethylenimines contain all secondary amines, whereas branched PEIs contain primary, secondary and tertiary amino groups. Due to their high cationic charge density at physiological pH, PEIs are able to form non-covalent complexes with DNA, siRNA and antisense oligodeoxynucleotide, and then brought into the cell via endocytosis. Once inside the cell, protonation of the amines results in an influx of counter-ions and a lowering of the osmotic potential, leading to bursts in the vesicle releasing the polymer-DNA complex (polyplex) into the cytoplasm. If the polyplex unpacks, then the DNA is free to diffuse to the nucleus; however, the long PEI chains have higher efficiency in gene transfection, and are more cytotoxic [69–93].

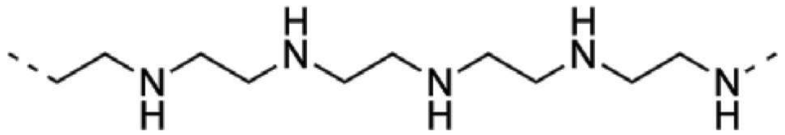

Figure 7. Structure of linear PEI.

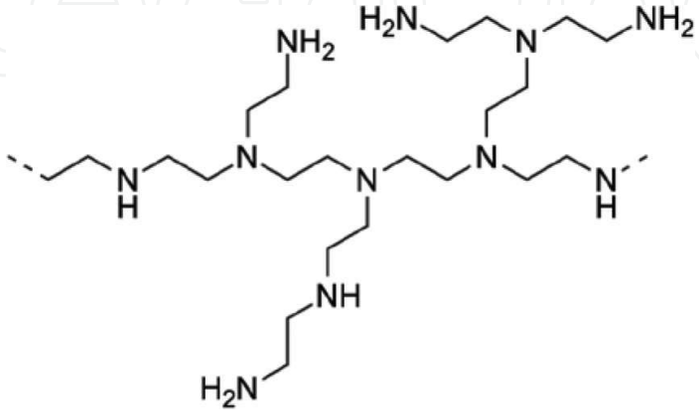

Figure 8. Structure of branched PEI.

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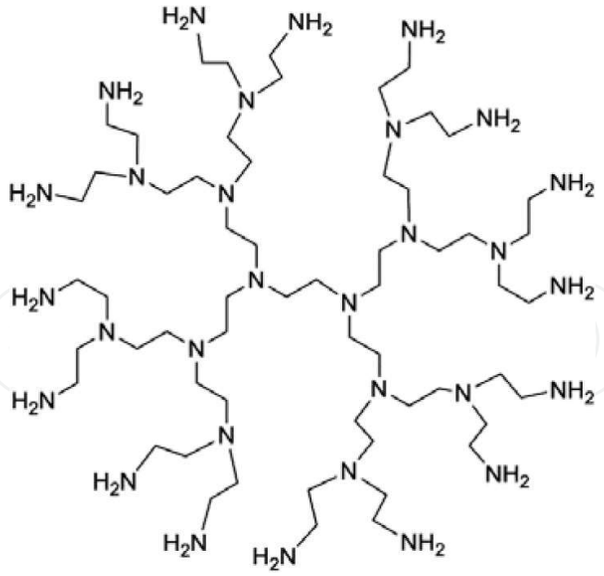

Figure 9. Structure of dendrimer PEI.

5. Cationic lipids

The four constituents are given as follows:

1. The cationic polar head group.
2. A hydrophobic chain that affects the physical properties of the lipid bilayer.
3. The space between two mentioned sections that improves chemical stability, biodegradability and gene transfection efficiency.
4. A backbone domain as a scaffold [19].

5.1. Monovalent cationic lipids

5.1.1. DOTMA

Chemically, it is N-[1-(2,3-dioleyloxy) propyl]-N,N,N-trimethylammonium chloride, as given in **Figure 10**, that consists of four different moieties: (1) a quaternary ammonium head group as the cationic head group, (2) a glycerol-based backbone, (3) two linkage bonds and (4) two hydrocarbon chains. Alternations can be made in the above moieties to reduce the toxicity and increase the gene transfection efficiencies. Replacement of a methyl group on the quaternary amine of DOTMA with a hydroxyl improves protein expression after gene transfection due to the replaced hydroxyl group in contact with the aqueous layer surrounding the liposome. Increase in the length of the aliphatic chain decreases the gene transfection and vice versa [94–98].

5.1.2. DOTAP

DOTAP, [1,2-bis(oleoyloxy)-3-(trimethylammonio) propane], as given in **Figure 11**, consists of a quaternary amine head group coupled to a glycerol backbone with two oleoyl chains.

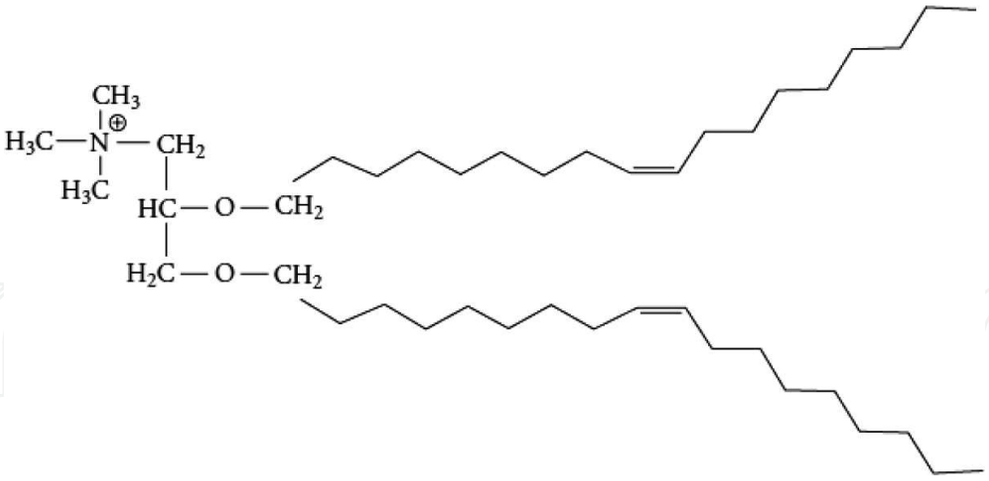

Figure 10. Structure of DOTMA.

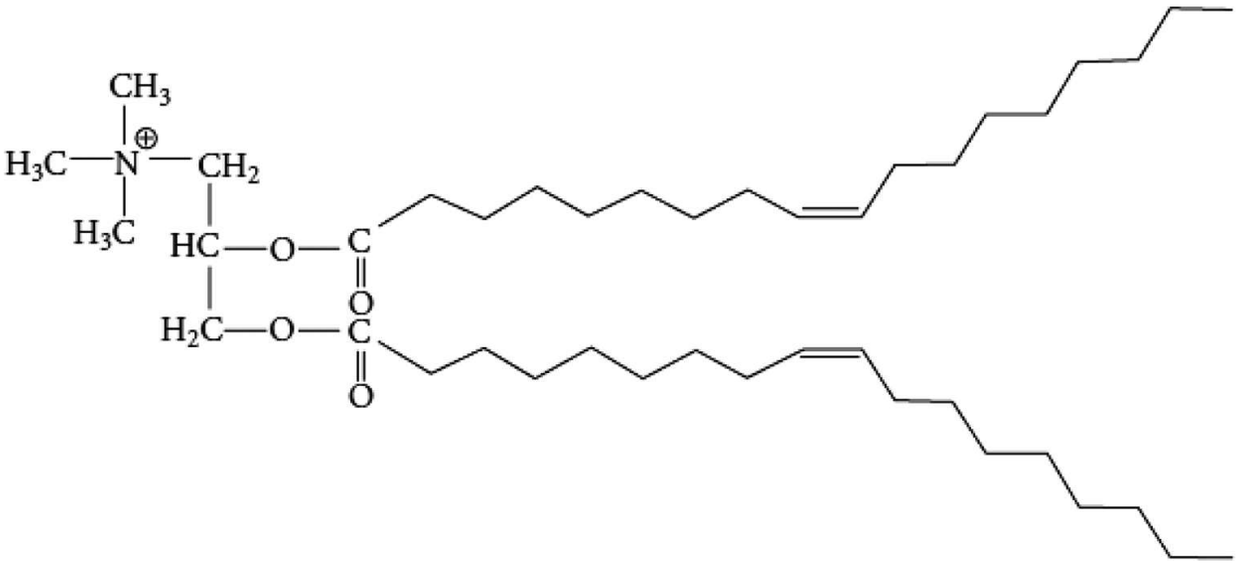

Figure 11. Structure of DOTAP.

The only differences between this molecule and DOTMA are that ester bonds link the chains to the backbone rather than ether bonds. The ester bonds present in the backbone are hydrolysable and lead to render the lipid biodegradable and reduce cytotoxicity. DOTAP cannot be used alone as a cationic liquid for gene delivery due to its dense positive charge, thereby preventing the ion exchange. Its gene-delivery efficiency can be changed by combining with other helper liquids [94, 99–103].

5.1.3. DC-Chol

3β[*N*-(*N*′,*N*′-dimethylaminoethane)-carbamoyl] cholesterol, as given in **Figure 12**, contains a cholesterol moiety attached by an ester bond to a hydrolysable dimethylethylenediamine. Due to the presence of cholesterol moiety, it is biocompatible and has good stability. The combination of DC-Chol and dioleoylphosphatidylethanolamine (DOPE) in the ratio 1:1

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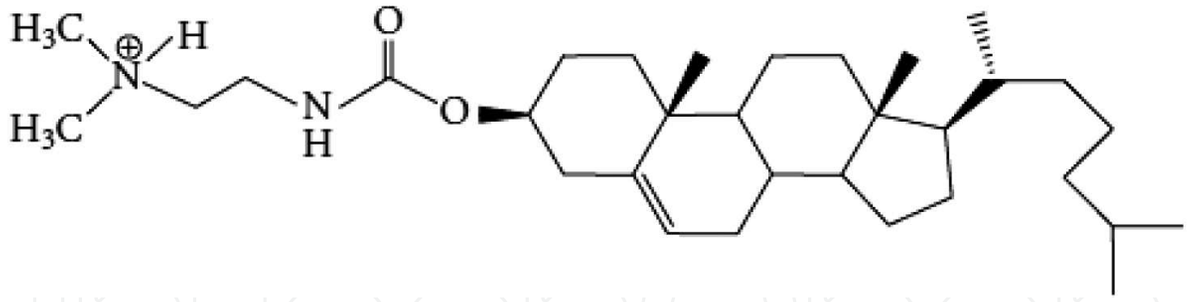

Figure 12. Structure of DC-Col.

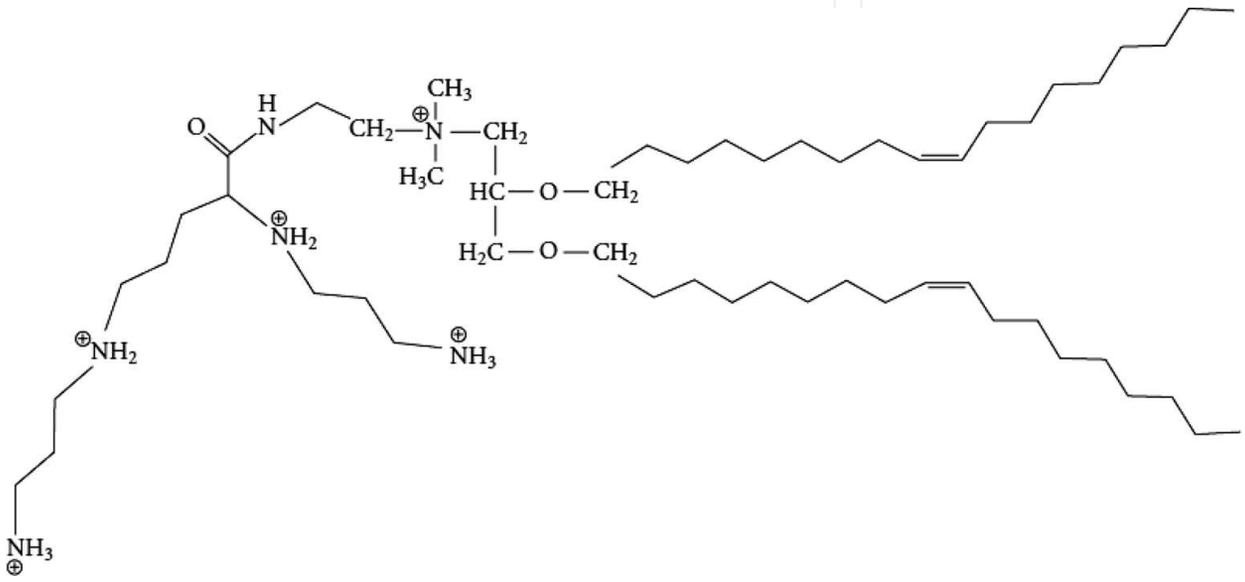

Figure 13. Structure of DOSPA.

reduces the lipoplex aggregation; it assists the DNA dissociation during gene delivery [94, 99, 100, 103, 104].

5.2. Multivalent cationic lipids

5.2.1. DOSPA

DOSPA is a derivative of DOTMA. Chemically, it is 2,3-dioleyloxy-N-[2(sperminecarboxamido) ethyl]-N,N-dimethyl-l-propanaminium trifluoroacetate, which is given in **Figure 13**. The difference between DOSPA and DOTMA is a spermine group, which is bound through a peptide bond to the hydrophobic chains. Spermine group allows more efficient packing of DNA due to its hydrogen bond interaction with the DNA [43, 94].

5.2.2. DOGS

DOGS, chemically it is di-octadecyl-amido-glycyl-spermine, structure of the DOGS is similar to DOSPA, as given in **Figure 14**. The molecular structures of both DOGS and DOSPA consist of a multivalent spermine head group and two 18-carbon alkyl chains. The saturated chains

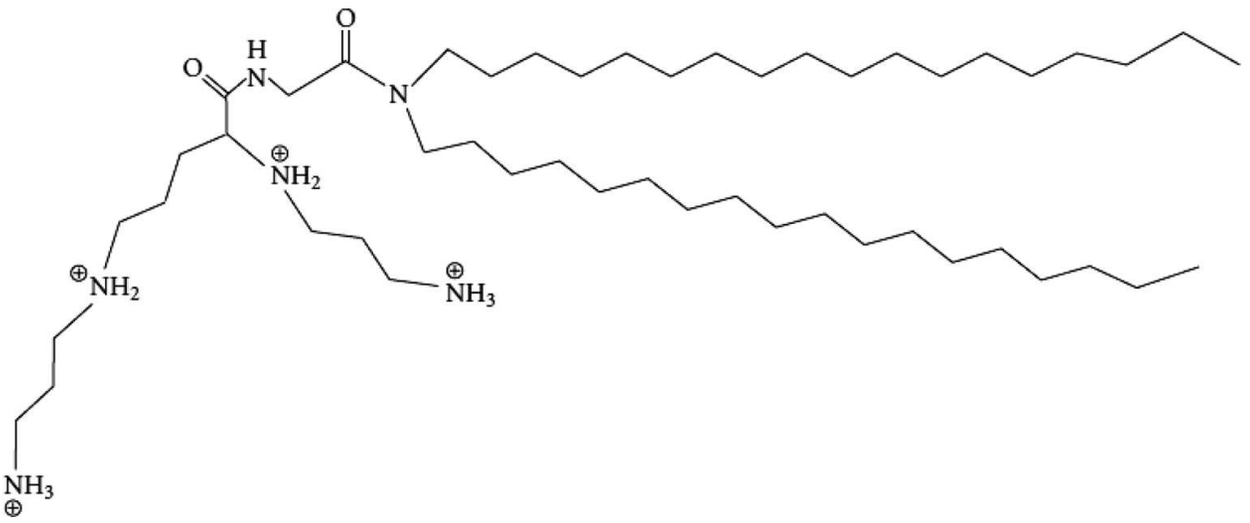

Figure 14. Structure of DOGS.

in DOGS are linked to the head group through a peptide bond. The packing ability of DNA by DOGS is due to its large head group molecule and the length of long unsaturated carbon chains. DOGS have efficient packing of DNA, due to its spermine head group. The presence of spermine head group in DOGS leads to efficient packing of DNA [94, 105–107].

6. Neutral lipids

The commonly used neutral lipids are dioleoylphosphatidylethanolamine (DOPE), as given in **Figure 15**, and dioleoylphosphatidylcholine (DOPC), as given in **Figure 16**. These neutral lipids are used in combination with the other cationic polymers. The gene transfection efficiencies of the cationic polymer are increased when it is used in combination with the helper neutral liquids. The increase in gene transfection efficiency is due to conformational shift to an inverted hexagonal packing structure like a honeycomb by DOPE at lower or acidic pH. The formation

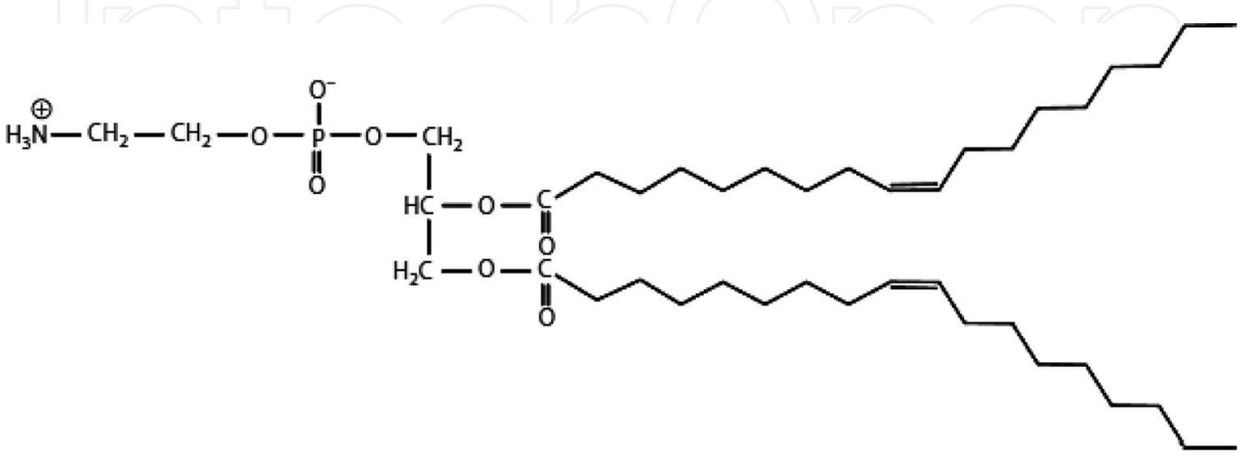

Figure 15. Structure of DOPE.

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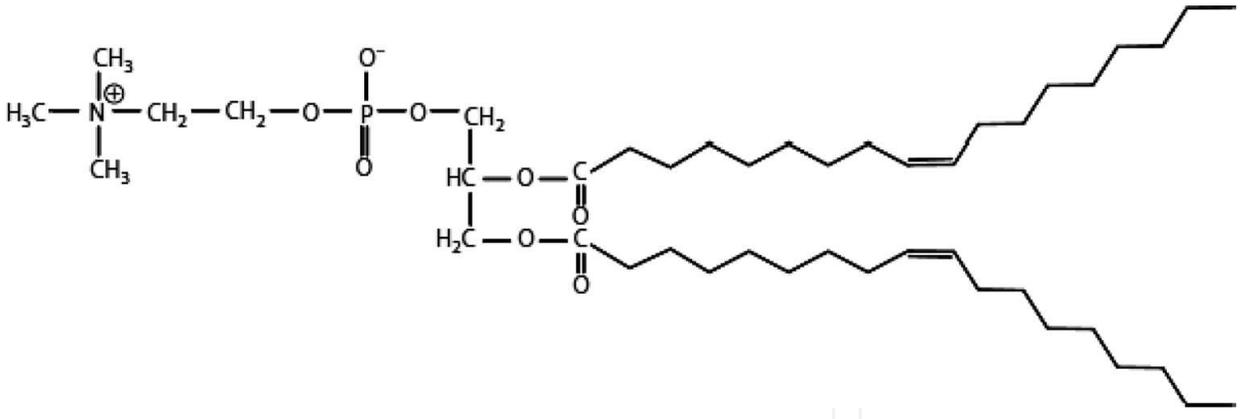

Figure 16. Structure of DOPC.

of inverted hexagonal-packing structure condenses the DNA inside by electrostatic interactions. During gene transfection, fusion and destabilization of the lipoplex occur which lead to the release of DNA from endosomal vesicles. Cationic polymers DOTAP, DC-Chol and other cholesterol derivatives have been incorporated with DOPE for gene transfection efficiency [94, 103, 108–114].

7. Poly(ethylene) glycol (PEG)

Chemically, poly(ethylene) glycol (PEG) ($C_{2n}H_{4n+2}O_{n+1}$) is a polyether or polymer of ethylene oxide.

The physical properties of PEG vary with respect to its chain length, whereas its chemical properties are almost the same. It is available in different molecular weights and different geometries such as branched PEG, star PEG and comb PEG. PEG is non-toxic and excreted through kidney. Degradation of the drug can be protected due to its surface modification property, and it has been extensively used as liposomal targeting by liposomal coating. The liposomes have longer circulation time in blood, reduced macrophage uptake, higher gene transfection efficiencies, larger available concentration and bioavailability [94, 115–120].

8. Conclusion

Nanotechnology is a science adapted in various research areas specifically in the drug-delivery system. At present, gene delivery system includes viral-based, non-viral-based and combined hybrid systems, which are widely used for the treatment of various diseases. To provide the desired concentration of the drug in the target site and therapeutic effect is critical of the drug-delivery system. Biopolymer is a biomaterial that has been utilized extensively for formulating genetic material into a nanoparticle either embedded or encapsulated within the polymeric matrix. Despite various biopolymers, choosing a suitable biopolymer, nanoparticle preparation procedure with desired properties can achieve the bio-distribution and effective

delivery of the genetic material into the target site and regulate the damaged genes to produce the required proteins.

Author details

Sakthivel Lakshmana Prabu[1]* and Kandasamy Ruckmani[1,2]

*Address all correspondence to: slaxmanvel@gmail.com

1 Department of Pharmaceutical Technology, Bharathidasan Institute of Technology, Anna University, Tiruchirapalli, Tamil Nadu, India

2 Centre for Excellence in Nanobio Translational Research (CENTRE), National Facility on Drug Design (NFDD), Professor and Head, Department of Pharmaceutical Technology, Bharathidasan Institute of Technology, Anna University, Tiruchirapalli, Tamil Nadu, India

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