13

Nanobiomaterials for Nonviral Gene Delivery

13.1 Introduction

Since its first clinical trial in 1990 (Anderson 1992), the field of gene therapy has grown exponentially and drawn more and more attention from the fields of biotechnology, pharmaceutical research, and medicine (Park et al. 2006; Ragusa et al. 2007). Despite the added attention, only a minor percentage of clinical trials utilizing gene delivery have reached phase III, which indicates the level of difficulty associated with the methods and the strict restrictions to such promising and at the same time risky treatments (Ragusa et al. 2007).

One important obstacle to gene therapy is the delivery of therapeutic polynucleotides past the plasma membrane and into the cells of interest. The delivery of naked DNA has yielded little success due mainly to limited cellular uptake; its use is only feasible in select tissues such as the skeletal muscle (Giannoukakis et al. 1999; Liu et al. 2001a). Because of this limitation in uptake, the development of efficient and safe delivery systems has been an essential component of gene therapy research. A good gene delivery vector should be able to effectively compact and protect DNA, bypass the immune system of the host, traverse the plasma membrane (typically through endocytosis), disrupt the endosomal membrane, and deliver the DNA into the nucleus (Mahato 1999). (In gene therapy, not only could DNA be delivered, but small interfering RNA [siRNA] molecules could be used instead for silencing the expression of specific host genes. The use of RNA entails different cellular goals versus DNA delivery. However, since this chapter is focused on gene delivery, siRNA delivery will not be discussed further.)
Gene delivery systems can be divided into two general categories: viral transduction systems and non-viral transfection systems. Although viral gene delivery vectors have an established history of efficacy, the nonviral delivery systems have merits in that there is limited-to-no induction of immune responses, there is virtually no limitation on the size of the genes that can be delivered, and the cost of production is relatively low (Lee and Kim 2005). For nonviral vectors, the size of the delivery complexes depends on the molecular weight of the vector, the ratio between the vector nitrogens and the DNA phosphates (termed the N:P ratio), and the salt concentration of the buffer solution. For example, complexes of poly(l-lysine) (PLL)/DNA with N:P ratios greater than 0.5 form either 25–50 nm toroids or 40–80 nm rods (Kwoh et al. 1999) and complexes of poly(ethyleneimine) (PEI)/DNA (using 25 kDa PEI) at N:P = 2.3:1 are homogenous 40–60 nm toroids (Tang and Szoka 1997). (The same group also reported that the size of PEI/DNA complexes ranged from 90 to 130 nm when dynamic light scattering was used, as opposed to the 40–60 nm seen when electron microscopy was used [Tang and Szoka 1997].) In general, the sizes of the complexes formed by the nonviral gene delivery vector and DNA fit into the nanoparticle category, which is usually defined as particles having diameters less than 100 nm. Using this definition, viral gene delivery vectors can also be considered nanoparticles due to the fact that viruses typically have a maximum dimension between 10 and 100 nm (www.nano.gov). In this chapter, we will focus upon the development of nonviral vectors used for gene therapy.

Apart from the polycations, another class of nonviral gene delivery materials is the cationic lipid. A solution of cationic lipids, often formed with neutral helper lipids, can be mixed with DNA to form a positively charged complex termed a lipoplex (Wasungu and Hoekstra 2006). Well-characterized and widely used commercial reagents for cationic lipid transfection include N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) (Felgner et al. 1987), [1,2-bis(oleoyloxy)-3-(trimethylammonio) propane] (DOTAP) (Leventis and Silvius 1990), 3β[N-(N,N’-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol) (Gao and Huang 1991), and dioctadecylamidoglycylspermine (DOGS) (Behr et al. 1989). Dioleoylphosphatidylethanolamine (DOPE), a neutral lipid, is often used in conjunction with cationic lipids because of its membrane destabilizing effects at low pH, which aid in endolysosomal escape (Farhood et al. 1995).

Many cationic lipid compounds have been formulated since the advent of DOTMA (Behr 1994; Farhood et al. 1994). Each lipid has different structural aspects that confer distinct characteristics to the lipid/DNA complex, which affect association with and uptake into the cell. However, the basic structure of cationic lipids mimics the chemical and physical attributes of biological lipids (Maurer et al. 1999). Cationic lipids used for gene delivery have a hydrophobic region that is often in the form of two fatty acid tails linked by a (glycerol) backbone molecule to a cationic head-group. The positive charge facilitates spontaneous electrostatic interaction with DNA as well as binding of the resulting lipoplexes to the negatively charged components of the cell membrane prior to cellular uptake (Nicolaou and Papahadjopoulos 1998). The use of a cation is a recurring theme for virtually all chemically mediated gene delivery vectors, including polymers, lipids, and nondegradable nanoparticles.

### 13.2 Cationic Polymers

DNA, when combined with sufficient amounts of cationic polymers, will condense into discrete entities known as polyplexes (Vuorimaa et al. 2008). 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. For example, in theory, the DNA could be completely complexed by the 25 kDa branched PEI at N:P ratios of 1.0, but agarose gel electrophoresis data imply that complete complexation does not occur below N:P = 2.0 (Zanta et al. 1997). Moreover, the surface charge concentrations (zeta potentials) of the polycation/DNA complexes, which can range from strongly negative to strongly positive (Erbacher et al. 1999), is also a function of the N:P ratio. For the branched 25 kDa PEI, the zeta potential is about −50 mV at N:P = 2, around 0 mV at N:P = 3, and around 20 mV at N:P = 10 (Zanta et al. 1997; Erbacher et al. 1999; Godbey et al. 1999a).
Positive zeta potentials indicate an excess of positive charges on the surface of the nanoparticles, which aids in their attachment to the negatively charged exterior of the plasma membrane, facilitating the internalization of the nanoparticles.

The most common cationic polymers used as nonviral gene delivery vectors include chitosan, PLL, PEI, poly(amide amine) (PAMAM) dendrimers, and select polypeptides.

13.2.1 Chitosan-Based Gene Delivery Systems

Chitosan is obtained by the alkaline deacetylation of chitin, which is the second most abundant polysaccharide in nature (Synowiecki and Al-Khateeb 2003). The main commercial sources of chitosan are the crustacean shell wastes of crabs, shrimps, and lobsters (Koping-Hoggard et al. 2001; Hejazi and Amiji 2003). Chitosan is a polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamines and N-acetyl-D-glucosamines, with different molecular weights (50–200 kDa), degrees of deacetylation (40%–98%), and viscosities (Illum 1998). Chitosan is biodegradable, biocompatible, and nontoxic at low molecular weights (10–50 kDa) (Lee et al. 2001). However, chitosan has shown concentration-dependent cytotoxicity in B16F10 cells in vitro (Carreño-Gómez and Duncan 1997). It has been suggested that the toxicity of chitosan is perhaps due to impurities in the chitosan polymers (Koping-Hoggard et al. 2001).

Chitosan is a robust vector with kinetics that differ from those of other polycations. In vitro transfections of Hela cells yielded positive results even in the presence of 10% serum, and gene expression increased over time to be 10 times more efficient than PEI over 96 h (Erbacher et al. 1998). In separate trials using 293 cells (Koping-Hoggard et al. 2001), it was found that the onset of gene expression using chitosan was slower than when PEI was used, but gene expression increased over time. After 120–144 h, chitosan yielded a maximal transgene expression in 293 cells that was lower, but statistically comparable, to that yielded by PEI. The group also reported that the transfection efficiency of chitosan in HT-1080 and Caco-2 cell lines was found to be much lower than that of PEI, which suggested that the transfection ability of chitosan was cell line dependent (Koping-Hoggard et al. 2001).

Because of the adhesive and transport properties of chitosan in the gastrointestinal (GI) tract, this polymer has also been used to form chitosan/DNA polyplexes for oral gene therapy applications (Roy et al. 1999; Chew et al. 2003). Chitosan/pCMV Arah2 (Arah2 is the dominant anaphylaxis-inducing antigen in mice sensitized to peanuts) has been administered into a strain of mouse from the Jackson Laboratory (AKR/J) mice as an oral immunization method for modulating peanut antigen-induced murine anaphylactic responses (Roy et al. 1999). Chitosan/pDerp1 (Derp1 is a major triggering factor for mite allergy) has been investigated for its potential as an oral vaccine against mite allergy with promising results (Chew et al. 2003). These two studies show that chitosan possesses characteristics suitable for oral vaccination and could potentially be used for oral gene delivery in general.

Derivatives of chitosan have been developed in an attempt to target specific cell types and improve transfection efficiencies. Examples of such modifications include conjugation with folate (Mansouri et al. 2006; Chan et al. 2007), thiolation (Lee et al. 2007), and glycolation (Yoo et al. 2005). These modifications reportedly did not affect the ability of chitosan to condense and compact DNA, but they did produce enhanced gene expression in the targeted cells.

13.2.2 PLL-Based Gene Delivery Systems

PLL is a well-known polycation that has been widely studied as a nonviral gene delivery vector since the first reported formation of PLL/DNA complexes (Laemmli 1975). PLL is a polypeptide of the essential amino acid L-lysine that can be produced via bacterial fermentation (Shima 1977). At physiological pH, each repeating unit of PLL carries a positive charge on the ε-amine of the side chain, a property that has been exploited to allow PLL to condense plasmid DNA to varying degrees depending upon salt concentration (Gonsho et al. 1994). However, aggregation and precipitation of PLL/DNA complexes have also been found to be dependent upon salt concentration (Liu et al. 2001b).
To combat aggregation, the surfactant dextran has been used to increase the solubility and stability of PLL/DNA complexes without considerably hindering the electrostatic interaction between PLL and DNA (Ferdous et al. 1998).

Although the structure of PLL appears to be suitable for gene delivery, unmodified versions of this polymer are associated with low transfection efficiencies and cytotoxicity. Partially gluconoylated PLL, by reaction with β-gluconolactone, has improved transfection efficiencies in HepG2 cells over those of unmodified PLL (Erbacher et al. 1997). The acylation with β-gluconolactone partially blocked the ε-amino groups of the PLL, and as a result the electrostatic interactions between the PLL and DNA were decreased, promoting dissociation between the plasmid and the carrier (Erbacher et al. 1997). Another modification to PLL has been the conjugation of poly(ethylene glycol) (PEG) at the ε-amino group, with the result of reduced toxicity and improved transfection efficiency (30-fold) in HepG2 cells (Erbacher et al. 1998a). Glycosylation has also been used in an attempt to decrease cytotoxicity (Boussif et al. 1999), and while improved cell survival was achieved with this approach, transfection efficiencies were low and remained comparable to unmodified PLL (Boussif et al. 1999).

Just as with other nonviral gene delivery vehicles, PLL had been conjugated with various ligands in an attempt to improve the specific cellular uptake of the targeted cells or tissues while at the same time reducing side effects to neighboring tissues. For example, lactose has been attached to PLL to target the asialoglycoprotein of hepatocytes (Midoux et al. 1993; Choi et al. 1998b). Folate has also been conjugated to PLL in an attempt to target the folate-overexpressing cancer cells (Cho et al. 2005).

### 13.2.3 PEI-Based Gene Delivery Systems

Branched PEI is produced by the acid-catalyzed polymerization of aziridine (Dick and Ham 1970). PEI has been employed in industry for years in processes such as shampoo manufacturing, paper production, and water purification. However, PEI was not, until 1995, introduced as a “versatile vector” for gene delivery (Boussif et al. 1995; Godbey et al. 2000). At that time, Boussif et al. (1995) used PEI as the vector to deliver plasmids coding for luciferase into various cell types including 3T3, HepG2, Cos-7, and chicken embryonic hypothalamic neurons, and into newborn mice. PEI has been a popular cationic gene delivery vehicle because of its relatively high transfection efficiency in a variety of cell lines (Boussif et al. 1996; Godbey et al. 2000; Oh et al. 2007; Yao et al. 2007; Ye et al. 2007). Like PLL, PEI/DNA complexes are prone to aggregate (Tang and Szoka 1997; Sharma et al. 2005), and overdosage of PEI is toxic to the cells. Many investigations have focused on the PEGylation of PEI in an attempt to reduce aggregation and the cytotoxicity (Ogris et al. 1999; Lee et al. 2001; Petersen et al. 2002; Shi et al. 2003). It has been shown that PEI cytotoxicity in 3T3 fibroblasts can be modulated by the degree of PEGylation, independent of the molecular weight of the PEG used (Petersen et al. 2002). This is consistent with observations that an overabundance of cations in gene delivery complexes is deleterious to cells.

In order for the transfecting complexes to reach cells of interest, many researchers have studied the conjugation of PEI with targeting moieties. An αβ3/αβ3 integrin-binding the tri-peptide Arginine-Glycine-Aspartate (RGD) peptide, ACDCRGDCFC, has been conjugated into PEI via a PEG spacer to target angiogenic human dermal microvascular endothelial cells (HDMEC) (Suh et al. 2002). The conjugated PEI/DNA complexes showed approximately five times greater transfection efficiencies in vascular endothelial growth factor (VEGF)-stimulated angiogenic HDMEC versus unconjugated PEI/DNA complexes. However, the conjugated PEI/DNA complexes showed much lower transfection efficiencies in angiostatic HDMEC than unconjugated PEI/DNA complexes, which suggested that the RGD conjugated PEI was highly selective toward angiogenic endothelial cells. The human epidermal growth factor receptor-2 (HER-2) has also been used for targeted gene delivery, in this case to target breast cancer cells (Chiu et al. 2004). The HER-2-conjugated PEI showed enhanced transfection efficiencies in HER-2 overexpressing human breast adenocarcinoma cells (Sk-BR3) as compared to unmodified PEI, but not in breast cancer cells expressing low levels of HER-2 (MDA-MB-231). Other molecules conjugated with PEI for targeting include anti-CD3 (Kircheis et al. 1997), transferrin (Ogris et al. 1998), and folate (Benns et al. 2001).
The linear form of PEI (L-PEI) is also being used for gene delivery. L-PEI also arises from cationic polymerization, but from a 2-substituted 2-oxazoline monomer (instead of aziridine). The polymerization product, for example, linear poly(N-formalethyleneimine) is then hydrolyzed to yield L-PEI. The linear form of PEI can also be obtained by the same process as that used to obtain branched PEI, but the reaction must take place at a relatively low temperature (reviewed and described in Tomalia and Killat 1985).

Linear PEI has been widely studied as a gene delivery vector, especially in the transfection of the lung (Goula et al. 1998; Goula et al. 2000; Uduehi et al. 2001a,b). It has been reported that L-PEI/DNA complexes can pass the capillary barrier in the lung to reach and transfect other pulmonary cell types with minimal-to-no toxicity (Goula et al. 1998; Goula et al. 2000). However, as separate investigations have shown, L-PEI-mediated gene delivery to rat lungs is associated with the moderate impairment of lung function (Uduehi et al. 2001a,b).

13.2.4 Dendrimer-Based Gene Delivery Systems

The first article using the term “dendrimer” was written by Tomalia et al. (1985) in which the preparation of PAMAM dendrimers was described in detail. At the same time though, Newkome et al. (1985), independently reported the synthesis of similar macromolecules that they termed arborols. “Dendrimer” is the term that is in general usage today.

Dendrimers are branched polymers that are synthesized in a stepwise fashion to control both monodispersity and the exact number of branching layers, or “generations” (Fréchet and Tomalia 2001). Dendrimers can be synthesized by either divergent or convergent methods (Tomalia and Fréchet 2002). For the divergent method, the dendrimer grows in a stepwise fashion outwards from a multifunctional core molecule (Figure 13.1). Slight structural defects can occur in larger molecules, especially at higher generation numbers. For the convergent method, the dendrimer is constructed beginning with the end groups and progressing inwards (Figure 13.1). Defective structures can be more readily separated with this method (Tomalia et al. 1985). Unlike hyperbranched polymers, dendrimers are polymerized in a tightly controlled, stepwise fashion to produce monodisperse sets of macromolecules. Because of their

![Diagram of Dendrimer Structures](#)

**FIGURE 13.1** Dendrimers can be constructed via divergent or convergent pathways. In the divergent pathway (left), the dendrimer is extended outward from a multifunctional core molecule, often ending with a functionalized terminal group. The convergent method (right) begins at the outer ends and polymerization extends toward what will be the interior of the dendrimer, ending with the addition of the core molecule.
globular shapes and the presence of internal cavities, dendrimers could be used to encapsulate guest molecules. Dendrimer/DNA complexes are often called dendriplexes to preserve terminology that is analogous with lipoplexes and polyplexes. PAMAM, and PAMAM derivatives have been investigated as gene delivery vectors both in vitro and in vivo (Bielsinska et al. 1996; Kukowska-Latallo et al. 2000; Kim et al. 2004; Huang et al. 2007). A commercially available dendrimeric formulation is sold under the name SuperFect™ (Qiagen, Valencia, CA). This reagent consists of activated dendrimers with a defined spherical architecture. The low pKₐ of the amines (3.9 and 6.9) afford the dendrimer the potential to buffer pH changes during the acidification of the endosome (Klajnert and Bryszewska 2001), which may contribute to the favorable transfection efficiencies associated with SuperFect/DNA complexes.

13.2.5 Peptide-Based Gene Delivery Systems

Poly(L-lysine) (PLL), as introduced earlier, was one of the first studied cationic peptides used to mediate gene delivery. However, as the length of PLL increases, so does the cytotoxicity. Moreover, the polydispersity of PLL complicates modifications with ligands, making the chemical synthesis of PLL conjugates hard to control (Martin and Rice 2007). To solve these problems, the synthesis of oligolysines was investigated and reported (Gottschalk et al. 1996; Wadhwa et al. 1997; Adami and Rice 1999; McKenzie et al. 2000; Yang et al. 2001; Kwok et al. 2003). A peptide with the sequence YKAK_WK (4 ≤ n ≤ 12) was designed to determine the minimum lysine required for DNA binding and gene expression (Gottschalk et al. 1996), and the results showed that an eight-lysine cluster was sufficient to condense DNA. Efficient gene expression in a variety of cell lines was also shown when an additional endosome-disruptive peptide was coupled to the lysine-containing sequence. Another peptide, CWKₙ (n = 3, 6, 8, 13, 16, 18, 26, and 36) was used to transfect HepG2 and Cos-7 cells (Wadhwa et al. 1997). Reports from this investigation showed a 40-fold reduction in particle size and a 1000-fold amplification in transfection efficiency for CWK_{18}/DNA condensates relative to K_{19}. Although oligopeptides containing lysine showed efficient gene transfection in vitro, they were not stable enough for in vivo transfection due to their low affinity for DNA. Glutaraldehyde (Adami and Rice 1999; Yang et al. 2001) and disulfide bonds (McKenzie et al. 2000; Kwok et al. 2003) have been used to cross-link the peptides in an attempt to stabilize the complexes, and results showed that the cross-linkage stabilized the peptide/DNA condensates and enhanced the metabolic stability of the carried DNA.

Another class of peptides that have been used for gene delivery was created with the goal of inducing endosome disruption. One of the oldest and most well-studied endosome-disruptive peptides is the INF peptide, which was derived from the amino-terminal sequence of the influenza virus hemagglutinin HA-2 (Wagner et al. 1992; Plank et al. 1994). The peptide has membrane perturbation activity that is triggered by an acidic environment. Other endosome-disruptive peptides include trans-activating transcriptional activator (TAT, a 86 amino acid peptide derived from the human immunodeficiency virus-1 [HIV-1]) (Frankel et al. 1988; Ruben et al. 1989), penetratin (a 16-mer peptide derived from the third α-helix of the homeodomain of Antennapedia) (Derossi et al. 1994), and transportan (a 27 amino acid peptide, containing the peptide sequence from the amino terminus of the neuropeptide galani) (Pooga et al. 1998). Both TAT and penetratin are arginine-rich peptides, and transportan consists of amphipathic helical peptides (Gupta et al. 2005). Synthetic amphipathic peptides have also been designed for endosome disruption, and include such molecules as model amphipathic peptide (MAP, an 18-mer peptide) (Oehlke et al. 1998), and GALA (a 30 amino acid peptide, containing a repeating unit of glutamic acid [G]-alanine [A]-leucine [L]-alanine [A]) (Subbarao et al. 1987).

13.3 Cationic Lipids

A cationic lipid is an amphipathic molecule that consists of a hydrophobic region and a hydrophilic region (Wasungu and Hoekstra 2006). The hydrophobic region usually consists of one or two hydrophobic fatty acid chains linked via a glycerol backbone to a cationic head group that can vary based
on how the lipid is being administered (in vitro or in vivo), and what cell lines are being transfected (Wasungu and Hoekstra 2006). A cholesterol-like moiety is sometimes used with or instead of a fatty acid. The hydrophobic group of the molecule allows for self-assembly into micelles or liposomes of varying morphologies, depending on the lipid being used (Tranchant et al. 2004).

There are a number of structures that are known to appear during polynucleotide compaction in the liposome. Each structure is formed in the most energetically favorable conformation based upon the characteristics of the lipids used in the system (Israelachvili 1991). The structure-packing parameter suggests what shape the amphiphile will take depending on the ratio of size variables. The packing parameter is

\[ P = \frac{v}{a l_c} \]

where
- \( v \) is the volume of the hydrocarbon
- \( a \) is the effective area of the headgroup
- \( l_c \) is the length of the lipid tail

This correlation predicts a range of structures according to the following conditions (Israelachvili 1991; Hsu et al. 2005) (Figure 13.2):

\[ P < \frac{1}{3} \rightarrow \text{spherical micelle} \]
\[ 1/3 \leq P < \frac{1}{2} \rightarrow \text{cylindrical micelle} \]
\[ 1/2 \leq P < 1 \rightarrow \text{flexible bilayers, vesicles} \]
\[ P = 1 \rightarrow \text{planar bilayers} \]
\[ P > 1 \rightarrow \text{inverted micelles (hexagonal (H_{II}) phase)} \]

Between 8 and 18 carbons commonly constitute the hydrocarbon tails of lipids used for gene delivery. The tails are typically saturated, but a single double bond is occasionally seen. The combination of hydrocarbon chains attached to glycerol can be symmetric or asymmetric. It has been shown that asymmetric lipids with both a shorter saturated carbon chain lipid and a long unsaturated carbon chain produce relatively high transfection efficiencies as compared to symmetric cationic lipids (Ferrari et al. 2002).

Hydrophobic tails are not the only liposomal features that play a role in effective gene delivery—ionizable head groups are also involved. Some examples are the multivalent cationic lipids 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-l-propanaminium trifluoroacetate (DOSPA) and DOGS (covered in Section 13.3.2), both of which have a functionalized spermine head group that confers the ability to act as a buffer, such as in the case where there is an influx of protons into a maturing endosome/endolysosome (Remy et al. 1994). The buffering could extend the amount of time needed to activate acid hydrolases, and could explain why some multivalent cationic lipids can exhibit higher transfection efficiencies versus their monovalent counterparts (Behr et al. 1989; Uchida et al. 2002).

### 13.3.1 Monovalent Cationic Lipids

#### 13.3.1.1 DOTMA

DOTMA, was one of the first synthesized and commercially available cationic lipids used for gene delivery (Figure 13.3). Its structure consists of two unsaturated oleoyl chains (C18:1 Δ9), bound by an ether bond to the three-carbon skeleton of a glycerol, with a quarternary amine as the cationic head group (Felgner et al. 1987). As compared to other methods of gene transfer used in the late 1980s, DOTMA proved to facilitate up to 100-fold more efficient gene delivery than the use of diethylaminoethyl (DEAE)-dextran coprecipitation or calcium phosphate (Felgner et al. 1987). The ability to entrap DNA or RNA
FIGURE 13.2  Relation of the packing parameter \( P = v/a l c \) to the predicted geometries of packed lipids. The physical interpretation of \( v \), \( a \), and \( l_c \) are shown for spherical micelles. The parameter \( a \) is shown slightly larger than the maximal cross-sectional area of the head groups for clarity of illustration only. \( P < \frac{1}{2} \) is typically seen for single-tailed lipids, while \( P \geq \frac{1}{2} \) is typical of two-tailed lipids, which are predicted to pack into bilayers or inverted micelles.

FIGURE 13.3  DOTMA.
in a liposome in a relatively simple fashion, with effective gene delivery, significantly influenced and improved the potential of nonviral agents for gene therapy (Felgner et al. 1987; Malone et al. 1989). The initial success of in vitro transfection of multiple cell lines with DOTMA sparked a number of attempts to improve the lipid formulation and resulted in the creation of many effective formulations including such notable lipids as DOTAP (Leventis and Silvius 1990) (see Section 13.3.1.2) and DC-Chol (Gao and Huang 1991) (Section 13.3.1.3).

Commercialization of DOTMA as Lipofectin® involved its coupling with DOPE (Section 13.3.3.1) in a 1/1 ratio due to the ability of DOPE to increase transfection efficiencies. Once commercialized, improvements in Lipofectin were desired, motivating others to add functional groups to the DOTMA. Many alterations made in the four major moieties of DOTMA (head group, linker, linkage bonds, and hydrocarbon chains) have reflected widespread efforts to reduce toxicity and increase transfection efficiencies (Leventis and Silvius 1990; Ren et al. 2000). Felgner et al. (1994) also experimented with novel lipid formulations by altering DOTMA and DOPE to obtain a more robust understanding of the mechanism of biological action. The structural changes included different combinations of side chains and alkyl attachments to the head groups as well as the replacement of a methyl group on the quaternary amine of DOTMA with a hydroxyl. Their report suggested that compounds with such a hydroxyl modification display improved transfection efficiencies over DOTMA. The stabilization of the bilayer vesicles was purported to occur as a result of the hydroxyl group remaining in contact with the aqueous layer surrounding the liposome. Compounds lacking this moiety were hypothesized to become entrenched in the aliphatic region, thus destabilizing the membrane. It was also indicated that the aliphatic chain length had a large effect on the efficacy of lipid vectors. As the lengths of the saturated chains were increased in the DOTMA analogs, transfection efficiencies decreased. This was thought to be due to increased bilayer stiffness, which may have prevented efficient fluid interactions with the endosomal membrane to thus hamper the release of the liposomes or pDNA from the endosomal compartments.

13.3.1.2 DOTAP

DOTAP, was first synthesized by Leventis and Silvius in 1990. The molecule consists of a quaternary amine head group coupled to a glycerol backbone with two oleyl chains. The only differences between this molecule and DOTMA are that ester bonds link the chains to the backbone rather than ether bonds. It was originally hypothesized that ester bonds, which are hydrolysable, could render the lipid biodegradable and reduce cytotoxicity (Figure 13.4).

The use of 100% DOTAP for gene delivery is inefficient due to the density of positive charges on the liposome surface, which possibly prevents counterion exchange (Zuidam and Barenholz 1998). DOTAP is completely protonated at pH 7.4 (which is not the case for all other cationic lipids) (Zuidam and

![FIGURE 13.4 DOTAP.](https://example.com/dotap.png)
Barenholz 1998), so it is possible that more energy is required to separate the DNA from the lipoplex for successful transfection (Zabner et al. 1995). Thus, for DOTAP to be more effective in gene delivery, it should be combined with a helper lipid, as seems to be the case for most cationic lipid formulations.

High temperature and long incubation times have been used to create lipoplexes that exhibit resistance to serum interaction (Yang and Huang 1998). Interestingly, this approach was only observed to affect monovalent cationic lipids such as DOTMA, DOTAP, or DC-Chol, as opposed to multivalent cationic lipids. The specific reasons for this phenomenon remain unclear. In fact, the specific mechanism behind serum inactivation of lipoplexes in general is as yet unexplained. Several hypotheses have been offered as to the mechanism, including the prevention of lipoplex binding to cell membranes by serum proteins (Yang and Huang 1997), the prevention of structural complex maturation by serum proteins binding to cationic charges on the lipoplexes (Yang and Huang 1998), and the disparity of endocytosis pathways—which have varying kinetics—that are used for lipoplex endocytosis, with the method of endocytosis being regulated by the size of the lipoplexes or aggregates of lipoplexes plus serum proteins (Marchini et al. 2009).

13.3.1.3 DC-Chol

3β[3N-(N,N′-dimethylaminoethane)-carbamoyl]cholesterol, or DC-Chol, was first synthesized by Gao and Huang (1991). DC-Chol contains a cholesterol moiety attached by an ester bond to a hydrolysable dimethylethylenediamine. Cholesterol was reportedly chosen for its biocompatibility and the stability it imparts to lipid membranes, an idea which was supported by desirable transfection efficiencies with reduced cytotoxicities in many cell lines (Gao and Huang 1991) (Figure 13.5).

In contrast to cationic liposomes containing fully charged quarternary amines (e.g., DOTMA and DOTAP), DC-Chol in a 1:1 lipid ratio with DOPE, contains a tertiary amine that is charged on 50% of the liposome surface at pH 7.4 (Zuidam and Barenholz 1997). This feature is thought to reduce the aggregation of lipoplexes leading to a higher transgene expression (Ajmani and Hughes 1999). The reduction in overall lipoplex charge can also aid in DNA dissociation during gene delivery (Zuidam and Barenholz 1998), which has been proven to be necessary for successful transfection (Zabner et al. 1995).

13.3.2 Multivalent Cationic Lipids

13.3.2.1 DOSPA

DOSPA is another cationic lipid synthesized as a derivative of DOTMA. The structure is similar to DOTMA except for a spermine group that is bound via a peptide bond to the hydrophobic chains. This cationic lipid, used with the neutral helper lipid DOPE at a 3:1 ratio, is commercially available as the transfection reagent Lipofectamine® (Figure 13.6).

In general, the addition of the spermine functional group allows for a more efficient packing of DNA in terms of liposome size. The efficient condensation is possibly due to the many ammonium groups in spermine. It has been shown that spermine can interact via hydrogen bonds with the bases of DNA in such a way as to be attracted on one strand and wind around the major groove to interact with complementary bases of the opposite strand (Jain et al. 1989).
13.3.2.2 DOGS

DOGS has a structure similar to DOSPA; both molecules have a multivalent spermine head group and two 18-carbon alkyl chains. However, the chains in DOGS are saturated, are linked to the head group through a peptide bond, and lack a quarternary amine. DOGS is commercially available under the name Transfectam®. This lipid has been used to transfect many cell lines with relatively low levels of toxicity (Behre et al. 1989) (Figure 13.7).

Much like the multivalent cationic lipid DOSPA, DOGS is very efficient at binding and packing DNA, a result of the spermine head group that so closely associates with DNA (Behre et al. 1989). Characterization of the head group of DOGS was determined to facilitate not only the efficient condensation of DNA but also the buffering of the endosomal compartment, which was thought to protect the delivered DNA from degradation by pH-sensitive nucleases (Remy et al. 1994). DOGS is a multifaceted molecule in terms of buffering capacity. At pH values lower than 4.6, all the amino groups in the spermine are protonated, while at pH = 8 only two are purportedly ionized, which promotes arrangement into a lamellar structure (Boukhniachvili et al. 1997). The packing ability of DOGS is due in part to the dynamics of the large head group molecule and the length of long unsaturated carbon chains.
13.3.3 Improvements to Cationic Lipids for Increased Transfection Efficiency

13.3.3.1 Use of DOPE and DOPC as Neutral Helper Lipids

Most liposomal formulations used for gene delivery consist of a combination of charged lipids and neutral helper lipids. The neutral helper lipids used are often DOPE, which is the most widely used neutral helper lipid, or dioleoylphosphatidylcholine (DOPC). Results have shown that the use of DOPE versus DOPC as the helper lipid yields higher transfection efficiencies in many cell types (Farhood et al. 1994; Simoes et al. 1998), thought to be due to a conformational shift to an inverted hexagonal packing structure that is imparted by DOPE at low pH (Figure 13.2). In contrast to the creation of repeated layers of DNA/lipids as is the case in lamellar packing, the inverted hexagonal packing structure is similar to that of a honeycomb of tubular structures that condense DNA inside the tubes through electrostatic interactions. The tubes aggregate due to van der Waals interactions between the lipid tails that spread out to encircle each tube. Fusion and destabilization of the lipoplexes during transfection are thought to occur due to the exposure of the endosomal membrane to invasive hydrocarbon chains (Chesnoy and Huang 2000). It has been shown that a hexagonal conformation allows for efficient escape of complexed DNA from endosomal vesicles via destabilization of the vesicle membrane (Hui et al. 1996; Zuhorn et al. 2005). With the lysosomotropic agent chloroquine inhibiting the activity of DOPE-containing lipoplexes, it is reasonable to assume that the membrane destabilizing hexagonal conformation associated with DOPE is brought about at acidic pH (Legendre and Szoka 1992; Farhood et al. 1995) (Figure 13.8).

In DOTAP-mediated DNA-binding studies, it was discovered that liposomes—formulated without DOPE—would not effectively complex with DNA to neutralize it until a 2:1 N:P ratio was reached.

![Diagram of DOPE and DOPC](image-url)

**FIGURE 13.8** DOPE (top) and DOPC.
due to an inability to displace counterions bound to the cationic lipid head groups (Zuidam and Barenholz 1998). In contrast, complexes with a 1:1 ratio of DOTAP/DOPE continuously neutralized and complexed with the negatively charged DNA at all charge ratios. This is possibly due to salt bridges more easily forming between the positively charged head groups of the cationic lipids and the phosphate groups of DOPE moieties. This association would force the primary amine of DOPE to stabilize itself in the plane of the liposome surface and allow for more close interactions with the negatively charged phosphate of the DNA. DOPE could also facilitate counterion release from the positively charged lipid head group, thus lowering the energy required for binding DNA (Zuidam and Barenholz 1998). Circular dichromism has been used to indicate that the use of DOPE as a helper lipid allows for much closer contact and packing of DNA helices (Zuidam and Barenholz 1998).

DC-Chol and other cholesterol derivatives have been incorporated into the lipoplex assembly for increased transfection efficiency in vivo (Bennett et al. 1995; Hong et al. 1997). Galactosylated cholesterol derivatives have been shown to lower cytotoxicity levels and improve transfection efficiencies in human hepatoma cells (Hep G2), likely due to the affinity of cellular receptors for galactosylated ligands (Kawakami et al. 1998). This result indicates that lipoplexes can be formulated for cell-specific uptake through the addition of specific ligands.

13.3.3.2 Poly(ethylene) Glycol

Recent improvements in lipofection have involved liposomal targeting and facilitated protection from degradation in vivo, both due to surface modifications with PEG. PEG presents many attractive qualities as a liposomal coating, such as availability in a variety of molecular weights, lack of toxicity, ready excretion by the kidneys, and ease of application (Metselaar et al. 2003). Methods of modifying liposomal surfaces with PEG include physical adsorption to the surface of the complex and covalent attachment onto premade liposomes (Immordino et al. 2006).

It has been shown by Kim et al. (2003), that PEGylated lipoplexes yield increased transfection efficiencies in the presence of serum as compared to liposomal transfection methods lacking surface attachments. Additionally, the PEGylated lipoplexes display improved stabilities and longer circulation times in blood. It is thought that the PEG forms a steric barrier around the lipoplexes, which stifles clearance due to reduced macrophage uptake (Immordino et al. 2006), and may allow the liposome to overcome aggregation problems through mutually repulsive interactions between the PEG molecules (Needham et al. 1992). These characteristics increase bioavailability, facilitating higher transfection efficiencies due to improved tissue distribution and larger available concentrations (Decastro et al. 2006).

Because of decreased immune responses and increased circulation times associated with PEG-modified liposomes, they are sometimes referred to as “stealth liposomes.” However, such liposomes lack specificity with regard to cellular targeting. Notably, Shi et al. (2002) found that PEGylation inhibited endocytosis of the lipoplexes in a fashion that was dependent upon the mole percentage of PEG on the liposome as well as the identity of certain functional groups that were conjugated to the lipoplexes. Additionally, upon incorporation into the cell, PEG worked to deter proper complex dissociation by stabilizing a lamellar phase of DNA packing. As a result of these findings, a need has arisen for the creation of novel PEG-containing liposomes whereby the attached PEG is removed following endocytosis via a hydrolysable connecting molecule.

Alternative formulations utilizing PEG and other polymers are being produced with the aim of creating steric protection. The goals of such a system include biocompatibility, a flexible structure, and solubility in physiological systems (Immordino et al. 2006). A report by Metselaar et al. (2003) on L-amino-acid-based polymers found an extended circulation time and reduced clearance by macrophages at levels similar to PEG-modified lipids. These oligopeptides are attractive alternatives to PEG due to advantages such as increased biodegradability and favorable pharmokinetics when lower concentration doses are used.

Liposomes can also be coupled to targeting moieties through the use of PEG to impart attraction to affected tissues for optimal routing and transfection. Targeting ligands are selected based upon specific target cell receptors. The target cells can be normal or transformed tumor cells. Examples are transferrin
(Ishida et al. 2001), a popular ligand for the delivery of anticancer drugs to solid tumors \textit{in vivo}, and haloperidol (Mukherjee et al. 2005), a ligand that associates with sigma receptors that are overexpressed in many types of cancer.

13.4 Nondegradable Nanoparticles

13.4.1 Magnetic Nanoparticles

One limitation of gene therapy is that often only low amounts of DNA reach cells of interest. The principle behind magnetofection is the use of magnetic nanoparticles, attached to gene delivery complexes, which are guided via an applied magnetic field to specific tissues, organs, or cells. Goals of magnetofection are to reduce the total amount of DNA used, to decrease the time needed for complexes to reach the targeted areas, and to enhance the percentage of cells that express the delivered genes. Figure 13.9 is a pictorial description of magnetofection in an \textit{in vitro} setting.

Magnetic nanoparticles used for gene delivery have been based on a concept established in 1978 by Widder et al., where magnetic micro- and nanoparticles were used for drug delivery (Widder et al. 1978). The use of magnetic microparticles for gene delivery was demonstrated 22 years later \textit{in vitro} in C12S cells and \textit{in vivo} in mice using adeno-associated viruses linked to magnetic microspheres via heparin (Dobson 2006). The use of magnetic nanoparticles linked to nonviral vectors (PEI, Lipofectamine, DOTAP-Cholesterol, PLL) was described shortly thereafter in 2002 (Scherer et al. 2002). Since these initial studies, this technique, now termed “magnetofection,” has been drawing more and more attention after successful transfections were demonstrated in various additional types of cells.

The magnetic nanoparticles used for gene or drug delivery are usually designed as follows: a magnetic core is coated by a protective layer, which can be further functionalized covalently or non-covalently with therapeutic agents such as carrier/DNA complexes (or other drugs). A schematic design for a magnetic nanoparticle is shown in Figure 13.10. The magnetic core can be made from a wide variety of materials having a range of magnetic properties. The most widely used cores are superparamagnetic iron oxide nanoparticles (IONPs), especially those using magnetite ($\text{Fe}_3\text{O}_4$) and maghemite ($\gamma$-$\text{Fe}_2\text{O}_3$). These materials are currently used as contrast agents in magnetic resonance imaging (MRI) and their pharmacokinetics and toxicities have been extensively studied (Dobson 2006; Ragusa et al. 2007).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{magnetofection.png}
\caption{Schematic representation of magnetofection \textit{in vitro}.}
\end{figure}
The magnetic nanoparticle core is fairly reactive, and is usually coated to prevent corrosion. The coating could also prevent the leaking of potentially toxic components into the body when applied in vivo. The coating materials can be silica; gold; natural polymers such as dextran; or synthetic polymers such as PEI, PLL, PEG, and polyvinyl alcohol (PVA) (McBain et al. 2008). Organic linkers are used to alter surface properties to suit various therapeutic agents. Commonly used organic linkers include amines, thiols, and aldehydes (McBain et al. 2008). However, in gene delivery, the most suitable coating surfaces for the magnetic particles are those that are strongly cationic because of the negatively charged DNA molecules that are to be delivered. Although organic linkers provide a good approach for the attachment of biomolecules, this approach is not always necessary because DNA could attach to positively charged coating polymers such as PEI or PLL through electrostatic interactions.

As mentioned earlier, the first investigation of magnetic nanoparticles linked to nonviral vectors such as PEI, PLL, and lipofectamine indicated that magnetofection could improve vector targeting and efficacy (Scherer et al. 2002). In 2004, the mechanism of magnetic INOPs coated with PEI entering cells was studied (Huth et al. 2004). The investigation showed that cellular uptake of the coated INOPs was similar to that of PEI polyplexes. It seemed that the magnetic field did not aid in the uptake process, it just quickened the gathering of vector/DNA complexes near the cell surface, which increased the chance of internalization of the complexes. In 2006, Kamau et al. (2006) coupled DNA to PEI-coated INOPs, and then the vector/DNA complexes were delivered to different cell lines (Hela, 293T, Cos-7) that were subsequently exposed to permanent or pulsating magnetic fields. The transfection efficiencies were found to be much higher than in cells not exposed to magnetic fields, with cellular uptake observed 5 min after exposure to the magnetic fields.

At present, magnetofection has been applied to transfect a number of cell types such as primary lung epithelial cells (Gersting et al. 2004) and human umbilical vein endothelial cells (HUVEC) (Krotz et al. 2003b), which are known to be resistant to transfection. Moreover, magnetofection has been used to deliver antisense oligonucleotides (Krotz et al. 2003a) and siRNA (Schillinger et al. 2005) to down-regulate gene expression.

The main advantage of magnetofection is rapid gene delivery, taking just a few minutes as compared to traditional transfection methods that can take hours. Moreover, in vivo, the magnetic field could not only enhance transfection but also target therapeutic genes to specific organs, which could save considerable amounts of transfection materials. Although magnetofection is relatively new, optimization of parameters such as those associated with the magnetic field have shown great promise for the advancement of this technique.

### 13.4.2 Gold Nanoparticles

Gold nanoparticles (GNPs or AuNPs) have been widely used for their optical properties, and have recently drawn attention as potential candidates as gene delivery vectors. The size of GNPs can range from a few to several hundred nanometers, and the synthesis processes in aqueous solutions...
as well as in organic solvents are very well established (Sperling et al. 2008). GNPs can be made very small to provide a high surface-to-volume ratio, and the surfaces of GNPs can be easily modified by conjugation with various ligands (Sperling et al. 2008). Due to the high binding affinity of thiol moieties to gold surfaces, thiol-modified ligands are frequently used for binding to the GNP surface via Au-sulfur bonds. Such modifications are usually called monolayer-protected clusters (MPCs) or mixed monolayer protected clusters (MMPCs). Since the gold core is essentially inert, GNPs are regarded as biocompatible in cell culture experiments. To date, there has been no acute cytotoxicity reported for GNP use.

The Rotello group has investigated the interaction between cationic GNPs and DNA, showing that tetraalkylammonium ligands conjugated to GNPs could completely inhibit in vitro DNA transcription by T7 RNA polymerase (McIntosh et al. 2001). Branched PEI (2 kDa)-conjugated GNPs have been used as gene delivery vectors to transfect Cos-7 (monkey kidney) cells, with transfection efficiencies varying with the PEI:gold ratio in the hybrid GNP-PEI. The hybrid GNP-PEI conjugate was about 12 times more efficient than PEI alone at the optimized ratio (Thomas and Klibanov 2003). β-Cyclodextrin was also attached to the oligo(ethylenediamino)-modified GNPs (OEA-CD-NP), and it was found that the modified GNPs could effectively bind and concentrate DNA (Wang et al. 2007). The data demonstrated that OEA-CD-NP could deliver plasmid DNA to breast cancer cells (MCF-7).

13.4.3 Silica Nanoparticles

Silica is a major component of sand and glass, and has been studied extensively in material science and engineering due to the variety of chemical and physical modifications possible. Pure silica nanoparticles without surface modifications cannot condense and deliver DNA. To address this issue, silica particles are often functionalized aminosilanes (Luo and Saltzman 2006; Ragusa et al. 2007). N-(6-aminohexyl)-3-aminopropyltrimethoxysilane and N-(2-aminoethyl)-3-aminopropyltrimethoxysilane have been used to modify silica, and the resulting surface-functionalized silica can condense and deliver DNA to Cos-1 cells with low toxicity (Kneuer et al. 2000). Amino-hexyl-aminopropyltrimethoxysilane (AHAPS) has also been used to functionalize silica, yielding a material that successfully transfected mouse lung in vivo (Ravi Kumar et al. 2004).

Amino-functionalized, organically modified silica (ORMOSIL) has been used for in vivo gene delivery in mouse brain (Bharali et al. 2005). Intraventricular injection of ORMOSIL/pEGFP complexes showed effective transfection and expression of enhanced green fluorescent protein (EGFP) in neuron-like cells in the periventricular brain regions and the subventricular zone. Moreover, the transfection of ORMOSIL/FGFR-1 (nucleus targeting fibroblast growth factor receptor type 1) caused cells to withdraw from the cell cycle, which resulted in neuronal differentiation. These studies provided groundwork for the application of ORMOSIL nanoparticles to in vivo gene transfer of the central nervous system.

13.5 Problems Associated with Nanobiomaterials for Nonviral Gene Delivery

As stated in the beginning of this chapter, nonviral gene delivery agents have the merits of relatively low induction of immune responses, virtually no limitation to the size of delivered genetic material, and a relatively low cost of production. However, there are some drawbacks associated with nonviral gene delivery vectors including their low transfection efficiencies and cytotoxicity.

In order to achieve effective gene expression, investigators must address a series of issues including the intrinsic stability of the vector/DNA complexes to be constructed; how the complexes will fare with respect to plasma membranes, endolysosomes, and nuclear envelopes; and cytosolic transport (Wiethoff and Middaugh 2003).

DNA complexed with cationic polymers or lipids can easily form large self-aggregates, which can be recognized and cleared by macrophages. Moreover, interactions between positively charged complexes
and negatively charged molecules in the extracellular milieu, such as serum albumin, can inhibit the delivery of genetic cargo (Zelphati et al. 1998). It has been hypothesized that cationic complexes are coated nonspecifically by negatively charged proteins, leading to reduced binding to the negatively charged surfaces of cells. Another hypothesis is that the cationic complexes dissociate due to interactions with anionic serum components that act to pull cationic molecules away from the carried DNA via electrostatic attractions (Zelphati et al. 1998).

The cellular uptake of cationic vector/DNA complexes is mediated by nonspecific binding between positive charges on the exteriors of the complexes and negatively charged components of the extracellular portion of the plasma membrane, with the complexes internalized via endocytosis. Endosomal escape is one of the major barriers to efficient gene delivery (Zabner et al. 1995). For complexes utilizing cationic lipids, the mechanism of endosomal escape seems to involve lipid–lipid interactions between the membranes of the endosomes and lipoplexes, leading to membrane disruption and DNA release into the cytoplasm (Xu and Szoka 1996). However, the exact mechanism of the process has not yet been defined. For complexes utilizing cationic polymers, the mechanism involved following endocytosis is even less clear. Several hypotheses have been proposed. One indicates that endosomal membrane disruption is caused by direct interaction between the negatively charged endosomal membrane and the cationic polymers (Zhang and Smith 2000). Another hypothesis is that of the “proton sponge” (Boussif et al. 1995), which states that as protons are pumped into the endolysosome by vesicular ATPases, the pH of the endolysosome is buffered by polypeptide amines, preventing the activation of acid hydrolases. In the meantime, chloride ions enter the endolysosome via chloride channels to relieve the developing ionic gradient. Water molecules then enter the endolysosome to relieve the ensuing osmotic gradient, and the endolysosome will swell to the point that its membrane becomes leaky, allowing the endocytosed polypeptides to be released into the cytoplasm. Still other research indicates a lack of interaction between certain types of polypeptides and lysosomes, with DNA being delivered into nuclei still attached to their delivery vehicles (Godbey et al. 1999b, 2000; Akinc and Langer 2002).

Cytotoxicity is another obstacle to the use of nonviral gene delivery vectors for gene therapy. For cationic lipids, cytotoxicity is associated with the cationic nature of the head groups. For example, quaternary amine headgroups are more toxic than groups that employ tertiary amines. Moreover, the type of linker bond that is used to join the polar and nonpolar regions of the lipids also plays a role in cytotoxicity, which is partially due to the end products created by cationic lipid degradation (Lv et al. 2006). For cationic polymers, cytotoxicity is interrelated to polymer structure. As an example, the cytotoxicity of PEI is related to factors, such as molecular weight, degree of branching, zeta potential, and particle size (Kircheis et al. 1999; Kunath et al. 2003).

Despite the fact that nonviral vectors are used for their reduced immunogenic properties, there are still vectors that do stimulate portions of the immune system. These vectors and their antigenic properties must be characterized before optimal gene delivery vehicles can be created and used in the clinic. While DOTAP has been widely used in vitro, this vector—and many other cationic liposomes—have been found to stimulate the immune system activation markers CD80 and CD86 despite a lack of pro-inflammatory cytokine secretion (Yan et al. 2007). Another report has indicated that the increased activation of these markers is correlated with unsaturated or shortened saturated hydrocarbon chains in comparison to a lower level of activation by liposomes containing lipids with longer or saturated acyl chains (Vangasseri et al. 2006). A recently designed shorter phospholipid, DiC14-amidine, contains hydrocarbon chains with 14 saturated carbons. DiC14-amidine was found to not only stimulate CD80/86 marker proteins, but also to activate production and secretion of pro-inflammatory cytokines (Tanaka et al. 2008). It is necessary to keep the immunogenic characteristics of the structure of DiC14-amidine and other cationic lipids in mind when designing new and ideally effective vectors that will not act as immune system agonists.

The complement system is a major aspect of the nonspecific immune system that must remain inactivated for efficient gene delivery. All of the cationic vectors examined, including DOTAP, DC-Chol/DOPE,
DOGS/DOPE, DOTMA/DOPE, and cationic polymer vectors, activate the complement cascade, with the lipopolymamines (DOGS) acting as one of the most potent activators (Plank et al. 1996). Of the cationic polymers, long-chain polyllysines activate the complement system very strongly. Attenuation of these polymers to a length between 19 and 28 segments decreases complement activation by orders of magnitude (Plank et al. 1996). Other data by Plank and Szoka indicate that cationic peptides can be very active in gene delivery and hardly activate the complement system if their lengths are under 10 repeats (Plank et al. 1996). Cationic polymers complexed with DNA such that they are electrically neutral do not activate the complement system. The trend of activation seems to be dependent upon charge ratio or charge density: uncomplexed multivalent cationic liposomes activate complement to a greater degree than uncomplexed monovalent cationic liposomes. However, when complexed with DNA, neither multivalent nor monovalent liposomes activate to the same extent as the uncomplexed forms of the lipids (Plank et al. 1996).

13.6 Conclusion

The use of nanobiomaterials for gene delivery presents great potential for future medical applications both in vitro and in vivo. In terms of transfection efficiency, nonviral gene delivery vectors are still inferior to their viral counterparts. However, they are associated with advantages in that there is limited induction of immune responses, there is virtually no limitation on the size of the genes that can be delivered, and the cost of production is relatively low. Although nonviral gene delivery vectors have been widely investigated, considerable improvements are still needed to meet the requirements associated with clinical use. Nevertheless, the rapid development of nanotechnology should help to realize this aim sooner.

References


