Expert Opinion

Lipid and polymeric carrier-mediated nucleic acid delivery

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Importance of the field: Nucleic acids such as plasmid DNA, antisense oligonucleotide, and RNA interference (RNAi) molecules, have a great potential to be used as therapeutics for the treatment of various genetic and acquired diseases. To design a successful nucleic acid delivery system, the pharmacological effect of nucleic acids, the physiological condition of the subjects or sites, and the physicochemical properties of nucleic acid and carriers have to be thoroughly examined.

Areas covered in this review: The commonly used lipids, polymers and corresponding delivery systems are reviewed in terms of their characteristics, applications, advantages and limitations.

What the reader will gain: This article aims to provide an overview of biological barriers and strategies to overcome these barriers by properly designing effective synthetic carriers for nucleic acid delivery.

Take home message: A thorough understanding of biological barriers and the structure–activity relationship of lipid and polymeric carriers is the key for effective nucleic acid therapy.

Keywords: lipid, liposomes, non-viral gene delivery, nucleic acid, oligonucleotides, polymer, siRNA

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1. Introduction

Gene therapy is defined as the insertion of genes into cells to replace, correct or make up the defective genes for treating acquired and genetic disorders. Gene therapy can help us target the origin of the disorder instead of using drugs to alleviate symptoms. In the last decade, the spectrum of functional genetic materials used in gene therapy was dramatically broadened and diversified. Notably, gene therapy caught the world's eyes again when two Nobel Prize Laureates, Andrew Fire and Craig Mello, demonstrated the mechanism and application of RNA interfering (RNAi) in 2006 [1]. This is inspite of the fact that accidental deaths of virus-based genetic therapeutics occurred during clinical trials and frustrated enthusiastic scientists all over the world.

Plasmid DNA, oligonucleotide (ODN) and small interfering RNA (siRNA) are the most commonly investigated nucleic acids for gene therapy. The applications and therapeutic outcomes of these nucleic acids may vary depending on their mechanisms of action. They are typically macromolecules with big hydrodynamic size and negative charge in aqueous solution. Therefore, several strategies are being explored for their enhanced gene transfer, including design of different synthetic carries, as discussed below, or the use of physical approaches, such as needle injection [2], coated microneedle [3], electroporation [4], gene gun [5], ultrasound [6] and hydrodynamic delivery [7]. As naturally occurring genetic molecules are fragile and easily degraded by nucleases, harsh conditions such as physical stress, heat, oxidants, acids and alkali should be avoided to minimize their degradataion.

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Article highlights.

- The efficacy of nucleic acid delivery depends greatly on their stability and biological barriers.
- Three basic components of cationic lipids are discussed in terms of their structure–activity relationship, which is important for the design and application of new cationic lipids.
- Properties of cationic polymers are influenced by their structure and molecular mass, resulting in the different stabilities, particle size, zeta potential and final efficacy of polyplexes.
- Non-cationic lipids and polymers are usually used for structural modification and reducing toxicity. Pluronic® (BASF, Florham Park, NJ, USA), thiourea derivatives and PLGA interact with nucleic acid by means of hydrogen bonds or other weak forces and can be used independently.
- Liposomes, bioconjugate and nanoparticles are suitable for nucleic acid delivery.
- Understanding the biological barriers, structure–activity relationship and physicochemical properties of lipid or polymeric carriers is critical for the development of a successful nucleic acid delivery system.

This box summarizes key points contained in the article.

2. Barriers to nucleic acid therapeutics

It is difficult to achieve the expected biological effects of nucleic acids by traditional delivery strategies owing to many biological barriers. Besides instability, several obstacles have to be surpassed before nucleic acids take action at their desired sites. First of all, nucleic acids should go across extracellular, cellular and intracellular biological membranes. Following systemic administration, these molecules have to go sequentially across the vasculature wall, intercellular tissue junction and cytoplasmic membrane of the target cells, escape from endosome, and then enter the nucleus. siRNAs also need to overcome most of these biological barriers, except nuclear membrane. If the target site is located in the central nervous system (CNS), the tight junctions between endothelial cells of CNS vessels, the so-called blood–brain barrier (BBB), have to be overcome. Second, these nucleic acids may face various enzymes and proteins during their delivery to the target cells, which may degrade them or trigger immune response. Unmodified siRNAs have been reported to be unstable in the presence of high concentrations of fetal bovine serum (FBS) [8]. The conserved sequence of nucleic acids can be recognized and bound by immune molecules and engulfed by immune cells and activate complement systems [9,10]. In some cases the therapeutic effects become negligible after multiple administrations of genetic materials [11]. Third, the low efficacy is also often related to their nonspecific biodistribution to non-target cells and tissues.

Kidney plays an important role in the disposition of nucleic acids after systemic administration. In general, macromolecules with molecular mass of < 30,000 Da are susceptible to glomerular filtration [12]. Cy3-labeled siRNAs accumulated predominantly in the kidney, whereas no accumulation was detected in other organs in 20 min post-intravenous injection [13]. The macromolecules that are too large to pass through the glomerular pores will probably accumulate in the liver because of its loose structure in the endothelium and high blood perfusion [12]. However, hydrodynamic injection could accumulate macromolecules in the highly perfused internal organs, such as the liver, regardless of their molecular size [7,14].

Considering the different barriers to nucleic acid-based therapeutics discussed above (**Figure 1**), a successful *in vivo* delivery strategy should be designed to satisfy the following major criteria: i) the method should protect nucleic acids from degradation by nucleases; ii) it should help nucleic acids cross the cell membrane, escape from endosome and finally enter either the nucleus or the cytoplasm, depending on their mechanisms of action; iii) it should have no or fewer side effects caused by either nucleic acids or the method itself; and iv) it should prolong their circulation time and prevent nonspecific disposition of nucleic acids to facilitate their delivery to the target cells. For *in vitro* delivery, the first three criteria have to be satisfied.

The nucleic acid delivery systems can be generally classified to two categories, viral and non-viral vectors. Several types of virus, including retrovirus, adenovirus and adeno-associated virus (AAV), have been modified for use as viral vectors. These vectors have unique advantages and disadvantages. Retroviral vectors can integrate into the host genome, leading to long-term gene expression even after a single administration. Adenoviral vectors can efficiently transduce both dividing and non-dividing cell types, but they may cause immunostimulation, which often limits their *in vivo* application. AAV also infects many non-dividing and dividing cell types, but has a limited DNA insertion capacity. Detailed discussion of viral vectors is beyond the scope of this review, but can be found elsewhere [15-17].

Unlike viral vectors, most non-viral vectors, including cationic liposomes (lipids), cationic polymers, cationic proteins (peptides), dendrimers, non-cationic polymers, nanoparticles and bioconjugates, are usually safe and easy to manufacture. In this article, some of the most popularly used lipid and polymer-based nucleic acid carriers and their components are discussed, including in terms of their characteristics, applications, advantages and limitations.

3. Cationic lipids

Cationic lipids are the most commonly used transfection reagents for nucleic acid delivery. Significant progress has been made in the design and functionalization of cationic lipids since the introduction of *N*-[1-(2,3-dioleoyloxy)propel]-*N,N,N*-trimethylammonium (DOTMA). Other commonly used cationic lipids for nucleic acid delivery include: 2,3-dioleyloxy-*N*-[2-spermine carboxamide] ethyl-*N,N*-dimethyl-1-propanammonium trifluoroacetate (DOSPA,

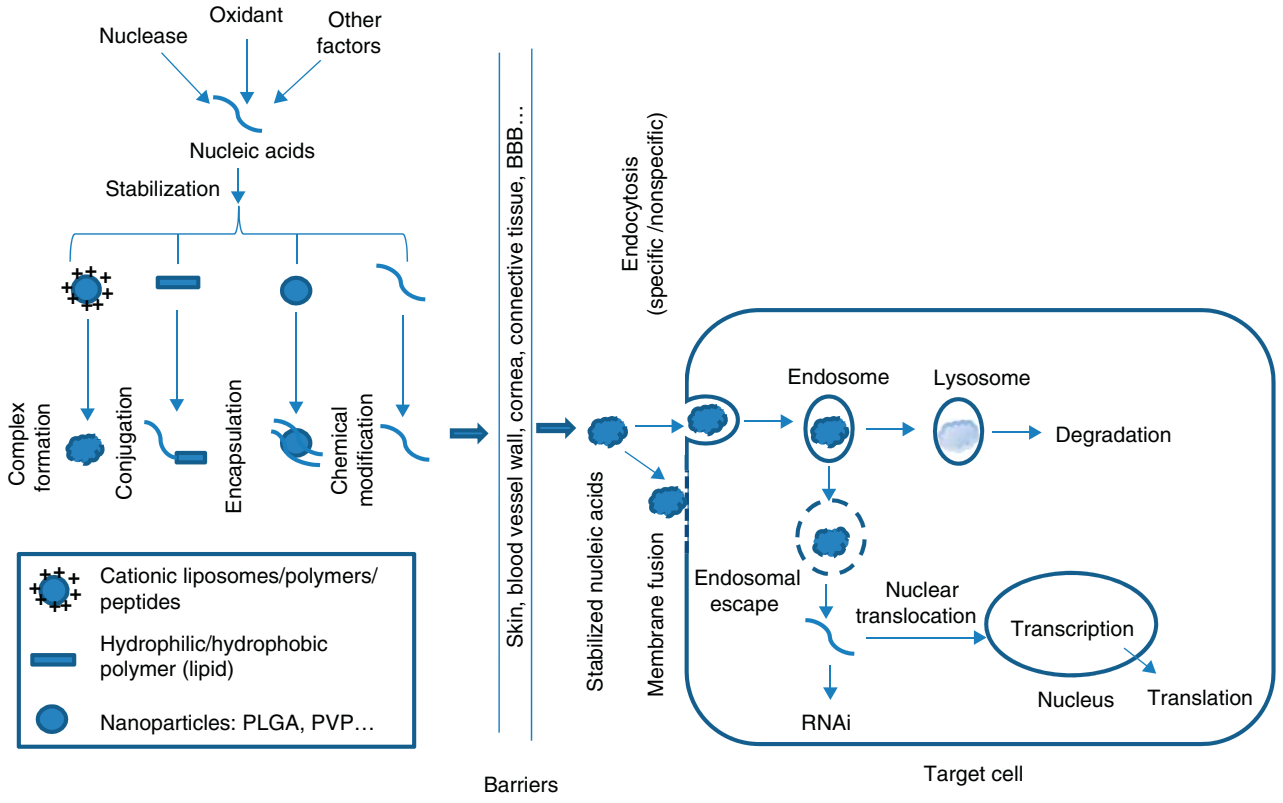


Figure 1. The barriers and strategies of nucleic acid delivery.

Lipofectamine); 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP); *N*-[1-(2,3-dimyristyloxy) propyl]-*N*,*N*-dimethyl-*N*-(2-hydroxyethyl) ammonium bromide (DMRIE), 3-β-[*N*-(*N*,*N*′-dimethylaminoethane) carbamoyl] cholesterol (DC-Chol); dioctadecyl amidoglyceryl spermine (DOGS, Transfectam); and imethyldioctadecylammonium bromide (DDAB) [18-22]. A few such lipids have been tested in initial clinical studies. However, many of these lipids, such as DC-Chol, DMRIE and GL-67, although effective *in vitro,* have proved inefficient *in vivo,* especially when compared with viral vectors [22,23]. Some other promising cationic lipids have also been studied. For example, various types of pyridinium lipid containing one or more pyridinium cationic head groups, which were designed by Mahato's [24] and other groups [25,26], showed high transfection efficiency and low cytotoxicity even at the high concentration of serum in the transfection medium [24].

Although the reported cationic lipids are structurally different, most of them contain three basic components, the cationic head group, the hydrophobic domain and the linker connecting the head group with hydrophobic group (**Table 1**). As the three parts of a cationic lipid significantly influence the transfection efficiency and cytotoxicity of the corresponding lipoplexes formed by cationic lipids (liposomes) and nucleic acids, a thorough understanding of these structural parameters is essential for the rational design of efficient cationic lipid-based transfection reagents.

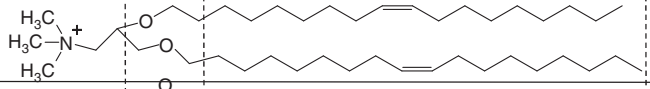
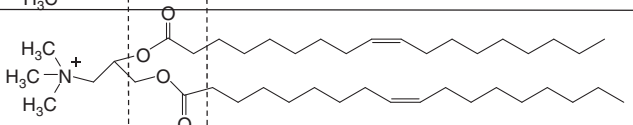
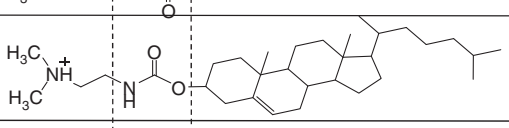
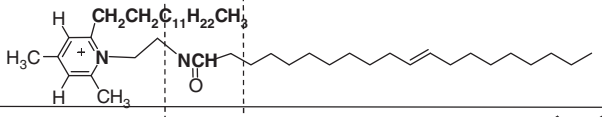
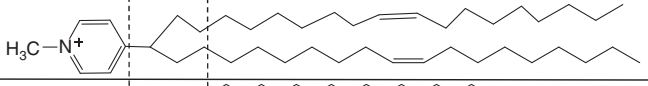
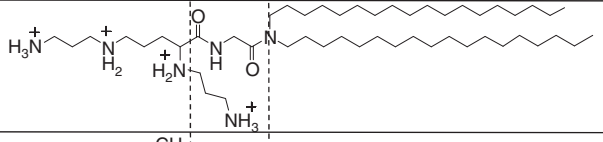
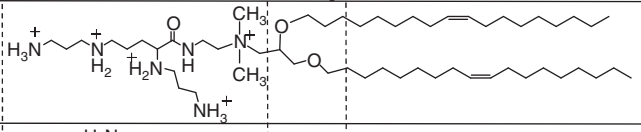
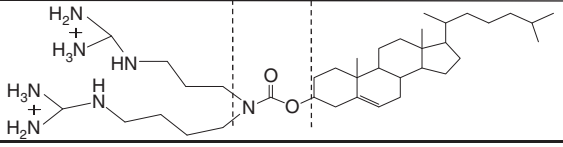
3.1 Cationic head group

The positively charged hydrophilic head group of cationic lipids usually consists of monoamine such as tertiary and quaternary amines, polyamine, amidinium, or guanidinium group. A pyridinium heterocyclic ring in the cationic amphiphile, first introduced in the 1940s, has been shown to have antiseptic and antibiotic properties. A series of pyridinium lipids have been developed, and some of them have been reported to reach or surpass the performance of commercially available transfection reagents both *in vitro* and *in vivo* [24,25,27]. In addition to pyridinium cationic lipids, other types of heterocylic head group have also been investigated, such as imidazole, piperizine and amino acid [28,29]. The main function of cationic head groups is to condense negatively charged nucleic acids by means of electrostatic interaction to slightly positively charged nanoparticles, leading to enhanced cellular uptake [30] and endosomal escape [31].

3.2 Hydrophobic lipid anchor group

The hydrophobic domains usually contain either simple aliphatic hydrocarbon chains such as fatty acid chains of various lengths and unsaturation states, or steroids such as cholesterol,

Table 1. The structures of commonly used cationic lipids.

Name	Cationic head	Linker	Hydrophobic anchor chain	Structural components
DOTMA				Monoamine, ether linker, two unsaturated fatty acid chains
DOTAP				Monoamine, ester linker, two unsaturated fatty acid chains
DC-cholesterol				Monoamine, carbamate linker, cholesterol
Pyridinium lipid				Pyridinium ring, amid linker, unsaturated fatty acid chains
SAINT 2				Pyridinium ring, aliphatic linker, two unsaturated fatty acid chains
DOGS				Polyamine, amid linker, two saturated fatty acid chains
DOSPA				Polyamine, ether linker, two unsaturated fatty acid chains
BGSC				Polyamine, carbamate linker, cholesterol

which helps in forming liposomes and exchanging lipids of cell membrane [32]. The type and length of aliphatic chain influence the transfection efficiency [24]. The most effective cationic lipids have two linear fatty acid chains such as DOTMA, DOTAP and SAINT-2. In general, cationic lipids containing one and more than two hydrophobic chains might cause either toxic or poor transfection [33], although tetraalkyl lipid chain surfactant, the dimer of *N,N*-dioleyl-*N,N*-dimethylammonium chloride (DODAC), has shown enhanced transfection over DODAC [34].

The degree of unsaturation and configuration of the hydrophobic chain also influence transfection efficiency and cytotoxicity. The degree of unsaturation in the lipid structure affects the fusogenicity of the lipid (L_α to H_{II} transition) [35]. The double bond in the fatty acid chain decreased phase transition temperature of the lipids [24]. The decrease in phase transition temperature increased fusogenicity, leading to an increase in transfection efficiency [36]. To investigate the relevance of the configuration of the double bond and cationic lipids' performance, new pyridinium cationic lipids were synthesized with one double bond (*cis* or *trans*) in their hydrophobic carbon chain. All the *trans*-orientated lipids regardless of their hydrophobic chain lengths ($C_{16:1}$, $C_{18:1}$ and $C_{20:1}$) enhanced the transfection efficiency compared with their *cis*-orientated counterparts (**Figure 2**) [24]. This trend was in good agreement with a previous report by van der Woude *et al.* [25]. The influence of aliphatic chain length on the performance of cationic lipids is a little complicated. Felgner *et al.* reported that the transfection efficiency increased with a decrease in the hydrophobic chain length from C_{18} to C_{14} in DOTMA derivatives [37]. In the study of pyridinium cationic lipids, it was found that the unsaturated lipid $C_{16:1}$ had better transfection ability compared with $C_{18:1}$ and $C_{20:1}$ [24,27]. A similar trend was also found in DOTAP derivatives [28]. However, transfection efficiencies were higher for the lipids with longer alkyl chain ($C_{18} > C_{16} > C_{14} > C_{12}$) in the study of trilysine-based Gemini surfactants with ε,ε-linkage [38]. Takahashi *et al.*

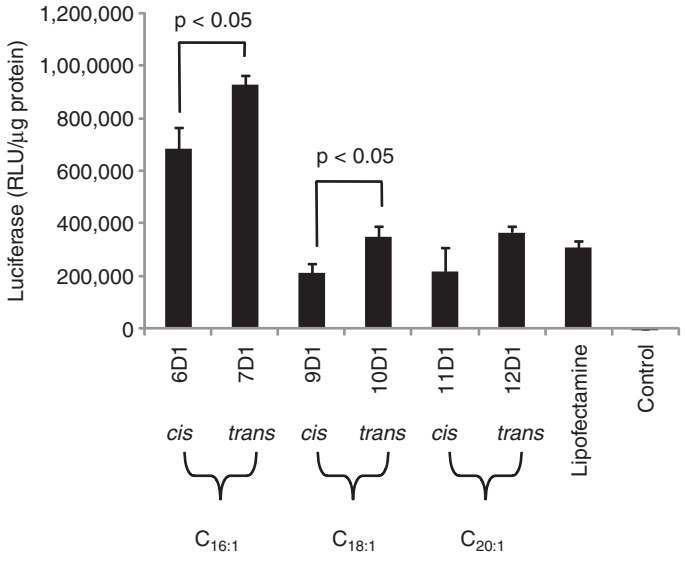


Figure 2. Influence of configuration (*cis* versus *trans*) of pyridinium lipids on transfection efficiency in CHO cells. Lipids 6 ($C_{16:1}$, amide linker, *cis*-isomer), 7 ($C_{16:1}$, amide linker, *trans*-isomer), 9 ($C_{18:1}$, amide linker, *cis*-isomer), 10 ($C_{18:1}$, amide linker, *trans*-isomer), 11 ($C_{20:1}$, amide linker, *cis*-isomer) and 12 ($C_{20:1}$, amide linker, *trans*-isomer) were used to prepare cationic liposomes with co-lipid DOPE at the molar ratio of 1:1. Lipoplexes were formed by mixing with luciferase plasmid at the charge ratio of 3:1(+/-). Luciferase gene expression in CHO cells was determined at 48 h after transfection. The dose of pcDNA3-Luc plasmid was 0.2 µg/well for 4×10^4 cells.

Reprinted with permission from [24].

BBB: Blood–brain barrier; D: DOPE.

also reported that polyamidoamine (PAMAM) dendron-bearing lipids with longer alkyl chain length showed higher transfection efficiency compared with their short chain counterparts [39]. The conflicting results with respect to hydrophobic chain length indicate that the transfection ability of cationic lipids is strongly influenced by both the cationic head group and its compatible hydrophobic group. A hypothesis based on 'dimensionless packing parameter, $p = V/a \bullet l$' [40] may explain why these experimental data showed conflict. Here, V denotes the hydrophobic chain volume, a the optimal cross-sectional head group area and l the length of the hydrophobic tails. When $\frac{1}{2} < p \leq 1$, the DNA/cationic liposomes complexes will undergo lamellar organization, L_α; whereas when $p > 1$, the complexes will prefer inverted structures, H_{II}. The L_α phase is more stable and resulted in a lower transfection potential compared with the H_{II} phase, which has a higher-order inverted hexagonal structure and is believed to have the ability to fuse with plasma membrane and trigger the endosomal escape, resulting in higher transfection efficiency [41,42].

3.3 Linker group

Stability, biodegradability and transfection efficiency of a cationic lipid depend greatly on the structure of the linker that binds the cationic head to the hydrophobic domain [43]. The most commonly used linkers include ether, ester, amide, or carbamate. Ether is stable and non-biodegradable when used as a linker. An enhanced gene transfection of cholesterol-based cationic lipids was observed when ether was used as the linker compared with that of the lipid containing either ester or urethane linker [44]. Both ester and amide are biodegradable linkages and show high transfection efficiencies in pyridinium lipids [26]. However, pyridinium lipids with amide linker were reported to be chemically stable with higher melting point but lower phase transition temperature, resulting in higher transfection efficiency compared with their ester linker counterparts (**Figure 3**) [24]; by contrast, ester was chemically not stable, but showed less toxicity [45]. Carbamate structurally shares the properties of both ester and amide and show high transfection efficiency and low toxicity when used as the linker in DC-Chol [21] and bis-guanidiniumspermidine-cholesterol (BGSC) [46]. As cleavable linkers show low cytotoxicity and can facilitate nucleic acid release from lipoplexes after their cleavage, the design of new types of cleavable linker is of interest, such as photosensitive [47], pH-sensitive [48] and redox-sensitive linkers [49].

Although the three basic components have been studied extensively, it should be mentioned that the performance of a cationic lipid in nucleic acid delivery is contributed by the entire molecule rather than the three independent parts. In summary, transfection efficiency of a cationic lipid greatly depends on: i) the extent of DNA or RNA condensation;

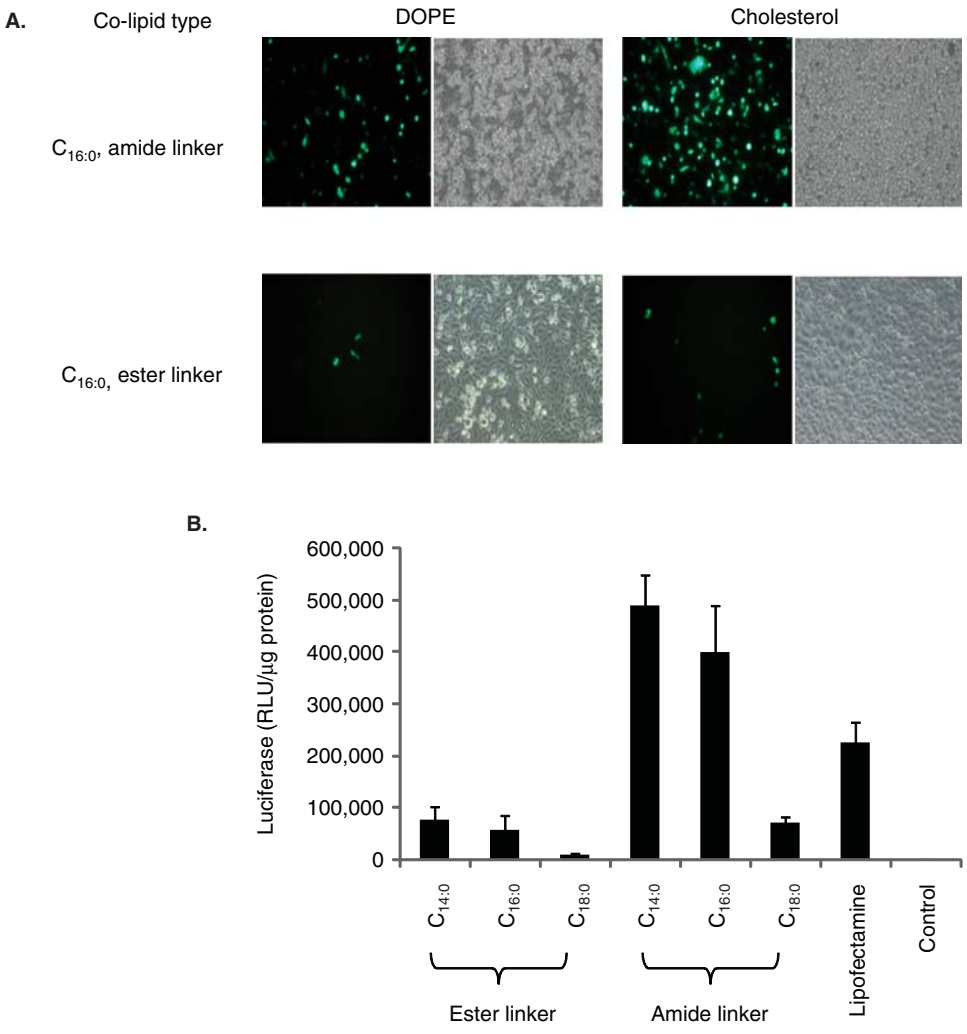


Figure 3. Influence of linker type (ester versus amide) of pyridinium lipids on transfection efficiency in CHO cells. A. $C_{16:0}$ lipid with amide linker and $C_{16:0}$ lipid with ester linker were used to prepare cationic liposomes with co-lipid DOPE and cholesterol at the molar ratio of 1:1. Lipoplexes were formed by mixing with GFP plasmid at the charge ratio of 3:1 (+/-). GFP gene expression in CHO cells was observed at 48 h after transfection using fluorescence microscopy. The dose of pCMS-EGFP plasmid was 0.4 μg/well for 4×10^4 cells. B. Lipids with ester linker and lipids with amide linker were used to prepare cationic liposomes with DOPE at the molar ratio of 1:1. Lipoplexes were formed by mixing with luciferase plasmid at the charge ratio of 3:1(+/-). Luciferase gene expression in CHO cells was determined at 48 h after transfection. The dose of pcDNA3-Luc plasmid was 0.2 μg/well for 4×10^4 cells.
Reprinted with permission from [24].

ii) enhanced cellular uptake resulting from ionic and/or hydrophobic interaction with biological surfaces; and iii) membrane fusion by means of transient membrane destabilization to achieve delivery into cytoplasm while avoiding degradation in the lysosomal compartment. However, for siRNAs, the complete condensation may not be achieved and the stability of siRNA/lipid complexes is relatively low compared with that of plasmid DNA/lipid complexes [50]. In this condition, other mechanisms such as temporary destabilization or pore forming of cell membrane may play a key role in siRNA transfer.

4. Cationic polymers

Cationic polymers readily form complexes with nucleic acids by means of electrostatic interaction and create a net positive charge under appropriate conditions. This facilitates cell attachment, subsequent internalization by endocytosis or membrane fusion, and endosomal escape by proton sponge [51]. The structures of cationic polymers are very different, including linear polymers such as chitosan and linear poly (ethyleneimine), branched polymers such as branched poly (ethyleneimine), circle-like polymers such as cyclodextrin,

network (crosslinked) type polymers such as crosslinked poly (amino acid) (PAA), and dendrimers. The design of new functional polymers has become a hot area of research. To understand the properties and applications of these polymers in nucleic acid delivery, in the following section some of the cationic polymers most commonly used as transfection reagents are reviewed.

4.1 Polyethylenimine

Polyethylenimine (PEI) is one of the most widely used synthetic carriers for nucleic acid delivery [51]. Based on its structure, PEI exists as either linear PEI or branched PEI. PEI is a highly positively charged polymer. Linear PEI contains all secondary amines in its backbone except the terminal groups. By contrast, branched PEI contains primary, secondary and tertiary amino groups at the estimated ratio of 1:1:1 [52]. The different types of amine group have different pK_a values and could be protonated in different levels at a given pH. This confers PEI with a superior buffering capacity over a wide range of pH. After endocytosis, unprotonated amines will be protonated at acidic pH in endosome, which increases the influx of protons, chloride ions and water into endosome. The increased osmotic pressure causes the endosome to swell and rupture, as a result of which the endosomal content is released [53]. This is the widely accepted 'proton sponge' hypothesis. PEI's buffering capacity may protect its cargoes from degradation in the lysosomes and lead to early endosomal escape during the endosome's maturation process and its subsequent fusion with lysosome. The transfection efficiency and cytotoxicity of PEI depend greatly on its molecular mass. An increase in its molecular mass results in an increase of its transfection efficiency as well as its cytotoxicity [54] and adherence on cell membrane [55]. The most suitable molecular mass of PEI for complexation with DNA segments is between 5 and 25 kDa [56], although some groups reported that high (800 kDa) [54] and low (2 kDa) [57] molecular mass PEIs also showed good transfection at their preferred N/P ratios.

In addition to the effect of molecular mass, different behaviors were found between branched and linear PEI. Branched PEI has relatively stronger electrostatic interaction with DNA than linear PEI, resulting in higher DNA condensing efficiency and zeta potential and smaller particle size at equivalent dose of DNA [58]. Choosakoonkriang *et al.* demonstrated a similar buffering capacity for 25 kDa branched and linear PEIs, which is in the pH range 6.5 – 10.0, whereas a higher buffering capacity was observed with branched but not linear PEI in the pH range 4.0 – 6.0, when they were titrated with HCl [59]. Kircheis *et al.* reported that the DNA complexes formed by 25 kDa branched PEI were ~ 180 nm whereas the complexes formed by 22 kDa linear PEI aggregated at the same N/P ratio and ionic strength [60]. The 25 kDa branched PEI/pDNA nanoparticles also significantly increased lucifearase gene expression compared with the nanoparticles formed by the same molecular mass linear PEI at N/P ratios of 10 and above in HEK293, COS7, HeLa and HepG2 cells [58]. It is noted that these transfection studies were performed under serum-free conditions. However, Seib *et al.* found that > 85% of associated linear PEI was internalized compared with only 50% of internalization rate in the associated branched PEI although the total cell association with branched PEI was higher than that of linear PEI during the cellular uptake study [61]. These studies indicate that branched PEI is more effective in *in vitro* transfection in the absence of serum whereas linear PEI is less sensitive to serum and thus is suitable for transfection in the presence of serum and for *in vivo* delivery. Goula *et al.* reported that systemic administration of linear PEI/pDNA complexes into mice caused higher luciferase gene expression in the lung than in other organs [62]. In another study, Bolcato-Bellemin *et al.* demonstrated that intravenous injection of the complexes formed by short sticky overhangs (ssiRNAs) and linear PEI into mice significantly silenced luciferase gene expression in the lung [63]. Although both branched and linear PEI were widely used, their application for *in vivo* nucleic acid delivery is still not well accepted because of their high cytotoxicity [64].

4.2 Chitosan

Chitosan is produced from partial deacetylation of the naturally occurring chitin. It is a linear polysaccharide, which is composed of D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit) linked with randomly distributed β-(1,4) glycosidic bonds. Biodegradability, biocompatibilities, low immunogenicity and low cytotoxicity render chitosan one of the most prominent carriers for nucleic acid delivery [65-67]. The size and zeta potential, morphology, stability and biological effect of its polyplexes are strongly dependent on chitosan's molecular mass, degree of deacetylation, N/P ratio and pH [68,69]. An increase in molecular mass of chitosan increases the size of its polyplexes, resulting in an increase of transfection efficiency [70].

MacLaughlin *et al.* studied chitosans of different molecular masses ranging from 7 to 540 kDa and found that the particle size of chitosan/DNA complexes prepared at an N/P ratio of 6:1 with a plasmid concentration of 100 μg/ml increased significantly from 100 to 500 nm along with the increase of chitosan's molecular mass [70]. A similar trend was found by Katas and Alpar when they used chitosan hydrochloride to condense siRNAs [67]. Huang *et al.* also reported that low-molecular-mass chitosan was less efficient at condensing plasmid DNA, resulting in unstable polyplexes compared with its high-molecular-mass counterparts [71]. This may be because high-molecular-mass chitosan interacts with DNA not only by means of electrostatic interaction but also by means of effective chain entanglement [72]. However, in some cases high molecular mass cannot guarantee high gene transfection and silencing because the strong interaction force inside polyplexes might hinder the release of loaded nucleic acids [73,74]. These facts emphasize the importance of a fine balance between extracellular protection and intracellular release to obtain expected biological effects. As the positive charge of

chitosan comes from deacetylation, the degree of deacetylation is important for nucleic acid delivery. The commonly used chitosans are highly deacetylated, probably 80% or above [66,67,70], to expose more charge for condensing nucleic acids. Low deacetylation caused low transfection efficiency [71], although a similar condensation effect as high degree of deacetylation could be achieved at a relatively high N/P ratio. Liu *et al.* studied chitosan's protonation and its complexation with DNA at different pHs and found that amine groups were almost completely protonated at a pH below its pK_a (~ 6.5), resulting in increased electrostatic interaction between chitosan and DNA [75]. By contrast, the amine groups were hardly protonated at pH > 7.5, resulting in poor DNA condensation [76]. However, the low transfection efficiency was observed at pH < 6.5, owing to the hindrance of endosomal escape and cargo release, although chitosan could be highly protonated [76]. In addition to charge density, other characteristics also influence the performance of chitosan, including solubility, degradation and crystallinity [77,78]. Proper modification of chitosan with other polymers or ligands can improve its performance in the delivery of nucleic acids.

4.3 Cyclodextrin-based cationic polymers

Cyclodextrins (CDs) are naturally occurring cyclic oligosaccharides constituted by 6 – 8 (+) glucopyranoside units, linked by α-1,4-linkages, and well known as α-, β-, or γ-CD, respectively. They are topologically represented as torus-like macrorings with relatively hydrophobic inner cavities, where various molecules can be encapsulated and form supramolecular inclusion complexes. Therefore, CDs and their derivatives have been studied extensively for understanding the mechanism of molecular recognition and as drug carriers to enhance solubilization, stabilization and absorption [79]. As nucleic acids are negatively charged hydrophilic macromolecules, they cannot be trapped in native CD cavities such as small molecules. To use CDs for nucleic acid delivery, they have to be incorporated by cations or modified by other cationic polymers. Davis's group developed a linear β-CD-containing cationic polymer by polymerization of a bifunctional β-CD monomer, (2-aminoethanethio)-β-CD derivative, via the cationic linkage, dimethylsuberimidate [80]. Another modification was available by conjugating multiple oligoethylenimine (OEI) arms onto the α-CD core to form a star-shaped cationic polymer [81]. These kinds of modification of CDs were reported to enhance *in vitro* transfection efficiency and lower cytotoxicity.

The most important feature of CD-containing polymers is their capability to form inclusion complexes, which can be used as functional moieties for further modifications. Grafting PEG-adamantane on the β-CD moieties of DNA polyplexes was reported to stabilize polyplexes, decrease cytotoxicity and enhance transfection efficiency [82]. Recently, a new class of cationic supramolecules, CD-based polyrotaxanes, has been designed for gene delivery [83,84]. The new cationic polyrotaxane was composed of multiple OEI-grafted β-CDs that were threaded on PPO block and blocked on the two ends of a Pluronic® (PEO-PPO-PEO) triblock copolymer [85]. Cationic polyrotaxanes effectively condensed plasmid DNA to nanoparticles and showed low cytotoxicity and high transfection efficiency in BHK-21 and MES-SA cells [84].

4.4 Dendrimers

Dendrimer consists of a central core molecule, from which several highly branched arms 'grow' to form a tree-like structure with a manner of symmetry or asymmetry. Dendrimers have a unique architecture and properties that make dendritic polymers the focus of much research in drug and gene delivery as well as diagnostics and bioengineering. The most popularly used dendrimers are polyamidoamine (PAMAM) and polypropylenimine (PPI) dendrimers. PAMAM dendrimers are commonly synthesized starting from a core molecule, ethylenediamine or ammonia, and then esterizing the core with methylacrylate followed by amidation of the ester with ethylenediamine. The resulting polymer after one reaction circle is termed a generation (e.g., G2, G3, etc.). If the reactions terminate just after esterization, the term 'half generation' (e.g., G2.5, G3.5, etc.) is given to the dendritic polymer. For PPI dendrimers, the branched PPI units will grow from the core molecule, butylenediamine (DAB), by Michael addition followed by hydrogenation of nitrile groups [86].

As a result of their unique architecture, dendrimers adopt a planar-elliptical shape or spherical shape depending on the number of generations. Usually the inner structure is loose owing to the low density of molecules compared with the compact structure in the outer layer, because of the high density of molecules. The space inside dendrimers is favorable for small molecules to be encapsulated in. However, it seems impossible for nucleic acids because of their large size. Cationic dendrimers with the positive outer surface are commonly used for nucleic acid delivery. Dendrimers interact with biological membranes by their pore-forming ability, which induces small, transient pores near the contact site. The pore-forming ability depends on the charge density and generation of dendrimers. The higher the charge density or generation, the higher the transfection efficiency [86]; however, large dendrimers with high generation commonly show higher cytotoxicity than the dendrimers with low generation [87]. In general, the toxicity of dendrimers is lower than that of PEI, PLL or DEAE-dextran [64]. Another important aspect of dendrimers is their pH buffering capacity, which can cause endosomal disruption and facilitate the release of nucleic acids from the complexes [53]. As dendrimers show higher transfection efficiency and low cytotoxicity, dendrimer-based transfection reagents have already been commercialized, including PolyFect and SuperFect (QIAGEN, Valencia, CA, USA), Starburst® PAMAM dendrimers and Priostar® dendrimers (Dendritic Nanotechnologies, Mount Pleasant, MI, USA), and Astramol™ (DSM, Netherlands).

Dendrimers have similar or superior performance compared with cationic polymers or lipids [88,89]. However, only ~ 1% of PAMAM dendrimers (G3 and G4) was detected in blood circulation compared with > 60% of dendrimers accumulated in the liver at 1 h after intravenous injection into rats [90]. To use dendrimers as an *in vivo* delivery system, some improvement has to be made to decrease their nonspecific distribution and increase their efficacies. Conjugation of hydrophilic α-CD to G2 to G4 PAMAM dendrimers significantly increased their transfection efficiency and decreased their cytotoxicity, caused by highly dense positive charge even at high N/P ratio [91]. Taratula *et al.* reported that coating of PPI G5 dendrimer/siRNA nanoparticles with PEG-luteinizing hormone-releasing hormone (LHRH) peptide could stabilize siRNA complexes in the presence of plasma. These nanoparticles efficiently accumulated in the tumor area compared with unmodified particles [92]. Quaternization of primary amino groups, especially external amine, was also explored in high generation of PPI dendrimers to reduce their cytotoxicity [93]. However, the transfection efficiency might be decreased owing to insufficient surface charge [94].

5. Non-cationic lipids and polymers

Non-cationic polymers or lipids cannot condense nucleic acids by means of electrostatic interaction and in most cases they work as the constituents in cationic vectors. For example, DOPE and cholesterol are the two most commonly used neutral co-lipids for preparing cationic liposomes. The incorporation of fusogenic lipids such as DOPE can improve endosomal escape by membrane fusion between the liposomal and endosomal bilayers [95]. DOPE has been shown to be a major driving force in promoting transfection efficiency of SAINT-2 cationic lipids, as it undergoes L_α to H_{II} transition when dispersed in a physiological buffer [96]. The use of cholesterol as a co-lipid has been reported to show enhanced *in vivo* gene expression, as cholesterol-containing liposomes could efficiently interact with cell membrane in the presence of serum [97].

Some new materials have been created by conjugating lipids to polymers. Branched PEI-cholesterol water-soluble lipopolymer was synthesized by Han *et al.* [98] and Wang *et al.* [99]. These branched PEI-cholesterol conjugates self-assemble into cationic micelles with a diameter of ~ 50 nm in aqueous buffer and show enhanced transgene expression and low cytotoxicity compared with PEI control in CT-26 colon adenocarcinoma and 293 T human embryonic kidney transformed cells [98,99]. PEG is an FDA-approved safe molecule that can also be used to modify lipids or polymers to improve their properties.

Besides the above discussion, non-ionic polymers were designed and used independently for nucleic acid delivery. Pluronic, also called poloxamer, is a series of block copolymers based on ethylene oxide and propylene oxide. It is widely used in drug and gene delivery [100]. Enhanced gene expression was observed post-injection of plasmid DNA with SP1017, which is the combination of Pluronics L61 and F127 into rat skeletal muscle compared with polyvinyl pyrrolidone (PVP) [101]. Further study showed that SP1017 could improve DNA distribution inside muscular tissue, resulting in increased bioavailability, although Pluronic did not increase *in vitro* gene transfection and expression because it interacted with DNA by means of a weak force such as hydrogen bonds rather than strong electrostatic interaction [101]. Owing to the strong hydrogen binding activity, thiourea derivatives were used to prepare the stable lipoplexes with nucleic acids [102]. Leblond *et al.* synthesized a new lipopolythiourea with a T-shaped bisthiourea head group and found that the ratio of mol thiourea functions/mol DNA phosphate groups (UT/P) significantly influenced the particle size of lipoplexes and the plasmid DNA could be efficiently condensed at high UT/P ratios. It was also noted that the introduction of hydrophilic groups, a diol or a tetraol, at the terminus of the thiourea part facilitated the formation of the DNA formulation [103]. Another frequently reported non-ionic polymer is poly(lactic-*co*-glycolic acid) (PLGA) or its derivatives, by which nucleic acids can be encapsulated into the core or absorbed on the surface of PLGA nanoparticles [104,105].

Bioconjugation technology to deliver oligonulceotides or siRNAs is also being widely investigated. Oligonucleotides and siRNAs are easier to conjugate with lipids or polymers because they are relatively small compared with plasmids [106], which can lose their supercoiled configuration. Bioconjugation of ODNs and siRNAs is discussed in the following section.

6. Design elements for effective nucleic acid delivery

To achieve efficient delivery of nucleic acids, a delivery system should be designed based on the cargo's properties and desired therapeutic effect using lipids, polymers, or their combination.

6.1 Liposomal delivery system

Liposomes, the microscopic bubbles of amphiphilic lipids surrounding an aqueous interior, have a history of ~ 40 years. Given their biocompatibility, biodegradability, low toxicity and immunogenicity, liposomes have attracted attention in the past three decades as pharmaceutical carriers of great potential [107]. Liposomes are the most widely used carriers for *in vitro* and *in vivo* nucleic acid delivery. Although in some cases cationic lipids are used alone, most cationic lipids are used to prepare cationic liposomes that contain at least two components, the cationic lipid and neutral co-lipid. The selection of co-lipids is important, as they can significantly influence the overall performance of cationic liposomes. The authors used DOPE and cholesterol as the co-lipid to prepare pyridinium-lipid-based cationic liposomes to transfer plasmid DNA in CHO cells and found that both DOPE and cholesterol could enhance transgene expression when used

with each favorite cationic lipid [24]. The cationic lipids are usually considered to be transfection inefficient if they cannot work well with co-lipids to improve gene transfection. However, Mukherjee *et al.* proposed a different strategy that involved combination of co-lipids (DOPE, cholesterol and DOPC) in equimolar ratio. This approach could improve the transfection efficiencies of cationic lipids that had proved transfection inefficient when formulated with each of these co-lipids alone [108].

There is a strong relationship between the morphology of lipoplexes and their performance. A few models were introduced to predict and mimic the morphologies of lipoplexes, including an external model (DNA is adsorbed onto the surface of cationic liposomes), internal model (DNA is surrounded by cationic liposomes), beads (cationic liposomes) on a string (DNA) model, and globular model [109]. It was recently agreed that the 'phase models' of lipoplexes were more helpful in understanding their transfection abilities, including lamellar structure (L_α), inverted hexagonal structure [76] and intercalated hexagonal structure (H_I) [109]. Many excellent research and review papers have been published to address these issues [42,96,109,110]. In general, L_α phase lipoplexes are relatively stable and have low transfection activity, whereas H_{II} phase lipoplexes are unstable and show high propensity of lipid fusion, resulting in high transfection efficiency. Besides liposomal components, the charge ratio (N/P ratio) of cationic lipids/nucleic acids, preparation methods, ionic strength and temperature may also influence the lipoplex formation and morphology. Charge ratio was one of the most studied issues, and was found to influence significantly the morphology and transfection ability of lipoplexes. At high N/P ratios, lipid/DNA complexes were found to adopt a globular structure with the effective DNA condensation [111], whereas at low N/P ratio, the beads on a string structure could be formed with the addition of DNAs [112]. Highly condensed positively charged particles are easier for cells to take up. However, if the concentration of cationic liposomes is too high, it may cause cytotoxicity. The effect of lipoplexes' particle size on transfection efficiency was controversial. However, generally, large lipoplexes are more efficient at transfecting nucleic acids *in vitro* because large particles lead to fast sedimentation, maximum contact with cell membrane, and easier dissociation of lipoplexes post endocytosis. By contrast, small particles are much safer and appropriate for *in vivo* delivery of nucleic acids [109].

To design an *in vivo* liposomal delivery system, surface modification of cationic liposomes was reported to be effective. Sato *et al.* successfully used vitamin A-coupled cationic liposomes to specifically deliver siRNAs to hepatic stellate cells in fibrotic rats, by which they almost completely resolved liver fibrosis and prolonged the rat lifespan [113]. PEGylation of stabilized plasmid lipid particle (SPLP) or stable nucleic acid lipid particle (SNALP) could neutralize the positively charged surface of cationic lipids and provide a neutral, hydrophilic coating using a diffusible PEG-lipid conjugate, PEG2000-C-DMA, resulting in long circulation time and low immune response [114,115]. Systemic administration of anti-HBV siRNAs containing PEGylated SNALP significantly inhibited HBV DNA and HBsAg expression in an HBV mouse model [115].

6.2 Oligonucleotide and siRNA conjugates

Oligonucleotides and siRNAs are readily modified by functional molecules including lipids [116,117], polymers [118,119] and nanoparticles [120], by means of non-degradable or degradable linkage, such as acid-labile ester [118,121] and reducible disulfide bond [122,123], to improve their stability and pharmacokinetic behaviors [118]. Both the 3′ and the 5′ termini of single-strand oligonucleotides were used for conjugation [118,121]. For siRNAs, however, it was known that the antisense strand of siRNA worked as the template in RNA-induced Silencing Complex (RISC) for RNAi. It was further shown that the 5′ terminus of the antisense strand is more important than the 3′ terminus and determines RNAi activity [124-126]. Therefore, the 3′ and 5′ ends of sense strands are preferred for conjugation [106].

Cholesterol is a commonly used lipid for ODN and siRNA conjugation. After conjugation, the hydrophobicity will be increased, resulting in a change of biodistribution. Cheng *et al.* conjugated triplex-forming oligonucleotide (TFO) with cholesterol at its 3′ terminus by means of a disulfide bond and found that conjugation with cholesterol had little effect on the triplex-forming ability of TFO with target duplex DNA and enhanced hepatic uptake of ODNs after intravenous injection into rats (**Figure 4A**) [116]. To make apolipoprotein B (apoB) siRNA conjugate, Soutschek *et al.* linked cholesterol with the phosphorothioated antisense RNA oligonucleotide at the 3′ terminus via aminocaproic acid-pyrrolidine linkage followed by annealing with the complementary sense strand oligonucleotide. Systemic administration of siRNA-cholesterol conjugate resulted in silencing of apoB mRNA in the liver and jejunum as well as reduction of the total cholesterol level [117]. Water-soluble polymers with the functional terminal groups were also used to conjugate with nucleic acids, such as PEG [118,123] and *N*-(2-hydroxypropyl) methacrylamide (HPMA) (**Figure 4B**) [119]. The authors conjugated galactosylated poly(ethylene glycol) (Gal-PEG) to ODN by means of an acid-labile ester linkage of β-thiopropionate. Gal-PEG-ODN was found to be stable when incubated with rat serum, while the ester linkage was cleaved and ODN dissociated from the conjugate when environmental pH was 5.5. Systemic administration of Gal-PEG-ODN significantly enhanced cellular uptake of ODNs in hepatocytes because galactose residue is the specific ligand for asialoglycoprotein receptors on the surface of hepatocytes, whereas the accumulation of ODNs in the liver was significantly inhabited by pre-injection of Gal-BSA (**Figure 5**) [118].

PEGylated siRNA was studied by Park's group [123,127] and Katoka's group [121,128]. Like PEGylated ODN, PEGylation can protect siRNA from nuclease digestion, prolong the systemic circulation and confer a targeting effect if a targeting

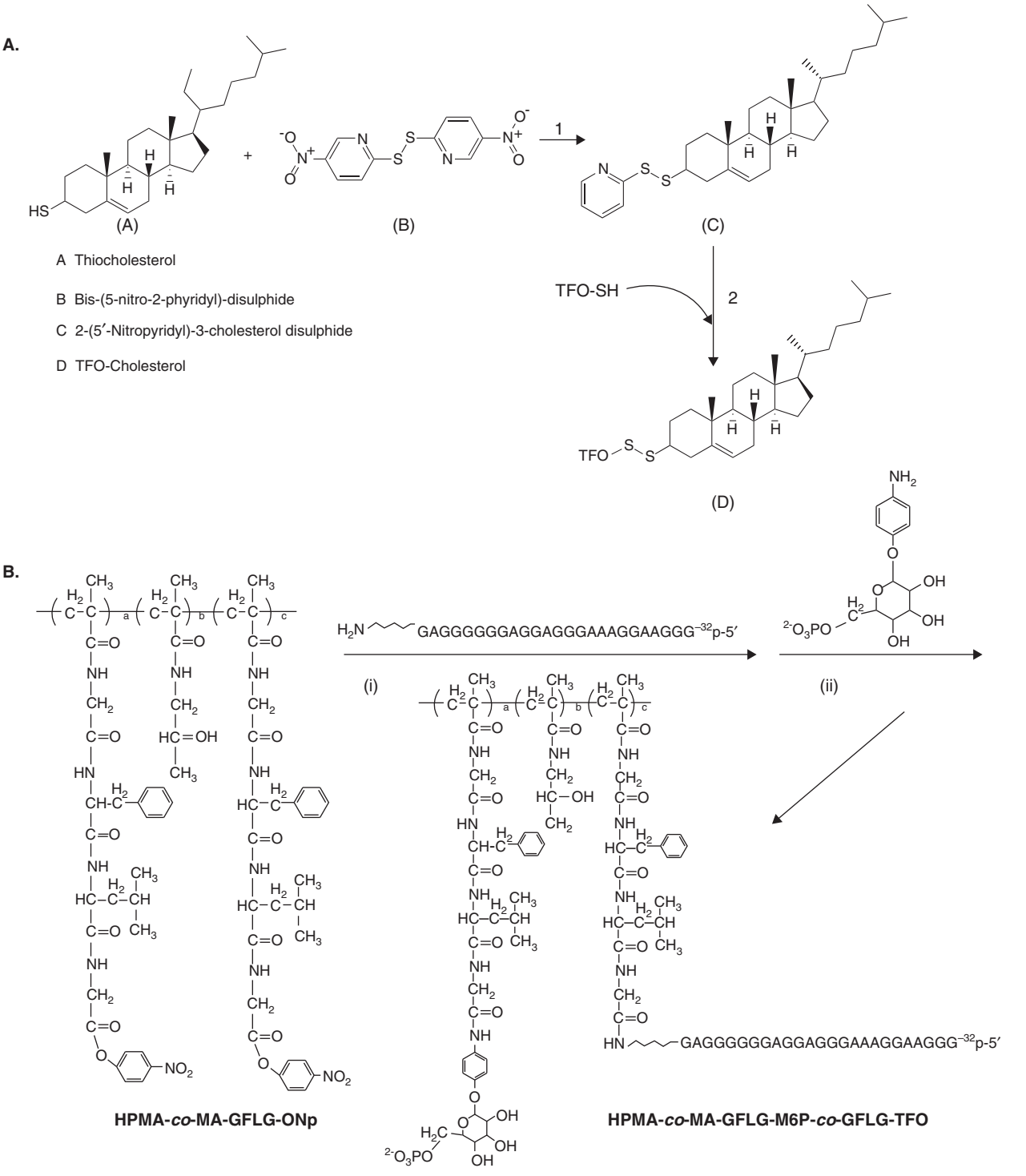


Figure 4. Conjugation of TFO to cholesterol (A) and HPMA (B). A. Thiocholesterol was reacted with bis-(5-nitro-2-pyridyl)-disulfide in pyridine at room temperature for 2 h to form 2-(5′-nitropyridyl)-3-cholesterol disulfide. Then, 2-(5′-nitropyridyl)-3-cholesterol disulfide was conjugated to TFO-SH in DMF under N_2 protection at 40°C for 24 h. B. Poly(HPMA-*co*-MA-GFLG-ONP) and TFO-NH_2 were reacted in the presense of *N,N′*-diisopropylethylamine (DIPEA) in DMSO at room temperature, followed by reaction with *p*-aminophenyl-6-phospho-α-D-mannopyranoside (papM6P) in the presense of DIPEA.

(**A**) Reprinted with permission from [116].

(**B**) Reprinted with permission from [119].

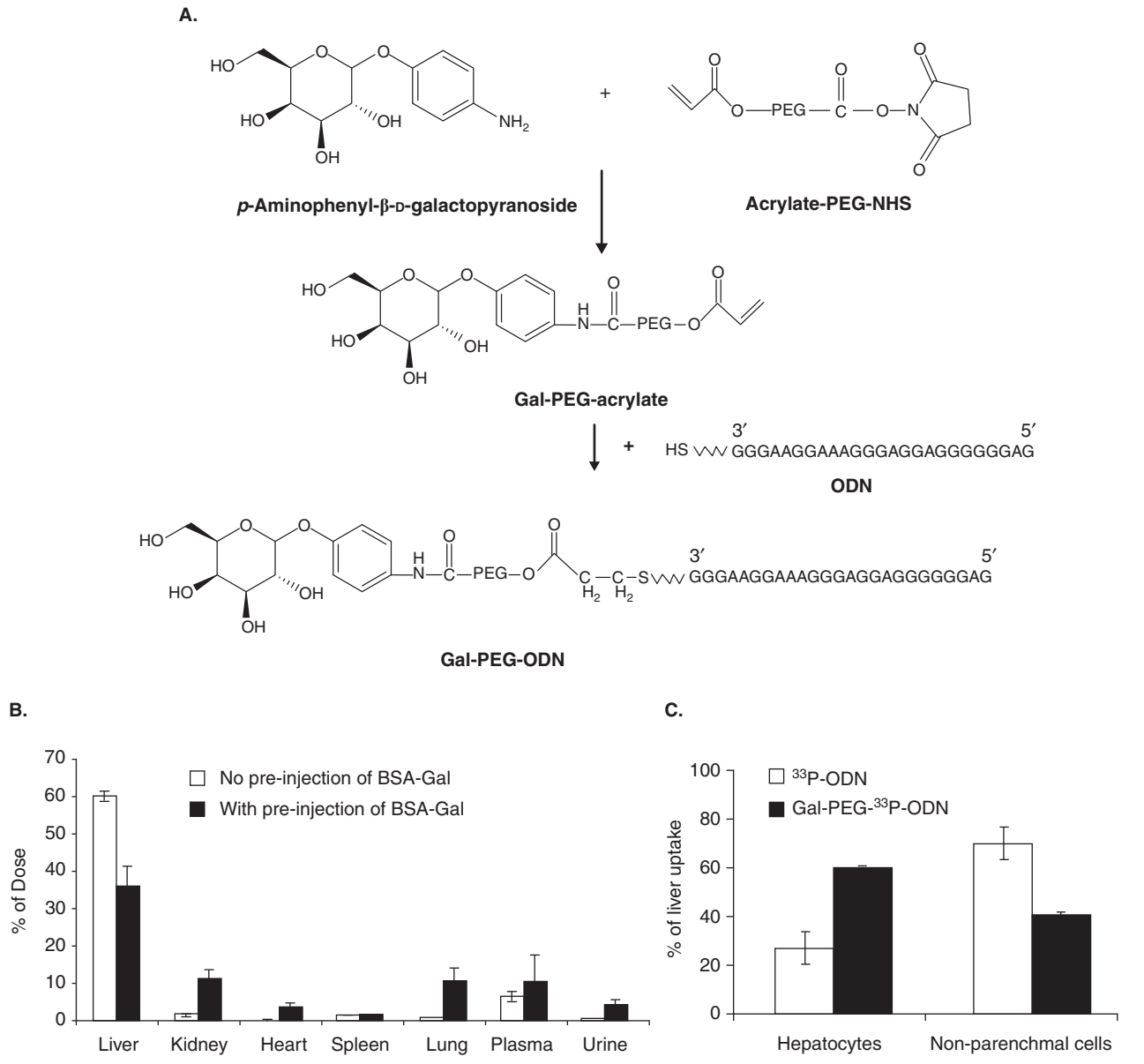


Figure 5. Synthesis scheme of Gal-PEG-ODN (A), biodistribution of Gal-PEG-ODN after intravenous injection (B) and hepatic localization of Gal-PEG-ODN (C). A. *p*-Aminophenyl-β-D-galactopyranoside reacted with acrylate-PEG-NHS in DMF. Then Gal-PEG-acrylate was conjugated to reduced 3′-thiol ODN by Michael addition in nuclease-free water. B. Gal-BSA (10 mg/kg) was injected intravenously into rats 2 min before the injection of Gal-PEG-^{33}P-ODN at a dose of 0.2 mg/kg. At 30 min post-injection, rats were killed, major organs were isolated and radioactivity was determined. C. The liver was perfused by collagenase/pronase digestion at 30 min post-injection of ^{33}P-ODN or Gal-PEG-^{33}P-ODN at a dose of 0.2 mg/kg. Parenchymal (hepatocytes) and non-parenchymal cells were separated and the associated radioactivity was measured.
Reprinted with permission from [118].

ligand is attached to PEG. In Park and co-workers' study, *N*-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) was first reacted with NH_2 at the 3′ terminus of the sense strand to produce a 2-pyridyl disulfide-activated siRNA, which could further react with the sulfhydryl group in PEG to form a disulfide bond. By contrast, Katoka and co-workers conjugated PEG to siRNA by means of acid-labile linkage. Both Park and Katoka's groups reported that PEGylated siRNA could spontaneously form polyelectrolyte complex (PEC) micelles with PEI [129] or PEGylated polyplexes with poly(L-lysine) (PLL) [128] via electrostatic interaction. After complex formation, siRNAs would be buried in the core and completely covered by hydrophilic PEG shell. This kind of nanoparticle was found to be significantly accumulated in

the tumor region in an *in vitro* three-dimensional tumor model [128] and tumor-bearing mice after systemic administration [129] owing to enhanced permeability and retention (EPR). To achieve efficient *in vivo* delivery of siRNA to hepatocytes, Dynamic PolyConjugate was designed by attaching PEG and the targeting ligand *N*-acetylgalactosamine (NAG) to a poly(vinyl ether) composed of butyl and amino vinyl ethers (PBAVE) by means of the reversible bifunctional maleamate linkages. Modification with PEG chain protected the polymer from nonspecific interaction and facilitated hepatocyte targeting. After endocytosis, the dissociation of PEG and NAG unmasked the positively charged amine groups on PBAVE, which triggered endosomal escape and the following siRNA release. Conjugation of siRNA to Dynamic PolyConjugate by means of disulfide bond was found to specifically silence two endogenous genes, apolipoprotein B (*apoB*) and peroxisome proliferator-activated receptor alpha (*ppara*), in mouse liver after systemic administration of this siRNA conjugate [130].

Although siRNA conjugates have been studied recently, it is still too early to see their benefits in the clinic. Most of these studies are still at an early stage and are focused on design and preparation. The therapeutic and side effects have to be tested thoroughly because the conjugate, in a sense, is more like a new drug molecule that has a different structure from its 'parents'.

6.3 Micro- and nanoparticle delivery system

Poly(DL-lactide-*co*-glycolide) (PLGA) is an FDA-approved polymer that is widely used for drug delivery. PLGA/nucleic acid particles could be made by conventional microsphere-making techniques, such as double emulsion (w/o/w) [104,131] and spray-drying techniques [105]. However, by these methods, the encapsulation efficiency of nucleic acids in PLGA particles was as low as < 1 μg/mg [105] because the hydrophilicity of nucleic acids was not compatible with the hydrophobic core of PLGA particles. Besides that, the hostile conditions of preparation and the decrease in pH during the hydrolysis of PLGA also inactivated the loaded nucleic acids [132]. Condensation of nucleic acids by cationic polymers before encapsulation in PLGA particles was reported to increase the encapsulation efficiency and protect the loaded nucleic acids from degradation. Capan *et al.* found complex formation with PLL (N/P 3:1 and 8:1) dramatically increased the encapsulation efficiency of supercoiled DNA in PLGA/DNA microparticles [133]. It was reported that other cationic materials, for example, PEI, DOTMA, DC-Chol, or CTAB, could be used to make cationic PLGA/DNA nanospheres by a modified o/w emulsion solvent-evaporation method [134]. Murata *et al.* prepared long-term sustained release PLGA microspheres encapsulating anti-VEGF siRNA/cationic polymer complexes (arginine or branched PEI) and observed the inhibition of tumor growth [104]. Modifcation of PLA moiety with cationic polymers can be an alternative strategy [135]. The hydrophobicity and molecular mass of PLGA also have profound influence on the encapsulation efficiency of nucleic acids [132]. Besides low encapsulation efficiency and inactivation of macromolecules, the slow drug release rate and burst release may hinder its application in delivery of nucleic acids.

7. Expert opinion

The success of gene therapy depends greatly on the delivery systems because of the nature of nucleic acids compared with that of small molecules. Cationic lipids and polymers are widely used transfection reagents for nucleic acid delivery. Each of them has its advantages and disadvantages. *In vivo* nucleic acid delivery is a complicated process that is influenced by many factors. For example, cationic lipids are excellent transfection reagents and can efficiently transfect nucleic acids *in vitro,* whereas they show low efficacies when administered intravenously [11] because of the immune response and nonspecific distribution. However, cationic lipid-formulated DNA vaccine was reported to enhance significantly the antigen-specific antibody responses compared with naked DNA vaccine via intramuscular injection, possibly owing to the induction of inflammatory cytokines such as IFN-γ, IL-12 and IL-6 [136]. PEGylation can significantly decrease nonspecific distribution and unexpected immune response. The cleavable linkage between PEG moiety and cationic lipid or polymer is preferred because the positively charged surface has to be exposed to trigger endocytosis and endosomal escape. Modification of gene carriers with ligands may be another efficient way to impart cell-specific delivery. The interaction, either electrostatic or other forces, between nucleic acids and cationic carriers should be strong enough to make sure the lipo(poly)-plexes are stable before cellular internalization. Thereafter, the complexes commonly locate in endosomes. The endosomal escape is an important step, which can be enhanced by the 'proton sponge effect' or lipid fusion. Then the loaded nucleic acids should be dissociated from the complexes and released in cytoplasm or translocated into the nucleus. Any problems in each step may cause the whole delivery process to fail. In this area, a great deal of work needs to be done so that we can have a comprehensive understanding of the nucleic acid delivery process. The commonly used cationic carriers can also be used for siRNA delivery. However, siRNA is a short molecule compared with DNA and has different behaviors when mixed with cationic carriers. The optimized delivery condition for DNA may not be suitable for siRNA delivery [50]. A variety of specific delivery systems have been developed to target siRNAs effectively to malignant cells. For example, lipid- or polymer-based siRNA conjugates have been shown to be effective *in vitro* and *in vivo* [106]. As most siRNA conjugates are not positively charged, they are more suitable for *in vivo* delivery.

The side effects of lipid and polymeric carriers have to be studied thoroughly, although they have been reported to be safer than viral vectors. The therapeutic applications of these carriers are still limited, although some exciting progress has been achieved in various research areas, including increased understanding of the biological processes involved in nucleic acid delivery, the technology to increase

the chemical stability and therapeutic efficacy of nucleic acids, and the innovative design of new functional materials and delivery systems. There is still a long way to go to design a perfect lipid or polymeric nucleic acid delivery system.

Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Fire A, Xu S, Montgomery MK, et al. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 1998;391(6669):806-11
2. Wolff JA, Malone RW, Williams P, et al. Direct gene transfer into mouse muscle in vivo. Science 1990;247(4949 Pt 1):1465-8
3. Gill HS, Prausnitz MR. Coated microneedles for transdermal delivery. J Control Release 2007;117(2):227-37
4. Barker GA, Diamond SL. RNA interference screen to identify pathways that enhance or reduce nonviral gene transfer during lipofection. Mol Ther 2008;16(9):1602-8
5. Yang NS, Sun WH. Gene gun and other non-viral approaches for cancer gene therapy. Nat Med 1995;1(5):481-3
6. Suzuki R, Takizawa T, Negishi Y, et al. Effective gene delivery with novel liposomal bubbles and ultrasonic destruction technology. Int J Pharm 2008;354(1-2):49-55
7. Liu F, Song Y, Liu D. Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. Gene Ther 1999;6(7):1258-66
8. Bramsen JB, Laursen MB, Nielsen AF, et al. A large-scale chemical modification screen identifies design rules to generate siRNAs with high activity, high stability and low toxicity. Nucleic Acids Res 2009;37(9):2867-81
9. Gao X, Kim KS, Liu D. Nonviral gene delivery: what we know and what is next. AAPS J 2007;9(1):E92-104
10. Judge AD, Sood V, Shaw JR, et al. Sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA. Nat Biotechnol 2005;23(4):457-62
11. Song YK, Liu F, Chu S, Liu D. Characterization of cationic liposome-mediated gene transfer in vivo by intravenous administration. Hum Gene Ther 1997;8(13):1585-94
12. Mahato RI, Takakura Y, Hashida M. Nonviral vectors for in vivo gene delivery: physicochemical and pharmacokinetic considerations. Crit Rev Ther Drug Carrier Syst 1997;14(2):133-72

• **A comprehensive review on physicochemical and pharmacokinetic properties of nonviral delivery systems.**

13. Santel A, Aleku M, Keil O, et al. A novel siRNA-lipoplex technology for RNA interference in the mouse vascular endothelium. Gene Ther 2006;13(16):1222-34
14. Suda T, Liu D. Hydrodynamic gene delivery: its principles and applications. Mol Ther 2007;15(12):2063-9
15. Seow Y, Wood MJ. Biological gene delivery vehicles: beyond viral vectors. Mol Ther 2009;17(5):767-77
16. Zaiss AK, Muruve DA. Immune responses to adeno-associated virus vectors. Curr Gene Ther 2005;5(3):323-31
17. Manjunath N, Wu H, Subramanya S, Shankar P. Lentiviral delivery of short hairpin RNAs. Adv Drug Deliv Rev 2009;61(9):732-45
18. 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):6982-6
19. Rose JK, Buonocore L, Whitt MA. A new cationic liposome reagent mediating nearly quantitative transfection of animal cells. Biotechniques 1991;10(4):520-5
20. Porteous DJ, Dorin JR, McLachlan G, et al. Evidence for safety and efficacy of DOTAP cationic liposome mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis. Gene Ther 1997;4(3):210-18
21. Gao X, Huang L. A novel cationic liposome reagent for efficient transfection of mammalian cells. Biochem Biophys Res Commun 1991;179(1):280-5
22. San H, Yang ZY, Pompili VJ, et al. Safety and short-term toxicity of a novel cationic lipid formulation for human gene therapy. Hum Gene Ther 1993;4(6):781-8
23. Scheule RK, St George JA, Bagley RG, et al. Basis of pulmonary toxicity associated with cationic lipid-mediated gene transfer to the mammalian lung. Hum Gene Ther 1997;8(6):689-707
24. Zhu L, Lu Y, Miller DD, Mahato RI. Structural and formulation factors influencing pyridinium lipid-based gene transfer. Bioconjug Chem 2008;19(12):2499-512
25. van der Woude I, Wagenaar A, Meekel AA, et al. Novel pyridinium surfactants for efficient, nontoxic in vitro gene delivery. Proc Natl Acad Sci USA 1997;94(4):1160-5
26. Ilies MA, Seitz WA, Johnson BH, et al. Lipophilic pyrylium salts in the synthesis of efficient pyridinium-based cationic lipids, gemini surfactants, and lipophilic oligomers for gene delivery. J Med Chem 2006;49(13):3872-87
27. Ilies MA, Seitz WA, Ghiviriga I, et al. Pyridinium cationic lipids in gene delivery: a structure-activity correlation study. J Med Chem 2004;47(15):3744-54
28. Heyes JA, Niculescu-Duvaz D, Cooper RG, Springer CJ. Synthesis of novel cationic lipids: effect of structural modification on the efficiency of gene transfer. J Med Chem 2002;45(1):99-114
29. Kumar VV, Pichon C, Refregiers M, et al. Single histidine residue in head-group region is sufficient to impart remarkable gene transfection properties to cationic lipids: evidence for histidine-mediated membrane fusion at acidic pH. Gene Ther 2003;10(15):1206-15
30. Miller CR, Bondurant B, McLean SD, et al. Liposome-cell interactions

in vitro: effect of liposome surface charge on the binding and endocytosis of conventional and sterically stabilized liposomes. Biochemistry 1998;37(37):12875-83

31. Tseng YC, Mozumdar S, Huang L. Lipid-based systemic delivery of siRNA. Adv Drug Deliv Rev 2009;61(9):721-31
32. Mahato RI, Rolland A, Tomlinson E. Cationic lipid-based gene delivery systems: pharmaceutical perspectives. Pharm Res 1997;14(7):853-9
33. Byk G, Dubertret C, Escriou V, et al. Synthesis, activity, and structure – activity relationship studies of novel cationic lipids for DNA transfer. J Med Chem 1998;41(2):229-35
34. Gaucheron J, Wong T, Wong KF, et al. Synthesis and properties of novel tetraalkyl cationic lipids. Bioconjug Chem 2002;13(3):671-5
35. Szule JA, Fuller NL, Rand RP. The effects of acyl chain length and saturation of diacylglycerols and phosphatidylcholines on membrane monolayer curvature. Biophys J 2002;83(2):977-84
36. Heyes J, Palmer L, Bremner K, MacLachlan I. Cationic lipid saturation influences intracellular delivery of encapsulated nucleic acids. J Control Release 2005;107(2):276-87
37. Felgner JH, Kumar R, Sridhar CN, et al. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. J Biol Chem 1994;269(4):2550-61
38. McGregor C, Perrin C, Monck M, et al. Rational approaches to the design of cationic gemini surfactants for gene delivery. J Am Chem Soc 2001;123(26):6215-20
39. Takahashi T, Kojima C, Harada A, Kono K. Alkyl chain moieties of polyamidoamine dendron-bearing lipids influence their function as a nonviral gene vector. Bioconjug Chem 2007;18(4):1349-54
40. Kumar VV. Complementary molecular shapes and additivity of the packing parameter of lipids. Proc Natl Acad Sci USA 1991;88(2):444-8

• **An excellent paper on application of packing parameter equation.**

41. Koltover I, Salditt T, Radler JO, Safinya CR. An inverted hexagonal phase of cationic liposome-DNA complexes related to DNA release and delivery. Science 1998;281(5373):78-81
42. Scarzello M, Chupin V, Wagenaar A, et al. Polymorphism of pyridinium amphiphiles for gene delivery: influence of ionic strength, helper lipid content, and plasmid DNA complexation. Biophys J 2005;88(3):2104-13
43. Mahato RI, Smith LC, Rolland A. Pharmaceutical perspectives of nonviral gene therapy. Adv Genet 1999;41:95-156
44. Ghosh YK, Visweswariah SS, Bhattacharya S. Nature of linkage between the cationic headgroup and cholesteryl skeleton controls gene transfection efficiency. FEBS Lett 2000;473(3):341-4
45. Aberle AM, Tablin F, Zhu J, et al. A novel tetraester construct that reduces cationic lipid-associated cytotoxicity. Implications for the onset of cytotoxicity. Biochemistry 1998;37(18):6533-40
46. Vigneron JP, Oudrhiri N, Fauquet M, et al. Guanidinium-cholesterol cationic lipids: efficient vectors for the transfection of eukaryotic cells. Proc Natl Acad Sci USA 1996;93(18):9682-6
47. Nagasaki T, Taniguchi A, Tamagaki S. Photoenhancement of transfection efficiency using novel cationic lipids having a photocleavable spacer. Bioconjug Chem 2003;14(3):513-16
48. Boomer JA, Thompson DH, Sullivan SM. Formation of plasmid-based transfection complexes with an acid-labile cationic lipid: characterization of in vitro and in vivo gene transfer. Pharm Res 2002;19(9):1292-301
49. Tang F, Hughes JA. Introduction of a disulfide bond into a cationic lipid enhances transgene expression of plasmid DNA. Biochem Biophys Res Commun 1998;242(1):141-5
50. Spagnou S, Miller AD, Keller M. Lipidic carriers of siRNA: differences in the formulation, cellular uptake, and delivery with plasmid DNA. Biochemistry 2004;43(42):13348-56
51. Boussif O, Lezoualc'h F, Zanta MA, 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(16):7297-301
52. von Harpe A, Petersen H, Li Y, Kissel T. Characterization of commercially available and synthesized polyethylenimines for gene delivery. J Control Release 2000;69(2):309-22
53. Sonawane ND, Szoka FC Jr, Verkman AS. Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. J Biol Chem 2003;278(45):44826-31

•• **An excellent paper describing "proton sponge" effect.**

54. Ogris M, Steinlein P, Kursa M, et al. The size of DNA/transferrin-PEI complexes is an important factor for gene expression in cultured cells. Gene Ther 1998;5(10):1425-33
55. Fischer D, Bieber T, Li Y, et al. 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(8):1273-9
56. Neu M, Fischer D, Kissel T. Recent advances in rational gene transfer vector design based on poly(ethylene imine) and its derivatives. J Gene Med 2005;7(8):992-1009
57. Shin JY, Suh D, Kim JM, et al. Low molecular weight polyethylenimine for efficient transfection of human hematopoietic and umbilical cord blood-derived CD34+ cells. Biochim Biophys Acta 2005;1725(3):377-84
58. Intra J, Salem AK. Characterization of the transgene expression generated by branched and linear polyethylenimine-plasmid DNA nanoparticles in vitro and after intraperitoneal injection in vivo. J Control Release 2008;130(2):129-38
59. Choosakoonkriang S, Lobo BA, Koe GS, et al. Biophysical characterization of PEI/DNA complexes. J Pharm Sci 2003;92(8):1710-22
60. Kircheis R, Wightman L, Schreiber A, et al. Polyethylenimine/DNA complexes shielded by transferrin target gene expression to tumors after systemic application. Gene Ther 2001;8(1):28-40

61. Seib FP, Jones AT, Duncan R. Comparison of the endocytic properties of linear and branched PEIs, and cationic PAMAM dendrimers in B16f10 melanoma cells. J Control Release 2007;117(3):291-300

62. Goula D, Becker N, Lemkine GF, et al. Rapid crossing of the pulmonary endothelial barrier by polyethylenimine/DNA complexes. Gene Ther 2000;7(6):499-504

63. Bolcato-Bellemin AL, Bonnet ME, Creusat G, et al. Sticky overhangs enhance siRNA-mediated gene silencing. Proc Natl Acad Sci USA 2007;104(41):16050-5

64. Fischer D, Li Y, Ahlemeyer B, et al. In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. Biomaterials 2003;24(7):1121-31

65. Lee MK, Chun SK, Choi WJ, et al. The use of chitosan as a condensing agent to enhance emulsion-mediated gene transfer. Biomaterials 2005;26(14):2147-56

66. Liu X, Howard KA, Dong M, et al. The influence of polymeric properties on chitosan/siRNA nanoparticle formulation and gene silencing. Biomaterials 2007;28(6):1280-8

67. Katas H, Alpar HO. Development and characterisation of chitosan nanoparticles for siRNA delivery. J Control Release 2006;115(2):216-25

68. Borchard G. Chitosans for gene delivery. Adv Drug Deliv Rev 2001;52(2):145-50

69. Kim TH, Jiang HL, Jere D, et al. and Cho CS. Chemical modification of chitosan as a gene carrier in vitro and in vivo. Prog Polym Sci 2007;32:726-53

70. MacLaughlin FC, Mumper RJ, Wang J, et al. Chitosan and depolymerized chitosan oligomers as condensing carriers for in vivo plasmid delivery. J Control Release 1998;56(1-3):259-72

71. Huang M, Fong CW, Khor E, Lim LY. Transfection efficiency of chitosan vectors: effect of polymer molecular weight and degree of deacetylation. J Control Release 2005;106(3):391-406

72. Kiang T, Wen J, Lim HW, Leong KW. The effect of the degree of chitosan deacetylation on the efficiency of gene transfection. Biomaterials 2004;25(22):5293-301

73. Li XW, Lee DK, Chan AS, Alpar HO. Sustained expression in mammalian cells with DNA complexed with chitosan nanoparticles. Biochim Biophys Acta 2003;1630(1):7-18

74. Yang X, Yuan X, Cai D, et al. Low molecular weight chitosan in DNA vaccine delivery via mucosa. Int J Pharm 2009;375(1-2):123-32

75. Liu W, Sun S, Cao Z, et al. An investigation on the physicochemical properties of chitosan/DNA polyelectrolyte complexes. Biomaterials 2005;26(15):2705-11

76. Ishii T, Okahata Y, Sato T. Mechanism of cell transfection with plasmid/chitosan complexes. Biochim Biophys Acta 2001;1514(1):51-64

77. Tomihata K, Ikada Y. In vitro and in vivo degradation of films of chitin and its deacetylated derivatives. Biomaterials 1997;18(7):567-75

78. Kas HS. Chitosan: properties, preparations and application to microparticulate systems. J Microencapsul 1997;14(6):689-711

79. Uekama K, Hirayama F, Irie T. Cyclodextrin drug carrier systems. Chem Rev 1998;98(5):2045-76

80. Gonzalez H, Hwang SJ, Davis ME. New class of polymers for the delivery of macromolecular therapeutics. Bioconjug Chem 1999;10(6):1068-74

81. Yang C, Li H, Goh SH, Li J. Cationic star polymers consisting of alpha-cyclodextrin core and oligoethylenimine arms as nonviral gene delivery vectors. Biomaterials 2007;28(21):3245-54

82. Pun SH, Bellocq NC, Liu A, et al. Cyclodextrin-modified polyethylenimine polymers for gene delivery. Bioconjug Chem 2004;15(4):831-40

83. Li J, Yang C, Li H, et al. Cationic supramolecules composed of multiple oligoethylenimine-grafted-cyclodextrins threaded on a polymer chain for efficient gene delivery. Advanced Materials 2006;18(22):2969-74

84. Yang C, Li H, Wang X, Li J. Cationic supramolecules consisting of oligoethylenimine-grafted alpha-cyclodextrins threaded on poly (ethylene oxide) for gene delivery. J Biomed Mater Res A 2009;89(1):13-23

85. Li J, Loh XJ. Cyclodextrin-based supramolecular architectures: syntheses, structures, and applications for drug and gene delivery. Adv Drug Deliv Rev 2008;60(9):1000-17

•• **A comprehensive review on CD-based delivery carriers.**

86. Dufes C, Uchegbu IF, Schatzlein AG. Dendrimers in gene delivery. Adv Drug Deliv Rev 2005;57(15):2177-202

•• **A comprehensive review on dendrimers.**

87. Zinselmeyer BH, Mackay SP, Schatzlein AG, Uchegbu IF. The lower-generation polypropylenimine dendrimers are effective gene-transfer agents. Pharm Res 2002;19(7):960-7

88. Gebhart CL, Kabanov AV. Evaluation of polyplexes as gene transfer agents. J Control Release 2001;73(2-3):401-16

89. Hollins AJ, Benboubetra M, Omidi Y, et al. Evaluation of generation 2 and 3 poly(propylenimine) dendrimers for the potential cellular delivery of antisense oligonucleotides targeting the epidermal growth factor receptor. Pharm Res 2004;21(3):458-66

90. Malik N, Wiwattanapatapee R, Klopsch R, et al. Dendrimers: relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of 125I-labelled polyamidoamine dendrimers in vivo. J Control Release 2000;65(1-2):133-48

91. Kihara F, Arima H, Tsutsumi T, et al. Effects of structure of polyamidoamine dendrimer on gene transfer efficiency of the dendrimer conjugate with alpha-cyclodextrin. Bioconjug Chem 2002;13(6):1211-19

92. Taratula O, Garbuzenko OB, Kirkpatrick P, et al. Surface-engineered targeted PPI dendrimer for efficient intracellular and intratumoral siRNA delivery. J Control Release 2009;140(3):284-93

93. Schatzlein AG, Zinselmeyer BH, Elouzi A, et al. Preferential liver gene expression with polypropylenimine

dendrimers. J Control Release 2005;101(1-3):247-58

94. Braun CS, Vetro JA, Tomalia DA, et al. Structure/function relationships of polyamidoamine/DNA dendrimers as gene delivery vehicles. J Pharm Sci 2005;94(2):423-36

95. Farhood H, Serbina N, Huang L. The role of dioleoyl phosphatidylethanolamine in cationic liposome mediated gene transfer. Biochim Biophys Acta 1995;1235(2):289-95

96. Smisterova J, Wagenaar A, Stuart MC, et al. Molecular shape of the cationic lipid controls the structure of cationic lipid/dioleylphosphatidylethanolamine-DNA complexes and the efficiency of gene delivery. J Biol Chem 2001;276(50):47615-22

97. Crook K, Stevenson BJ, Dubouchet M, Porteous DJ. Inclusion of cholesterol in DOTAP transfection complexes increases the delivery of DNA to cells in vitro in the presence of serum. Gene Ther 1998;5(1):137-43

98. Han S, Mahato RI, Kim SW. Water-soluble lipopolymer for gene delivery. Bioconjug Chem 2001;12(3):337-45

99. Wang DA, Narang AS, Kotb M, et al. Novel branched poly(ethylenimine)-cholesterol water-soluble lipopolymers for gene delivery. Biomacromolecules 2002;3(6):1197-207

100. Kabanov AV, Batrakova EV, Alakhov VY. Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery. J Control Release 2002;82(2-3):189-212

101. Lemieux P, Guerin N, Paradis G, et al. A combination of poloxamers increases gene expression of plasmid DNA in skeletal muscle. Gene Ther 2000;7(11):986-91

102. Tranchant I, Mignet N, Crozat E, et al. DNA complexing lipopolythiourea. Bioconjug Chem 2004;15(6):1342-8

103. Leblond J, Mignet N, Leseurre L, et al. Design, synthesis, and evaluation of enhanced DNA binding new lipopolythioureas. Bioconjug Chem 2006;17(5):1200-8

104. Murata N, Takashima Y, Toyoshima K, et al. Anti-tumor effects of anti-VEGF siRNA encapsulated with PLGA microspheres in mice. J Control Release 2008;126(3):246-54

105. Jensen DM, Cun D, Maltesen MJ, et al. Spray drying of siRNA-containing PLGA nanoparticles intended for inhalation. J Control Release 2010;142(1):138-45

106. Jeong JH, Mok H, Oh YK, Park TG. siRNA conjugate delivery systems. Bioconjug Chem 2009;20(1):5-14

•• **A comprehensive review on siRNA conjugate.**

107. Klibanov AL, Maruyama K, Torchilin VP, Huang L. Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. FEBS Lett 1990;268(1):235-7

108. Mukherjee K, Sen J, Chaudhuri A. Common co-lipids, in synergy, impart high gene transfer properties to transfection-incompetent cationic lipids. FEBS Lett 2005;579(5):1291-300

109. Ma B, Zhang S, Jiang H, et al. Lipoplex morphologies and their influences on transfection efficiency in gene delivery. J Control Release 2007;123(3):184-94

110. Zuhorn IS, Oberle V, Visser WH, et al. Phase behavior of cationic amphiphiles and their mixtures with helper lipid influences lipoplex shape, DNA translocation, and transfection efficiency. Biophys J 2002;83(4):2096-108

111. Wiethoff CM, Gill ML, Koe GS, et al. The structural organization of cationic lipid-DNA complexes. J Biol Chem 2002;277(47):44980-7

112. Felgner PL, Ringold GM. Cationic liposome-mediated transfection. Nature 1989;337(6205):387-8

113. Sato Y, Murase K, Kato J, et al. Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone. Nat Biotechnol 2008;26(4):431-42

114. Ambegia E, Ansell S, Cullis P, et al. Stabilized plasmid-lipid particles containing PEG-diacylglycerols exhibit extended circulation lifetimes and tumor selective gene expression. Biochim Biophys Acta 2005;1669(2):155-63

115. Morrissey DV, Lockridge JA, Shaw L, et al. Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. Nat Biotechnol 2005;23(8):1002-7

116. Cheng K, Ye Z, Guntaka RV, Mahato RI. Enhanced hepatic uptake and bioactivity of type alpha1(I) collagen gene promoter-specific triplex-forming oligonucleotides after conjugation with cholesterol. J Pharmacol Exp Ther 2006;317(2):797-805

117. Soutschek J, Akinc A, Bramlage B, et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature 2004;432(7014):173-8

118. Zhu L, Ye Z, Cheng K, et al. Site-specific delivery of oligonucleotides to hepatocytes after systemic administration. Bioconjug Chem 2008;19(1):290-8

119. Yang N, Ye Z, Li F, Mahato RI. HPMA polymer-based site-specific delivery of oligonucleotides to hepatic stellate cells. Bioconjug Chem 2009;20(2):213-21

120. Derfus AM, Chen AA, Min DH, et al. Targeted quantum dot conjugates for siRNA delivery. Bioconjug Chem 2007;18(5):1391-6

121. Oishi M, Nagasaki Y, Itaka K, et al. Lactosylated poly(ethylene glycol)-siRNA conjugate through acid-labile beta-thiopropionate linkage to construct pH-sensitive polyion complex micelles achieving enhanced gene silencing in hepatoma cells. J Am Chem Soc 2005;127(6):1624-5

122. Muratovska A, Eccles MR. Conjugate for efficient delivery of short interfering RNA (siRNA) into mammalian cells. FEBS Lett 2004;558(1-3):63-8

123. Kim SH, Jeong JH, Lee SH, et al. PEG conjugated VEGF siRNA for anti-angiogenic gene therapy. J Control Release 2006;116(2):123-9

124. Schwarz DS, Hutvagner G, Du T, et al. Asymmetry in the assembly of the RNAi enzyme complex. Cell 2003;115(2):199-208

125. Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. Cell 2003;115(2):209-16

126. Schwarz DS, Hutvagner G, Haley B, Zamore PD. Evidence that siRNAs function as guides, not primers, in the Drosophila and human RNAi pathways. Mol Cell 2002;10(3):537-48

127. Kim SH, Jeong JH, Lee SH, et al. LHRH receptor-mediated delivery of siRNA using polyelectrolyte complex micelles self-assembled from siRNA-PEG-LHRH conjugate and PEI. Bioconjug Chem 2008;19(11):2156-62

128. Oishi M, Nagasaki Y, Nishiyama N, et al. Enhanced growth inhibition of hepatic multicellular tumor spheroids by lactosylated poly(ethylene glycol)-siRNA conjugate formulated in PEGylated polyplexes. ChemMedChem 2007;2(9):1290-7

129. Kim SH, Jeong JH, Lee SH, et al. Local and systemic delivery of VEGF siRNA using polyelectrolyte complex micelles for effective treatment of cancer. J Control Release 2008;129(2):107-16

130. Rozema DB, Lewis DL, Wakefield DH, et al. Dynamic polyconjugates for targeted in vivo delivery of siRNA to hepatocytes. Proc Natl Acad Sci USA 2007;104(32):12982-7

131. Ravi Kumar MN, Bakowsky U, Lehr CM. Preparation and characterization of cationic PLGA nanospheres as DNA carriers. Biomaterials 2004;25(10):1771-7

132. Walter E, Moelling K, Pavlovic J, Merkle HP. Microencapsulation of DNA using poly(DL-lactide-co-glycolide): stability issues and release characteristics. J Control Release 1999;61(3):361-74

133. Capan Y, Woo BH, Gebrekidan S, et al. Preparation and characterization of poly (D,L-lactide-co-glycolide) microspheres for controlled release of poly(L-lysine) complexed plasmid DNA. Pharm Res 1999;16(4):509-13

134. Takashima Y, Saito R, Nakajima A, et al. Spray-drying preparation of microparticles containing cationic PLGA nanospheres as gene carriers for avoiding aggregation of nanospheres. Int J Pharm 2007;343(1-2):262-9

135. Maruyama A, Ishihara T, Kim JS, et al. Nanoparticle DNA carrier with poly(L-lysine) grafted polysaccharide copolymer and poly(D,L-lactic acid). Bioconjug Chem 1997;8(5):735-42

136. Hermanson G, Whitlow V, Parker S, et al. A cationic lipid-formulated plasmid DNA vaccine confers sustained antibody-mediated protection against aerosolized anthrax spores. Proc Natl Acad Sci USA 2004;101(37):13601-6

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