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Biopolymer in Gene Delivery

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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

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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**.

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.

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-alkylation. 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 PLL 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.

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-dioleoyloxy) 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 lipid 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 lipids [94, 99–103].

5.1.3. DC-Chol

3β [*N,N'*-dimethylethanolamine)-carbamoyl] cholesterol, as given in **Figure 12**, contains a cholesterol moiety attached by an ester bond to a hydrolysable dimethylethanolamine. 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

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-1-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 lipids. 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.

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.

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References

- [1] Ward MA, Georgiou TK. Thermoresponsive polymers for biomedical applications. *Polymers*. 2011; 3: 1215–1242.
- [2] Twaites BR, Alarcon CDH, Lavigne M, Saulnier A, Pennadam SS, Cunliffe D, et al., Thermoresponsive polymers as gene delivery vectors: Cell viability, DNA transport and transfection studies. *J Control Release*. 2005; 108: 472–473.
- [3] Mahapatra A, Singh DK. Biodegradable nanoparticle are excellent vehicle for site directed in-vivo delivery of drugs and vaccines. *J Nanobiotech*. 2011; 9: 55.
- [4] Mishra B, Vuppu S, Rath K. The role of microbial pullulan, a biopolymer in pharmaceutical approaches: A review. *J Appl Pharm Sci*. 2011; 1(6): 45–50.
- [5] Eliyahu H, Barenholz Y, Domb AJ. Polymers for DNA delivery. *Molecules*. 2005; 10: 34–64.
- [6] Pack DW, Hoffman AS, Pun S, Stayton PS. Design and development of polymers for gene delivery. *Nature Rev Drug Discov*. 2005; 4: 581–593.
- [7] Dang JM, Leong KW. Natural polymers for gene delivery and tissue engineering. *Adv Drug Del Rev*. 2006; 58(4): 487–499.
- [8] Nitta SK, Numata K. Biopolymer based nanoparticles for drug/gene delivery and tissue engineering. *Int J Mol Sci*. 2013; 14: 1629–1654.
- [9] Harris TJ, Green JJ, Fung PW, Langer R, Anderson DG, Bhatia SN. Tissue specific gene delivery via nanoparticle coating. *Biomaterials*. 2010; 31(5): 998–1006.
- [10] Labhasetwar V, Song C, Levy RJ. Nanoparticle drug delivery system for restenosis. *Adv Drug Del Rev*. 1997; 24(1): 63–85.

- [11] Erdal C, Ali DS, Emre SC. Gene delivery systems: Recent progress in viral and non-viral therapy. In: Recent Advances in Novel Drug Carrier Systems, Ali DS (Ed.), 2012, InTech, Croatia, DOI: 10.5772/53392. <http://www.intechopen.com/books/recent-advances-in-novel-drug-carrier-systems/gene-delivery-systems-recent-progress-in-viral-and-non-viral-therapy>
- [12] Liang GF, Zhu YL, Sun B, Hu FH, Tian T, Li SC, et al. PLGA-based gene delivering nanoparticle enhance suppression effect of miRNA in HePG2 cells. *Nanoscale Res Lett.* 2011; 6: 447.
- [13] Jin S, Ye K. Mediated drug delivery and gene therapy. *Biotechnol Prog.* 2007; 23: 32–41.
- [14] Georgiou TK, Vamvakaki M, Patrickios CS, Yamasaki EN, Phylactou LA. Nanoscopic cationic methacrylate star homopolymers: Synthesis by group transfer polymerization, characterization and evaluation as transfection reagents. *Biomacromolecules.* 2004; 5 (6):2221–2229.
- [15] Godbey WT, Mikos AG. Recent progress in gene delivery using non-viral transfer complexes. *J Cont Release.* 2001; 72 (1–3):115–125.
- [16] Xu FJ, Zhang ZX, Ping Y, Li J, Kang ET, Neoh KG. Star-shaped cationic polymers by atom transfer radical polymerization from beta-cyclodextrin cores for nonviral gene delivery. *Biomacromolecules.* 2009; 10 (2):285–293.
- [17] Cloninger MJ. Biological applications of dendrimers. *Curr Opin Chem Biol.* 2002; 6 (6):742–748.
- [18] Tang MX, Redemann CT, Szoka C. *In vitro* gene delivery by degraded polyamidoamine dendrimers. *Bioconjug Chem.* 1996; 7 (6):703–714.
- [19] Dizaj SM, Jafari S, Khosroushahi AY. A sight on the current nanoparticle-based gene delivery vectors. *Nanoscale Res Lett.* 2014; 9: 252.
- [20] Kabanov AV, Kabanov VA. DNA complexes with polycations for the delivery of genetic material into cells. *Bioconjug Chem.* 1995; 6: 7–20.
- [21] Ledley FD. Pharmaceutical approach to somatic gene therapy. *Pharm Res.* 1996; 13: 1595–1614.
- [22] Vijayanatham V, Thomas T, Thomas TJ. DNA nanoparticles and development of DNA delivery vehicles for gene therapy. *Biochemistry.* 2002; 41:14085–14094.
- [23] Armstrong, TKC, Girouard LG, Anchordoquy TJ. Effects of PEGylation on preservation of cationic lipid/DNA complexes during freeze-thawing and lyophilization. *J Pharm Sci.* 2002; 91:2549–2558.
- [24] Oyewumi MO, Rice KG. DNA nanoparticle gene delivery systems. In: Nanoparticle Technology for Drug Delivery. Gupta RB, Kompella UB (eds.). Marcel Dekker, New York, Vol. 159, 2006; pp. 361–379.
- [25] Boas U, Heegaard PM. Dendrimers in drug research. *Chem Soc Rev.* 2004; 33: 43–63.

- [26] Cheng Y, Wang J, Rao T, He X, Xu, T. Pharmaceutical applications of dendrimers: Promising nanocarriers for drug delivery. *Front Biosci.* 2008; 13: 1447–1471.
- [27] Dufes C, Uchegbu IF, Schatzlein AG. Dendrimers in gene delivery. *Adv Drug Deliv Rev.* 2005; 57: 2177–2202.
- [28] Gao Y, Gao G, He Y, Liu T, Qi R. Recent advances of dendrimers in delivery of genes and drugs. *Mini Rev Med Chem.* 2008; 8: 889–900.
- [29] Gillies ER, Frechet JM. Dendrimers and dendritic polymers in drug delivery. *Drug Discov Today.* 2005; 10: 35–43.
- [30] Svenson S, Tomalia DA. Dendrimers in biomedical applications--reflections on the field. *Adv Drug Deliv Rev.* 2005; 57: 2106–2129.
- [31] Tomalia DA, Baker H, Dewald J, Hall M, Kallos G, Martin S, et al. Dendritic macromolecules: Synthesis of starburst dendrimers. *Macromolecules.* 1986, 19: 2466–2468.
- [32] Klajnert B, Bryszewska M. Dendrimers: Properties and applications. *Acta Biochem Polonica.* 2001; 48: 199–208.
- [33] Tomalia DA, Baker H, Dewald JR, Hall M, Kallos G, Martin S, Roeck J, Ryder, J., Smith P. A new class of polymers: Starburst-dendritic macromolecules. *Polym J.* 1985; 17: 117–132.
- [34] Fischer M, Vogtle F. Dendrimers: From design to applications—A progress report. *Angew Chem Int Ed.* 1999; 38: 884–905.
- [35] Zimmerman SC, Zeng F, Reichert DEC, Kolotuchin SV. Self-assembling dendrimers. *Science.* 1996; 271: 1095–1098.
- [36] Frechet JMJ. Functional polymers and dendrimers: Reactivity, molecular architecture, and interfacial energy. *Science.* 1994; 263: 1710–1715.
- [37] Tomalia DA, Dvornic PR. What promise for dendrimers? *Nature.* 1994; 372: 617–618.
- [38] Twyman LJ, Beezer AE, Esfand R, Hardy MJ, Mitchell JC. The synthesis of water soluble dendrimers, and their application as possible drug delivery systems. *Tetrahedron Lett.* 1999; 40: 1743–1746.
- [39] Waite CL, Sparks SM, Uhrich KE, Roth CM. Acetylation of pamam dendrimers for cellular delivery of sirna. *BMC Biotechnol.* 2009; 9: 38.
- [40] Biswas S, Vladimir P. Torchilin. Dendrimers for siRNA delivery. *Pharmaceuticals.* 2013; 6: 161–183.
- [41] Medina SH, El-Sayed MEH. Dendrimers as carriers for delivery of chemotherapeutic agents. *Chem Rev.* 2009; 109: 3141–3157.
- [42] Tomalia DA, Frechet JMJ. Discovery of dendrimers and dendritic polymers: A brief historical perspective. *J Polym Sci Part A: Polym Chem.* 2002; 40(16): 2719–2728.

- [43] Vuorimaa E, Urtti A, Seppänen R, Lemmetyinen H, Yliperttula M. Time-resolved fluorescence spectroscopy reveals functional differences of cationic polymer-DNA complexes. *J Am Chem Soc.* 2008; 130(35): 11695–11700.
- [44] Guo P, Coban O, Snead NM, Trebley J, Hoeprich S, Guo S, et al. Engineering RNA for targeted siRNA delivery and medical application. *Adv Drug Deliv Rev.* 2010; 62: 650–666.
- [45] Nimesh S, Gupta N, Chandra R. Cationic polymer based nanocarriers for delivery of therapeutic nucleic acids. *J Biomed Nanotechnol.* 2011; 7: 504–520.
- [46] Synowiecki J, Al-Khateeb NA. Production, properties, and some new applications of chitin and its derivatives. *Crit Rev Food Sci Nutr.* 2003; 43(2): 145–171.
- [47] Illum L. Chitosan and its use as a pharmaceutical excipient. *Pharm Res.* 1998; 15(9): 1326–1331.
- [48] Hggard MK, Tubulekas I, Guan H, Edwards K, Nilsson M, Varum KM, et al., Chitosan as a nonviral gene delivery system. Structure property relationships and characteristics compared with polyethylenimine in vitro and after lung administration in vivo. *Gene Ther.* 2001; 8(14): 1108–1121.
- [49] Lee M, Nah JW, Kwon Y, Koh JJ, Ko KS, Kim SW. Water-soluble and low molecular weight chitosan-based plasmid DNA delivery. *Pharm Res.* 2001; 18(4): 427–431.
- [50] Lee D, Zhang W, Shirley SA, Kong X, Hellermann GR, Lockey RF, et al. Thiolated chitosan/DNA nanocomplexes exhibit enhanced and sustained gene delivery. *Pharm Res.* 2007; 24(1): 157–167.
- [51] Mathur NK, Narang CK. Chitin and chitosan, versatile polysaccharides from marine animals. *J Chem Educ.* 1990; 67:938–42.
- [52] Hudson SM, Jenkins DW. Chitin and chitosan. In: *Encyclopedia of Polymer Science and Technology.* Mark HF (ed.), New York, NY: Wiley; 2003. pp. 569–80.
- [53] Krajewska B. Application of chitin- and chitosan-based materials for enzyme immobilizations: A review. *Enzyme Microbiol Technol.* 2004; 35: 126–139.
- [54] Kanke M, Katayama H, Tsuzuki S, Kuramoto H. Application of chitin and chitosan to pharmaceutical preparations. *Chem Pharm Bull.* 1989; 37: 523–25.
- [55] Kato Y, Onishi H, Machida Y. Application of chitin and chitosan derivatives in the pharmaceutical field. *Curr Pharm Biotechnol.* 2003; 4: 303–309.
- [56] Anthonsen MW, Varum KM, Smidsrod O. Solution properties of chitosans: Conformation and chain stiffness of chitosans with different degrees of N-acetylation. *Carbohydr Polym.* 1993; 22: 193–201.
- [57] Rinaudo M. Chitin and chitosan: Properties and applications. *Prog Polym Sci.* 2006; 31: 603–632.

- [58] Alvarenga ES. Characterization and properties of chitosan. In: *Biotechnology of Biopolymers*. Elnashar M (ed.), InTech. 2011; pp. 91–108.
- [59] Tungtong S, Okonogi S, Chowwanapoonpohn S, Phutdhawong W, Yotsawimonwat S. Solubility, viscosity and rheological properties of water-soluble chitosan derivatives. *Maejo Int J Sci Technol*. 2012; 6(2): 315–322.
- [60] Pillai CKS, Paul W, Sharma CP. Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Prog Polym Sci*. 2009; 34: 641–678.
- [61] Laemmli UK. Characterization of DNA condensates induced by poly(ethylene oxide) and polylysine. *Proc Nat Acad Sci USA*. 1975; 72(11): 4288–4292.
- [62] Gonscho A, Irie K, Susaki H, Iwasawa H, Okuno S, Sugawara T. Tissue-targeting ability of saccharide-poly(L-lysine) conjugates. *Biol Pharm Bull*. 1994; 17(2): 275–282.
- [63] Shima S, Sakai H. Polylysine produced by *Streptomyces*. *Agric Biol Chem*. 1977; 41: 1807–1809.
- [64] Liu G, Molas M, Grossmann GA, Pasumarthi M, Perales JC, Cooper MJ, et al. Biological properties of poly-L-lysine-DNA complexes generated by cooperative binding of the polycation. *J Biol Chem*. 2001; 276(37): 34379–34387.
- [65] Ferdous A, Watanabe H, Akaike T, Maruyama A. Comb-type copolymer: Stabilization of triplex DNA and possible application in antigene strategy. *J Pharm Sci*. 1998; 87(11): 1400–1405.
- [66] Krikorian V, Kurian M, Galvin ME, Nowak AP, Deming TJ, Pochan DJ. Polypeptide-based nanocomposite: Structure and properties of poly(L-lysine)/Na⁺-montmorillonite. *J Poly. Sci Part B: Poly Phys*. 2002; 40: 2579–2586.
- [67] Liu G, Molas M, Grossmann GA, Pasumarthi M, Perales JC, Cooper MJ, et al. Binding of the polycation complexes generated by cooperative biological properties of poly-L-lysine-DNA. *J Biol Chem*. 2001; 276: 34379–34387.
- [68] Toncheva V, Wolfert MA, Dash PR, Oupicky D, Ulbrich K, Seymour LW, Schacht EH. Novel vectors for gene delivery formed by self-assembly of DNA with poly(L-lysine) grafted with hydrophilic polymers. *Biochim Biophys Acta*. 1998; 1380: 354–368.
- [69] Boussif O, Zanta MA, Behr JP. Optimized galenics improve in vitro gene transfer with cationic molecules up to 1000-fold. *Gene Ther*. 1996; 3(12): 1074–1080.
- [70] Godbey WT, Barry MA, Saggau P, Wu KK, Mikos AG. Poly(ethylenimine)-mediated transfection: A new paradigm for gene delivery. *J Biomed Mater Res*. 2000; 51(3): 321–328.
- [71] Yao YH, Liu YB, Feng Y, Feng X, Xu DM, Yao SD. Synthesis and screening of polyethylenimine as a carrier for gene transfer into cultured human tumor cells. *Ai Zheng*. 2007; 26(7): 790–794.
- [72] Sharma VK, Thomas M, Klibanov AM. Mechanistic studies on aggregation of polyethylenimine-DNA complexes and its prevention. *Biotechnol Bioeng*. 2005; 90(5): 614–620.

- [73] Ogris M, Steinlein P, Kursa M, Mechtler K, Kircheis R, Wagner E. The size of DNA/transferrin-PEI complexes is an important factor for gene expression in cultured cells. *Gene Ther.* 1998; 5(10): 1425–1433.
- [74] Lee H, Jeong JH, Park TG. A new gene delivery formulation of polyethylenimine/DNA complexes coated with PEG conjugated fusogenic peptide. *J Con Rel.* 2001; 76(1–2): 183–192.
- [75] Aoki K, Furuhashi S, Hatanaka K, Maeda M, Remy JS, Behr JP, et al. Polyethylenimine-mediated gene transfer into pancreatic tumor dissemination in the murine peritoneal cavity. *Gene Ther.* 2001; 8: 508–514.
- [76] Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, et al. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: Polyethylenimine. *Proc Natl Acad Sci USA.* 1995; 92: 7297–7301.
- [77] Lungwitz U, Breunig M, Blunk T, Gopferich A. Polyethylenimine-based non-viral gene delivery systems. *Eur J Pharm Biopharm.* 2005; 60: 247–266.
- [78] Zintchenko A, Philipp A, Dehshahri A, Wagner E. Simple modifications of branched PEI lead to highly efficient siRNA carriers with low toxicity. *Bioconjug Chem.* 2008; 19: 1448–1455.
- [79] Lee M, Kim SW. Polyethylene glycol-conjugated copolymers for plasmid DNA delivery. *Pharm Res.* 2005; 22: 1–10.
- [80] Nimesh S. Polyethylenimine as a promising vector for targeted siRNA delivery. *Curr Clin Pharmacol.* 2012; 7: 121–130.
- [81] Petersen H, Fechner PM, Martin AL, Kunath K, Stolnik S, Roberts CJ, Fischer D, Davies MC, Kissel T. Polyethylenimine-graft-poly(ethylene glycol) copolymers: Influence of copolymer block structure on DNA complexation and biological activities as gene delivery system. *Bioconjug Chem.* 2002; 13(4): 845–854.
- [82] Godbey WT, Wu KK, Mikos AG. Poly (ethylenimine)-mediated gene delivery affects endothelial cell function and viability. *Biomaterials.* 2000; 22: 471–480.
- [83] Godbey WT, Wu KK, Mikos AG. Size matters: Molecular weight affects the efficiency of poly(ethylenimine) as a gene delivery vehicle. *J Biomed Mater Res.* 1999; 45: 268–275.
- [84] Fischer D, Bieber T, Li Y, Elsässer HP, Kissel T. A novel non-viral vector for DNA delivery based on low molecular weight, branched polyethylenimine: Effect of molecular weight on transfection efficiency and cytotoxicity. *Pharm Res.* 1999; 16:1273–1279.
- [85] Furgeson DY, Chan WS, Yockman JW, Kim SW. Modified linear polyethylenimine-cholesterol conjugates for DNA complexation. *Bioconjugate Chem.* 2003; 14: 840–847.
- [86] Furgeson DY, Yockman JW, Janat MM, Kim SW. Tumor efficacy and biodistribution of linear polyethylenimine-cholesterol/DNA complexes. *Mol Ther.* 2004; 9: 837–845.
- [87] Thomas M, Lu J, Ge Q, Zhang C, Chen J, Klivanov AM. Full deacylation of polyethylenimine dramatically boosts its gene delivery efficiency and specificity to mouse lung. *Proc Natl Acad Sci USA.* 2005; 102: 5679–5684.

- [88] Jeon O, Yang HS, Lee TJ, Kim BS. Heparin-conjugated polyethylenimine for gene delivery. *J Control Release*. 2008; 132: 236–242.
- [89] Xu Z, Shen G, Xia X, Zhao X, Zhang P, Wu H, et al. Comparisons of three polyethylenimine-derived nanoparticles as a gene therapy delivery system for renal cell carcinoma. *J Transl Med*. 2011; 9: 46.
- [90] Felt O, Buri P, Guruy R. Chitosan: A unique polysaccharide for drug delivery. *Drug Dev Ind Pharm*. 1998; 24: 979.
- [91] Tang MX, Szoka FC. The influence of polymer structure on the interactions of cationic polymers with DNA and morphology of the resulting complexes. *Gene Ther*. 1997; 4: 823–832.
- [92] Fischer D, Bieber T, Li YX, Elsasser HP, Kissel T. A novel non-viral vector for DNA delivery based on low molecular weight, branched polyethylenimine: Effect of molecular weight on transfection efficiency and cytotoxicity. *Pharm Res*. 1999; 16: 1273–79.
- [93] Von Harpe A, Petersen H, Li YX, Kissel T. Characterization of commercially available and synthesized polyethylenimines for gene delivery. *J Control Release*. 2000; 69: 309–322.
- [94] Zhang X, Balazs DA, Godbey WT. Nanobiomaterials for nonviral gene delivery. In: *Nanobiomaterials Hand Book*, Balaji Sitharaman (ed.). CRC press, Taylor & Francis, 2011, pp.13–1 to 13–25.
- [95] Ren T, Song YK, Zhang G, Liu D. Structural basis of DOTMA for its high intravenous transfection activity in mouse. *Gene Therapy*. 2000; 7: 764–768.
- [96] Leventis R, Silvius JR. Interactions of mammalian cells with lipid dispersions containing novel metabolizable cationic amphiphiles. *Biochim Biophys Acta*. 1990; 1023: 124–32.
- [97] Gao X, Huang L. A novel cationic liposome reagent for efficient transfection of mammalian cells. *Biochem Biophys Res Commun*. 1991; 179: 280–285.
- [98] Malone RW, Felgner PL, Verma IM. Cationic liposome-mediated RNA transfection. *Proc Natl Acad Sci USA*. 1989; 86: 6077–6081.
- [99] Zuidam NJ, Barenholz Y. Electrostatic and structural properties of complexes involving plasmid DNA and cationic lipids commonly used for gene delivery. *Biochim Biophys Acta*. 1998; 1368: 115–128.
- [100] Zabner J, Fasbender AJ, Moninger T, Poellinger KA, Welsh MJ. Cellular and molecular barriers to gene transfer by a cationic lipid. *J Biol Chem*. 1995; 270: 18997–9007.
- [101] Yang JP, Huang L. Time-dependent maturation of cationic liposome-DNA complex for serum resistance. *Gene Ther*. 1998; 5: 380–387.
- [102] Marchini C, Montani M, Amici M, Amenitsch H, Marianecci C, Pozzi D, et al. Structural stability and increase in size rationalize the efficiency of lipoplexes in serum. *Langmuir*. 2009; 25: 3013–3021.

- [103] Gao X, Huang L. A novel cationic liposome reagent for efficient transfection of mammalian cells. *Biochem Biophys Res Commun*. 1991; 179(1): 280–285.
- [104] Zuidam NJ, Barenholz Y. Electrostatic parameters of cationic liposomes commonly used for gene delivery as determined by 4-heptadecyl-7-hydroxycoumarin. *Biochim Biophys Acta*. 1997; 1329(2): 211–222.
- [105] Behr JP, Demeneix B, Loeffler JP, Perez-Mutul J. Efficient gene transfer into mammalian primary endocrine cells with lipopolyamine-coated DNA. *Proc Natl Acad Sci USA*. 1989; 86(18): 6982–6986.
- [106] Boukhnikachvili T, Aguerre-Chariol O, Airiau M, Lesieur S, Ollivon M, Vacus J. Structure of in-serum transfecting DNA-cationic lipid complexes. *FEBS Lett*. 1997; 409(2): 188–194.
- [107] Remy JS, Sirlin C, Vierling P, Behr JP. Gene transfer with a series of lipophilic DNA-binding molecules. *Bioconjug Chem*. 1994; 5(6): 647–654.
- [108] Farhood H, Gao X, Son K, Yang YY, Lazo JS, Huang L, et al. Cationic liposomes for direct gene transfer in therapy of cancer and other diseases. *Ann N Y Acad Sci*. 1994; 716: 23–34.
- [109] Farhood H, Serbina N, Huang L. The role of dioleoyl phosphatidylethanolamine in cationic liposome mediated gene transfer. *Biochim Biophys Acta*. 1995; 1235(2): 289–295.
- [110] Chesnoy S, Huang L. Structure and function of lipid-DNA complexes for gene delivery. *Annu Rev Biophys Biomol Struct*. 2000; 29: 27–47.
- [111] Hui SW, Langner M, Zhao YL, Ross P, Hurley E, Cha K. The role of helper lipids in cationic liposome-mediated gene transfer. *Biophys J*. 1996; 71(2): 590–599.
- [112] Kawakami S, Yamashita F, Nishikawa M, Takakura Y, Hashida M. Asialoglycoprotein receptor-mediated gene transfer using novel galactosylated cationic liposomes. *Biochem Biophys Res Commun*. 1998; 252(1): 78–83.
- [113] Legendre JY, Szoka Jr FC. Delivery of plasmid DNA into mammalian cell lines using pH-sensitive liposomes: Comparison with cationic liposomes. *Pharm Res*. 1992; 9(10): 1235–1242.
- [114] Zuhorn IS, Bakowsky U, Polushkin E, Visser WH, Stuart MC, Engberts JB, Hoekstra D. Nonbilayer phase of lipoplex-membrane mixture determines endosomal escape of genetic cargo and transfection efficiency. *Mol Ther*. 2005; 11(5): 801–810.
- [115] Decastro M, Saijoh Y, Schoenwolf GC. Optimized cationic lipid-based gene delivery reagents for use in developing vertebrate embryos. *Dev Dyn*. 2006; 235(8): 2210–2219.
- [116] Ishida O, Maruyama K, Tanahashi H, Iwatsuru M, Sasaki K, Eriguchi M, et al., Liposomes bearing polyethyleneglycol-coupled transferrin with intracellular targeting property to the solid tumors in vivo. *Pharm Res*. 2001; 18(7): 1042–1048.

- [117] Huang RQ, Qu YH, Ke WL, Zhu JH, Pei YY, Jiang C. Efficient gene delivery targeted to the brain using a transferrin conjugated polyethyleneglycol-modified polyamidoamine dendrimer. *FASEB J.* 2007; 21(4): 1117–1125.
- [118] Kim JK, Choi SH, Kim CO, Park JS, Ahn WS, Kim CK. Enhancement of polyethylene glycol (PEG)-modified cationic liposome-mediated gene deliveries: Effects on serum stability and transfection efficiency. *J Pharm Pharmacol.* 2003; 55(4): 453–460.
- [119] Kim TI, Seo HJ, Choi JS, Jang HS, Baek JU, Kim K, et al. PAMAM-PEG-PAMAM: Novel triblock copolymer as a biocompatible and efficient gene delivery carrier. *Biomacromolecules.* 2004; 5(6): 2487–2492.
- [120] Metselaar JM, Bruin P, de Boer LW, de Vringer T, Snel C, Oussoren C, et al. A novel family of L-amino acid-based biodegradable polymer-lipid conjugates for the development of long-circulating liposomes with effective drug-targeting capacity. *Bioconjug Chem.* 2003; 14(6): 1156–1164.