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Cationic Liposomes in Different Structural Levels for Gene Delivery

Yinan Zhao, Defu Zhi and Shubiao Zhang
SEAC-ME Key Laboratory of Biochemical Engineering
College of Life Science, Dalian Nationalities University
China

1. Introduction

Over the last few decades, as a promising strategy for the treatment of many refractory diseases, such as inherited diseases (Martin-Rendon & Blake, 2003) and acquired immuno-deficiency syndrome (AIDS) (Fanning et al., 2003), gene therapy, the objective to allow a gene to express the protein coded in the target cells and consequently to treat disease by the protein secreted from cells transfected, has become an invaluable experimental tool to study gene function and its regulation (El-Aneed, 2004). The success of gene therapy critically depends on suitable transfection vectors, which have the high efficiency transfer of genes to target cells as well as a favorable safety profile. Broadly, these vectors are mainly classified into two categories: viral and non-viral (Liu & Huang, 2002). Currently, viral vectors based on many different viruses such as adenovirus and retrovirus have achieved some success particularly in cancer gene therapy (Williams et al., 2010), and their performance and pathogenicity have been evaluated in animal models. However, several issues including difficulty in production, limited opportunity for repeated administrations due to acute inflammatory response, and delayed humeral or cellular immune responses need to be addressed, so that their clinical potential can be fully realized (Love et al., 2010). Thus it is necessary to develop more efficient and flexible security system in the category of vectors for gene delivery. Synthetic non-viral vectors are potential alternatives to viral vectors, and may help to overcome some of these problems (Zhang et al., 2010). Among these, cationic compounds (mainly including cationic lipids and cationic polymers) are believed to cause less safety problems due to their relative simplicity, and have been the most extensively studied.

Since the first description of successful in vitro transfection with cationic lipid by Felgner et al. in 1987 (Felgner et al., 1987), numerous cationic lipids have been synthesised and used for delivery of nucleic acids into cells during the last 20 years. Cationic liposomes are composed of lipid constituents, and have improved the gene delivery efficacy owing to their typical bilayer structure. Some helper lipids such as dioleoylphosphatidyl choline (DOPC) or dioleoylphosphatidyl ethanolamine (DOPE), typically neutrally lipids (Zuhorn et al., 2005), are often employed with cationic lipids, and play very important role during the formation of lipoplexes by combining cationic liposomes and genes, as they could determine the morphologies of lipoplexes.
Cationic polymer, which condenses DNA by ionic interaction (at physiological pH), form a particulate complex, polyplex, capable of gene transfer into the targeted cells (El-Aneed, 2004). They can condense the negatively charged DNA to a relatively small size and reduce its susceptibility to nuclease so that they may be favorable for improving transfection efficacy. The introduction of polycations (such as poly-L-lysine and protamine) in cationic liposomes, as co-polymer may provide a synergistic effect on the transfection efficiency and a promising solution to the problem frustrating us (Gao & Huang, 1996; Li & Huang, 1997).

In this chapter, we review the effect of cationic liposomes in different structural levels for gene delivery, and help furthering the understanding of the mechanism governing the formation and behaviour of cationic liposomes in gene delivery. The first level for studying on the structure–activity relationship of cationic lipids is the synthesis of new vectors, as well as attempts to improve transfection efficiencies and decrease cytotoxicity, through hydrophilic, hydrophobic and linker domain modifications. The second one is the study on morphologies of lipoplexes through the effects of helper lipids and particle sizes. The last one is the hybridized utilization of non-viral vectors including the complexes of cationic liposomes and polymers, conjugates of lipids and peptides and of targeting moieties and lipids.

2. Chemical structure of cationic compounds

To understand the relationship between chemical structure and gene delivery, a large series of cationic lipids have been developed. In general, a cationic lipid used for gene therapy is constituted of three basic domains: a hydrophilic cationic headgroup, a hydrophobic domain, and a linker bond which joins the hydrophilic and hydrophobic regions (Fig. 1.) (Gao & Hui, 2001). The chemical structure of cationic lipids determines the physical parameters of the liposome and is an essential factor in both transfection activity and cytotoxicity levels. The effect of chemical structure modifications on gene delivery is discussed in detail through analyzing the large amount of literatures and exemplified thereinafter.

2.1 Headgroup domain

Since the first description of successful in vitro transfection with cationic lipid—DOTMA (Fig. 1.) by Felgner et al. in 1987 (Felgner et al., 1987), progress has been made in the design and analysis of each domain. The effect of transfection efficiency and cytotoxicity is associated with the cationic nature of the vectors, which is mainly determined by the structure of its hydrophilic headgroup. In general, the different types of headgroups fall into the following categories: primary, secondary, tertiary amines or quaternary ammonium salts (of which polyamines have often seen the most success) and guanidinium, amidine, as well as heterocyclic ring (Heyes et al., 2002). In addition, other charged groups that have been shown capable of binding plasmids have since been employed. For example, Floch et al. (Floch et al., 2000) showed that cationic lipids (Fig. 2.) characterized by a cationic charge carried by a phosphorus or arsenic atom instead of a nitrogen atom, led to an increased transfection efficiency (up to 7 times according to the cell lines tested) and reduced toxicity.

In hydrophilic headgroup, tertiary amine or quaternary ammonium groups are the most frequently used in many of the established cationic lipids (Felgner et al., 1987; Gao & Huang, 1991; Leventis & Silvius, 1990). A typical example that modified to the chemical
structure of headgroup was described by Felgner et al. (Felgner et al., 1994). They synthesized a series of 2,3-dialklyoxy quaternary ammonium compounds containing a hydroxyl moiety (Fig. 3), which were more efficient in transfection compared with DOTMA that lacks a hydroxyl group on the quaternary amine. In our previous work, we also synthesized a series of cationic lipids containing a hydroxyl moiety on the quaternary amine for liposome-mediated gene delivery (Fig. 4). Several cationic liposomes with relatively higher transfection efficiency were selected after in vitro transfection studies of the prepared cationic liposomes, whose biological performance was superior or parallel to that of the commercial transfection agents, Lipofectamine2000 and DOTAP. It is suggested that the headgroup hydration can be decreased by the incorporation of a hydroxyalkyl chain capable of hydrogen bonding to neighbouring headgroups while it improves the compaction of DNA by several mechanisms, for example, DNA can form hydrogen bonds with the lipid, and the hydroxyl group can enhance the membrane hydration. Accordingly, several groups have synthesised cationic lipids that varied the chain length of the hydroxyalkyl moiety, while keeping the remaining structure unchanged, and observed that the activity of lipid increased with the decrease in the hydroxyalkyl chain length. It shows that a decrease in the number of carbon atoms in the hydroxyalkyl chain, providing more rigidity to the terminal hydroxyl group, are very efficient in compacting DNA, which is responsible for the more observed transfection activity (Felgner et al., 1994; Bennett et al., 1997).

![DOTMA diagram](image_url)

Fig. 1. Representative structure of the cationic lipid DOTMA. Examples of cationic lipid structural components: hydrophilic headgroup, linker bond, and hydrophobic domain.
Fig. 2. Alternative cations in the lipid headgroup.

Fig. 3. Double-chain cationic lipids containing a hydroxyl moiety on the quaternary amine.

Fig. 4. Transfection comparisons of lipids synthesized and commercial reagents.

A number of cationic lipids that spread the positive charge of the cationic head by delocalizing it into a heterocyclic ring have been designed in an attempt to improve transfection efficacy and to lower toxicity. Heterocyclic cationic lipids with morpholine or piperazine polar heads conjugated to cholesterol directly or via a spacer (Fig. 5.) have been reported to compare favourably with linear polyamine head groups with similar or greater number of charges (Gao & Hui, 2001). And then, the use of imidazole or pyridine rings have been also reported to display higher transfection efficiency and reduced cytotoxicity when compared with classical transfection systems (Ilies et al., 2003; Medvedeva et al., 2009).
Guanidine and its salts are important intermediates for organic synthesis and medicine; they have also been proposed for making cationic lipids for gene delivery. Yingyongnarongkul et al. (Yingyongnarongkul et al., 2004) studied a library of aminoglycerol–diamine conjugate-based cationic lipids with urea linkage between varying length of diamines and hydrophobic chains, and found two compounds with bis-guanidinium and one tail had transfection activity superior to that of the commercial lipid transfection reagent effective and merit further investigation. In addition, several cationic lipids containing guanidine (Floch et al., 2000), amidine (Lensink et al., 2009), or cyclic guanidine (Frederic et al., 2000) in the head group have been studied.

As a way to not only bind DNA but to compact DNA, polyvalent cationic lipids known as lipopolyamines and lipospermine were synthesized, e.g., DOSPA, DOGS (Behr et al., 1989), are claimed to be more efficient than single-charged lipids such as DOTMA, DOTAP, DC-Chol, DMRIE (Ferrari et al., 1998). The choice of headgroup may depend on the desired and specific function of the cationic lipid, but for gene transfection it is essential that the headgroups of cationic lipid interact strongly with the minor groove of DNA via its multivalent headgroup, and has the ability to efficiently condense DNA. In addition, a few parameters, such as the shape (linear, T-shaped, globular, branched) and the length of the multivalent headgroup, and the distance between two consecutive nitrogen atoms in the polyamine, are also important in transfection efficiency and cytotoxicity (Byk et al., 1998; Fujiwara et al., 2000).

In summary, the choice of cationic headgroups has expanded into the use of natural architectures and functional groups with recognized DNA binding modes. Multivalent cationic lipids are more likely to be the most efficient in compacting DNA; however, they are prone to formation of micelles typically contributing to increased toxicity (Pedroso de Lima et al., 2003). The incorporation of a heteroatomic group as the substitution of the linear amine headgroup, such as pyridinium and guanidine, can improve the compaction of DNA by the positive charge of the cationic head, and then transfection efficiency is increased and toxicity is decreased significantly.

2.2 Hydrophobic domain

The hydrophobic domains represent the non-polar hydrocarbon moieties of cationic lipids and are usually made of two types of hydrophobic moieties—aliphatic chains, steroid domain. Transfection efficiency and toxicity of cationic lipids can be affected by structural variations in the hydrophobic domain such as length, the specific type of chemical bonds, and the relative position of the hydrocarbon chains (Zhi et al., 2010).

Cationic lipids with aliphatic chains have been very thoroughly researched. The chains are either linear and saturated or linear and mono-unsaturated and used in liposomal vectors.
ranging from C5:0 to C18:1, but oleyl, lauryl, myristyl, palmityl and stearyl, have been the most researched ones (Niculescu-Duvaz et al., 2003). A common variation is the use of branched (Ferrari et al., 2002), acetylenic (Fletcher et al., 2006) chains and cis-monounsaturated alkyl chains (Bennett et al., 1997).

It is commonly believed that cationic lipids have one to four hydrocarbon chains. Several studies have also shown that incorporating aliphatic chains with different numbers can improve transfection efficiency potentially by promoting endosomal escape (Felgner et al., 1987; Tang & Hughes, 1999a; Gaucheron et al., 2002; Zhi et al., 2010). Cationic lipids with double-chain hydrocarbons in the hydrophobic domain represent the majority of cationic lipids synthesized so far. Cationic lipids containing two aliphatic chains such as DOTMA and DOTAP, are among the most active lipids for systemic gene delivery. However, Tang et al. (Tang & Hughes, 1999a) demonstrated that 6-lauroxyhexyl ornithinate (LHON) with one tail was more efficient and of lower cytotoxicity compared to DOTAP. Generally speaking, for aliphatic chains, single-tailed and three-tailed cationic lipids are better known as surfactants because of their ability to form micelles in solution, but they are more toxic and less efficient than their double-tailed counterparts. Usually, cationic lipids with double-tailed hydrocarbons are capable of forming liposomes by themselves or with a helper phospholipid. Therefore, most of the aliphatic chains in the cationic lipids are double-tailed.

It is generally agreed that the length and saturation of the aliphatic chains incorporated into cationic lipids significantly affect their transfection efficiency. In order to gain chain length-activity correlation, Felgner et al. (Felgner et al., 1994) studied a series of hydroxyethyl quaternary ammonium lipids with myristoyl (diC14:0, DMRIE), palmitoyl (diC16:0, DPRIE), stearoyl (diC18:0, DSRIE), and oleyl (diC18:1, DORIE) chains. They observed that a comparison of vectors based solely on the lengths of the two aliphatic chains led to identify the order C14:0 > C18:1 > C16:0 > C18:0. Our study on double-chain cationic lipids also showed increasing transfection efficiency with decrease of the chain length (Liu et al., 2008). It was therefore proposed that cationic lipids with shorter chain length (for saturated chains) were generally important for acquiring high transfection efficiency, since they are responsible for membrane fluidity and good lipid mixing within the bilayer. Beyond that, the best chains in terms of benefit to transfection are frequently the unsaturated ones. The overwhelming majority of results showed that the unsaturated C18:1 oleyl was the optimal aliphatic chain, which was frequently the best choice for good transfection (Fletcher et al., 2006).

Some new vectors were designed to covalently connect some special moieties in the hydrophobic chains, in order to get the relationship between hydrophobic chains and transfection efficiency. Jacopin et al. (Jacopin et al., 2001) synthesized a glycosylated analogue (Fig. 6.) of the dialkylamidoglycylcarboxyspermines, which formed stable particles at low charge ratio and was efficient for gene delivery. Many groups also reported a few other glycosylated cationic bolaamphiphiles similar to the compound (Fabio et al., 2003; Brunelle et al., 2009). In addition, the fluorinated part of the hydrophobic chain can also influence the transfection efficiency of cationic lipids in vivo and in vitro. Many varieties of fluorinated cationic lipids have been developed as transfecting agents, which are very efficient in compacting DNA and delivering genes into cells in vivo and in vitro (Gaucheron et al., 2001a, 2001b, 2001c, 2001d).

In the steroid groups, cholesterol is by far the most frequently encountered and used as an alternative to aliphatic chains because of its rigidity, as well as its endogenous biodegradability and fusion activity. An example is cationic lipid ‘GL-67’ (Fig. 7.), which has been found to be particularly efficient for gene transfer to cultured cells and in murine lungs.
(Lee et al., 1996). Other steroid compounds used as hydrophobic moieties for cationic lipids include vitamin D (Ren et al., 2000), bile acids (Randazzo et al., 2009), antibiotic (Kichler et al., 2005), cholestane and lithocholic acid (Fujiwara et al., 2000).

![Glycosylated analogue of lipopolyamines.](image)

![Chemical structure of GL-67.](image)

To summarise, the hydrophobic domain of cationic lipids mainly includes aliphatic chains and steroid domain, which determines the phase transition temperature and the fluidity of the bilayer, and influences the stability and toxicity of liposomes, the DNA protection from nucleases, and the DNA release from complex.

### 2.3 Linker bond

For lipids without a backbone, the linker bond that acts as a connector between the hydrophobic and cationic headgroup domains can affect the transfection efficiency, biodegradability and stability of cationic lipids. Linker bonds are commonly ether, ester (Leventis & Silvius, 1990), amide (Behr et al., 1989) or urethane (or carbamate) (Lee et al., 1996) groups (Kovyova & Tenchov, 2010), but other groups such as redox-sensitive disulphide (Byk et al., 1998, 2000) have also been employed (Fig. 1.). Cationic lipids with ether bonds such as DOTMA in the linker domain generally render better transfection efficiency, but they are too stable to be biodegraded thus may cause higher toxicity. Compared with ether bonds, although cationic lipids with ester bonds such as DOTAP are more biodegradable and associated with less cytotoxicity in cultured cells (Leventis & Silvius, 1990; Choi et al., 2001), those with ester may also decrease the stability of liposomes in systemic circulation.

The chemistry of the linker has most often been of the carbamate or amide variety, both of which are chemically stable and biodegradable, and cationic lipids with these linkers could be used as efficient gene delivery carriers (Ren et al., 2001; Liu et al., 2005a, 2005b, 2008). A typical example of cationic lipid with carbamate linker is DC-Chol, which was the first lipid used in clinical trials because of its combined properties of transfection efficiency, stability, and low toxicity (Gao & Huang, 1995). As well known, when incorporating a carbamate group into the linker, it may therefore be hoped that the pH drop will act as a trigger,
disconnecting the hydrophobic and hydrophilic portions of the lipoplex, and thereby to release DNA after entering endosomes in cell because of the pH decreasing (Liu et al., 2005a, 2008). We synthesized a series of carbamate-linked cationic lipids for liposome-mediated gene delivery, which proved to have good gene transfection properties (Fig. 4.).

It is familiar to chemists that compounds comprising redox-sensitive disulphide bonds is stable chemically as long as no reducing agents, and it is expected that these disulphide-linked lipids can keep stable in the circulation system while decomposing to release DNA after entering endosomes in cells (in a similar manner as the pH-sensitive systems) (Tang & Hughes, 1999b). Byk et al. (Byk et al., 1998, 2000) prepared a series of lipopolyamines that harbor a disulfide bridge within different positions in the backbone of the lipids as biosensitive function. They found that an early release of DNA during or after penetration into cells, probably promoted by reduction of a disulfide bridge placed between the polyamine and the lipid, implied a total loss of transfection efficiency.

In addition, structural variations at the linker region such as length, the specific type of chemical bonds and the relative position of the hydrocarbon chains can affect the transfection efficiency, biodegradability and stability of cationic lipids (Fujiwara et al., 2000). The level of hydration and toxicity of the lipid can also be determined by the length of the linker (Floch et al., 2000). In a word, the use of linkers incorporating functional groups that are cleavable on shorter time scales and under specific stimuli is however of emerging interest, as DNA release may here be facilitated by a triggered decomplexation mechanism.

From the chemistry point of view, the structure of cationic compounds is an important factor for their transfection activity and toxicity. Some common conclusions can be achieved by comparing the different structures and their transfection activity in the same family or different families of lipids. The transfection efficiency is not only determined by one domain of cationic lipids, but also depends on the combination of them (Tang & Hughes, 1999a). In general, it seems when researchers design cationic compounds for gene delivery, the balances between the opposite factors including fluidity and rigidity, symmetry and asymmetry, saturation and unsaturation, linearity and branching, short chain and long chain, hydrophilicity and lipophilicity of compounds should be taken into serious consideration.

3. Helper lipids and morphology of lipoplexes

Neutrally charged helper lipids such as DOPE, DOPC (Fig. 8.), are often employed with cationic lipids in order to gain high transfection efficiency (Felgner & Ringold, 1989). When cationic liposomes are mixed with DNA, lipoplexes are formed with heterogeneous morphologies including beads on a string structure (Felgner & Ringold, 1989), spaghetti or meatballs structure (Sternberg et al., 1994), multilamellar structure \( L_{\infty}^{c} \) inverted hexagonal phase structure \( H_{\infty}^{c} \) (Kolturk et al., 1998), a map-pin structure (Sternberg et al., 1998) and a sliding column phase (O’Hern & Lubensky, 1998). Helper lipids play very important role during the formation of lipoplexes by combining cationic liposomes and genes, as they could determine the morphologies of lipoplexes. It has shown lipoplex size is very important for gene transfer to actively endocytosing cells (Ross & Hui, 1999), as such the influences on transfection efficiency: DNA ratio, types of liposomes, incubation time in polyanion containing media, and time of serum addition, are channeled mostly through their influences on lipoplex size.
3.1 DOPE

DOPE often presents a super synergistic effect when used in cationic liposomes, because DOPE destabilized lipid bilayers, and it was believed to be involved in endosomal disruption (Litzinger & Huang, 1992), allowing the release of DNA into the cytosol (Farhood et al., 1995) and leading to mixed bilayers (Scarzello et al., 2005). Most studies have shown that lipoplexes containing the non-bilayer-phase-preferring lipid DOPE or cholesterol would promote H_{cII} organization (Zuhorn et al., 2005). A transition from the L_{c} phase to the H_{cII} phase could be expected by increasing weight fraction of DOPE, via controlling the spontaneous radius of curvature “Ro” of the lipid layers, favored by the elastic free energy (Safinya, 2001). Another helper lipid, cholesterol, could also promote H_{cII} organization as DOPE. It has been proved that in vivo applications cholesterol was a more effective helper lipid than DOPE (Lasic, 1997).

Koltov et al. (Koltov et al., 1998) disclosed the reason in the level of phase transition through synchrotron small-angle X-ray scattering (SAXS) and optical microscopy to show the phase transition from L_{c} to H_{cII} induced by DOPE via controlling the spontaneous curvature C_{o} = 1/R_{o} of the lipid monolayer. It has been concluded that DOPE facilitates endosomal escape by forming an unstable inverted hexagonal phase at the endosomal pH that destabilizes both the complex and the endosomal membrane. But in a recent study (Leal et al., 2010), they developed CL-siRNA complexes with a novel cubic phase nanostructure exhibiting efficient silencing at low toxicity by using glycerol monooleate other than DOPE as the helper lipid. The inverse bicontinuous gyroid cubic nanostructure was unequivocally established from synchrotron X-ray scattering data, while fluorescence microscopy revealed colocalization of lipid and siRNA in complexes.

 Tubes of lipoplexes containing DOTAP/MOG, DOTAP or DOTAP/PC, and DOTAP/DOPE were observed in freeze-fracture electron micrographs. The tubes were extremely short and appeared bead-like in lipoplexes containing DOTAP/MOG, slightly longer in those containing DOTAP or DOTAP/PC, and extensively elongated in DOTAP/DOPE lipoplexes (Xu et al., 1999). The spaghetti-like structures, occurring at DNA: lipid concentrations which were used during transfection and their diameter came closest to the diameter of the nuclear pores, may be the active cationic lipoplexes (Zhdanov et al., 2002). In the study of the structure and morphology of DC-Chol–DOPE/DNA complexes it was found the existence of cluster-like aggregates made of multilamellar DNA/lipid domains coexisting with other multilamellar lipoplexes or, alternatively, with DNA-coated vesicles (Amenitsch et al., 2010). The further study showed that DC-Chol–DOPE/DNA lipoplexes preferentially used a raft mediated endocytosis, while DOTAP–DOPE/DNA systems were mainly internalized by not specific fluid phase macropinocytosis. Most efficient multicomponent lipoplexes, incorporating different lipid species in their lipid bilayer, can use multiple endocytic pathways to enter cells. Their data demonstrated that efficiency of endocytosis was
regulated by shape coupling between lipoplex and membrane lipids to suggest that such a shape-dependent coupling regulated efficient formation of endocytic vesicles thus determining the success of internalization (Marchini et al., 2010).

Kato et al. (Kato et al., 2010) observed the effect of phase separation of the membrane by changing PE from DOPE to dipalmitoyl ethanolamine (DPPE), which corresponded to a change from a homogeneous single phase to two segregated phases of liquid-ordered and liquid-disordered states on the membrane. This study further proved that helper lipids could change the morphologies of lipoplexes through the mutual interaction with DNA based on their chemical structures. Several helper lipids such as dilauroyl phosphatidylethanolamine (C12:0), dimiristoyl phosphatidylethanolamine (C14:0), dipalmitoyl phosphatidylethanolamine (C16:0), diphytanoyl phosphatidylethanolamine (C16:0, branched), distearoyl phosphatidylethanolamine (DSPE, C18:0) were compared with DOPE (C18:1) to show that the branched and unsaturated species combined with cationic lipids acted in physical synergism to increase transfection efficiency (Heinze et al., 2010).

3.2 DOPC
Ewert et al. (Ewert et al., 2004) demonstrated that $\sigma_M$, the average membrane charge density of the CL-vector, was a key universal parameter that governed the transfection behavior of $L_c^C$ complexes in cells. DOPC favors the formation of $L_c^C$ type of lipoplexes, in which, a system of DOPC/DOTAP-DNA lipoplex showed a strong dependence on the molar fraction of neutral lipid DOPC ($\Phi_{DOPC}$) and therefore membrane charge density $\sigma_M$. The transfection efficiency started low for $0.5 < \Phi_{DOPC} < 0.7$ and increased dramatically to a similar value, at $\Phi_{DOPC} = 0.2$, with $H_{II}^C$ lipoplex achieved by the DOPE/DOTAP-DNA. In contrast to $L_c^C$ complexes, $H_{II}^C$ complexes containing DOPE exhibited no dependence on $\sigma_M$. The transfection efficiency increased exponentially with a linear increase of $\sigma_M$ for the MVL5/DOPC/DNA lipoplex bearing $L_c^C$ (Ewert et al., 2002). And then, they found that the curve of transfection efficiency versus $\sigma_M$ assumed a bell-shape with increasing $\sigma_M$ using MVL type of cationic lipids (Ahmad et al., 2005). Ewert et al. (Ewert et al., 2006) also found that hexagonally arranged tubular lipid micelles ($H_{II}^C$) surrounded by DNA rods were formed though DOPC was used in the dendritic lipid-based cationic liposome.

Later it has been proved that the enhanced transfection efficiency was supported by a mesoscale computer modeling of cationic lipids in $L_c^C$ phase at high concentrations of cationic lipid (Farago et al., 2006). Recently, a study (Kedika & Srilakshmi, 2011) showed that DOPC was a more efficacious colipid than DOPE. The difference in the transfection efficiencies of lipoplexes in the presence of colipids DOPE and DOPC was explained as the uptake of the lipoplexes in the presence of DOPE took place mainly from the fusion of the lipoplexes with the plasma membrane, whereas “endocytosis” facilitated uptake in the presence of DOPC. Many researchers have agreed membrane charge density $\sigma_M$ is a universal parameter governing the transfection efficiency of $L_c^C$ lipoplexes (Ewert et al., 2005a, 2005b; Lin, 2003). But for the question, which morphology among $L_c^C$ governed mainly by DOPC and $H_{II}^C$ governed mainly by DOPE is favored in terms of transfection efficiency, we still need to carry out more research.

3.3 Lipoplex sizes
Another parameter of morphologies affecting transfection efficiency is lipoplex sizes, for the important role of lipoplex sizes in determining the nature of the entry pathway by endocytosis (Wasungu & Hoekstra, 2006). Though it is difficult to unify the size effect of
lipoplexes on the transfection activity so far, most transfection complexes fall within an average size range of 100–300nm. The lipoplex particles can be categorized as small (≤100nm), medium (100–200nm), large (200–1000nm) or giant (≥1000nm). (Donkuru et al., 2010).

Some times large lipoplexes sizes could be more efficient to transfer genes because large particles lead to maximum contact with cells (Kennedy et al., 2000), the formation of large intracellular vesicles which are more easily disrupted, thus releasing DNA into the cytoplasm (Escriou et al., 1998), phagocytic activity accompanied by endosomal escape (Xu et al., 1999) and faster sedimentation and better cellular trafficking (Lee et al., 2003). At the same time, some reports supported that particles with smaller size would gain high transfection efficiency (Pitard et al., 1997; Kneuer et al., 2006). The requirement for efficient transfection may be different in vivo and in vitro. Compared with in vitro delivery, small particles tend to have high transfection efficiency in vivo because of the ability of small particles to traverse narrow capillary networks. Large particles typically have low in vivo transfection efficiencies, while 200-400nm is the optimal size for lipoplexes in vitro (Zhdanov et al., 2002; Kennedy et al., 2000). Measurement of the endosomal uptake of fluorescent dextran beads of various sizes clarified that particles smaller than 200 nm were predominantly taken up by means of clathrin mediated endocytosis; with increasing the size, a shift to another mechanism occurred, so that particles larger than 500 nm were taken up predominantly by caveolae mediated pathways (Rejman et al., 2004). Carriere et al. (Carriere et al., 2002) have proved that lipofection inhibition by serum was largely due to the serum inhibition of lipoplex size growth, and may be overcome by using large, stable lipoplexes. Lipoplexes of over 700 nm mean diameter induced efficient transfection in the presence or absence of serum (Turek et al., 2000), but lipoplexes of less than 250 nm in size showed efficient transfection only in the absence of serum. It was reported that the particle sizes may be one of the factors that were contributed to serum resistance of EDL (ethanol-dried lipid-DNA) lipoplexes, and the large cationic lipoplexes may delay the dissociation of DNA with lipid, thereby enhancing DNA transfection efficiency (Lian & Ho, 2003).

Although a general rule is not obtained until now, there is no doubt that high transfection would be gained from large lipoplexes when endocytosis is dominant, because large particles facilitate membrane contact and fusion. When cells are not actively endocytosing, either small particles may have high transfection efficiency, or lipoplex sizes don’t correlate with lipofection efficiency. The possibility of a final agreement on the lipoplexes size effect may be very small, as the other conditions of every transfection case could be different. The controllable assembly of lipoplexes may provide a solution to this problem.

4. Hybrid vectors based on cationic lipids

The hybridized utilization of non-viral vectors also provides an alternative solution to the delivery of genes. We could hybridize cationic liposomes and polymers; introduce peptides and targeting moieties into lipids for approaching the requirements of gene therapy (Zhang et al., 2010).

4.1 Hybrids of cationic liposomes and polymers

Cationic polymers could combine with DNA to form a particulate complex, polyplex, capable of gene transfer into the targeted cells (El-Anned, 2004), because most of them are
completely soluble in water. Therefore, they have the obvious advantage of compressing DNA molecules to a relatively small size (Gershon et al., 1993; Ruponen et al., 1999). But they do not contain a hydrophobic moiety (Elouahabi & Ruysschaert, 2005), this may hinder the transfection efficiency and cause cytotoxicity to some degree.

Liposome-mediated gene transfer could be improved by natural polycations such as protamine sulfate (PS), poly(L-lysine) (PLL), and spermine (Li & Huang, 1997; Cheng et al., 2009). The addition of poly(L-lysine) and protamine dramatically reduced the particle size of the complex formed between DNA and cationic liposomes and rendered DNA resistant to the nuclease (Gao & Huang, 1996). These polycations could form a complex with DNA and condense DNA from extended conformation to highly compact structure into 30-100 nm in size. A type of hybrid vectors were developed by Huang et al. (Gao & Huang, 1996; Lee & Huang, 1996) in which poly(lysine)-condensed DNA was entrapped into folate-targeted cationic liposomes (LPD). They found LPD vectors to be more efficient and less cytotoxic compared to conventional cationic liposomal vectors. Later, they modified LPDs through different cationic liposomes wherein LPDs were used to deliver antisense oligodeoxynucleotide and siRNA (Li & Huang, 2006; Chen et al., 2009; Gao & Huang, 2009).

As a cationic polymer, PEI is commonly used for the delivery of genes. The hybrid usage with cationic liposomes provides a promising way to the field of gene transfer. In a study, the combination of PEI and DOTAP-Chol caused more than 10-fold increase in the transfection efficiency and less toxicity in many cells compared with using polymer or liposome alone (Lee et al., 2003). Nearly at the same time, PEI2K-DNA-Dosper complexes showed much more cellular uptake of DNA than PEI2K-DNA complexes and two times higher transfection than Dosper-DNA complexes. It has been hypothesized that Dosper improved the cellular uptake of PEI2K-DNA complexes and PEI2K improved a transfer of the complexes from lysosomes to nucleus (Lampela et al., 2003).

In recent years, chitosan-based carriers have become one of the non-viral vectors that have gained increasing interest as a safer and cost-effective delivery system for gene materials, as they have beneficial qualities such as low toxicity, low immunogenicity, excellent biocompatibility as well as a high positive charge density (Zhang et al., 2007; Mao et al., 2010). Katas et al. (Katas & Alpar, 2006) may be the first group to investigate the use of chitosan to deliver siRNA in vitro. Two types of cell lines, CHO K1 and HEK 293 were used to reveal that preparation method of siRNA association to the chitosan played an important role on the silencing effect. Chitosan-TPP nanoparticles with entrapped siRNA were shown to be better vectors as siRNA delivery vehicles compared to chitosan-siRNA complexes possibly due to their high binding capacity and loading efficiency. We have combined chitosans and cationic liposomes to form a ternary lipopolyplex which could facilitate the delivery of genes into cells more efficiently than the utilization of a lipoplex or a polyplex alone (Fig. 9.). The confocal microscopy method proved that exogenous DNA molecules entered the nucleus through the nuclear membrane other than via the NPC. The results explored novel ways based on the hybrid vectors to enhance the pDNA delivery with chitosans and with further suitable intracellular mechanism, allowing the development of nonviral gene delivery and may provide the most exciting solution for hybrid biomaterials design used for beneficial candidates for gene therapy.

### 4.2 Conjugates of peptides and lipids

One of the most challenge things for gene delivery by cationic liposome method is the toxicity of cationic lipids originated in the cationic nature. The replacement of cationic head
groups has been a major trend with other more biocompatible groups, such as peptides, in recent years, as the cationic lipids with quaternary ammonium head groups can become cytotoxic by interacting with critical enzymes such as PKC (Bottega & Epand, 1992). The commonly used headgroups are peptides consisting of amino acids, such as lysine, arginine, histidine, ornithine and tryptophan. Therefore, the conjugates of peptides and lipids are much less toxic, whilst keeping the same transfection efficiency (Behr et al., 1989; Ahn et al., 2004).

The first polypeptide cationic liposome prepared by a polycondensation reaction was described by Folda et al. (Folda et al., 1982). A subset of lipitoids with a repeated side chain trimer motif conjugated with dimyristoyl phosphatidyl-ethanolamine (DMPE) mediated DNA were also found to transfer cells with high efficiency (Huang et al., 1998). A compound which contained cholesterol and a dipeptide consisting of glycine and sterically protected arginine has been proved to be suitable for in vitro transfection in the presence of 10% sera more efficiently than other cholesterol derivatives (Sochanik et al., 2000). Peptide-based gemini surfactants GS could lead to an increase in levels of gene expression in vitro compared to well-established non-viral reagents (McGregor et al., 2001).

Obata et al. (Obata et al., 2008) have proved that the lysine- or arginine-type lipids exhibited higher gene expression efficiencies than that of Lipofectamine2000, with COS-7 cells. A series of new lipophilic peptides possessing a cationic tripeptide headgroup were effective non-viral vectors for gene delivery. Then, they also synthesized a series of cationic amino acid-based lipids having a spacer between the cationic head group and hydrophobic moieties and examined the influence of the spacer on a liposome gene delivery system. (Obata et al., 2009). At present, they are investigating the ability of cationic liposomes composed of 1,5-dihexadecyl N-arginy1-L-glutamate (Arg-Glu2C16) to carry nucleic acids into neuronal cells. Arg-Glu2C16 as a model cationic amino acid-based lipid, had a high capability as a gene carrier, even for neuronal transfection (Obata et al., 2010). Coles et al. (Coles et al., 2010) has synthesized positively charged peptide-based carriers which could interact with DNA improved by performing isothermal titration calorimetry and particle size and zeta potential experiments. The particle sizes of the carrier/DNA complexes varied over the different charge ratios from 200-800nm. The utilization of lipophilic carriers is a promising approach to improve the bioavailability of gene delivery.

Some peptide head groups could endow additional functions to the lipids, such as membrane-disturbing ability. In a hybrid molecule, the covalent coupling of an amphipathic and membrane-disturbing peptide to a lipid moiety might create a stable and efficient
peptide-based gene transfer system. The luciferase activity induced by the dioleoylamilittin/DNA complex was 5-500-fold higher than that induced by a cationic lipid/DNA complex, depending on the cationic lipid and the cell-line (Legendre et al., 1997). Later, a membrane-disrupting peptide derived from the influenza virus was covalently linked to different polymethacrylates using N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) as a coupling agent to increase the transfection efficiency of polyplexes based on these polymers. In vitro transfection and toxicity were tested in COS-7 cells, and these experiments showed that the polyplexes with grafted peptides had a substantially higher transfection activity than the control polyplexes, while the toxicity remained unchanged (Funhoff et al., 2005).

4.3 Conjugates of targeting moieties and lipids

For a number of gene therapy applications, targeted gene delivery systems have attracted great attention due to their potential in directing the therapeutic genes to the target cells, and it may help minimize adverse effects such as cytotoxicity or immune reactions, as well as maximizing the efficacy of the therapeutic response. The targeted delivery of the lipoplexes may be achieved through the addition of targeting moieties (e.g., ligands) into liposomes by direct formulation, with no covalent bond to any lipid (Seol et al., 2000); conjugated to helper lipid (Dauty et al., 2002), or connected directly to the cationic lipids (Kawakami et al., 2000a; Gaucheron et al., 2001c). For targeted lipoplexes, modified with a targeting moiety such as folate (Dauty et al., 2002), galactose (Kawakami et al., 2000a; Gaucheron et al., 2001c), mannose (Kawakami et al., 2000b), antibodies (Duan et al., 2008) and transferrin (Seol et al., 2000; Sakaguchi et al., 2008), the uptake can be receptor mediated and enhanced (Zhang et al., 2010).

Targeting of the folate receptor (FR) had received much attention in recent years, since the folate receptor is a tumor marker over expressed in large numbers of cancer cells, including cancers of the ovary, kidney, uterus, testis, brain, colon, and in addition, folic acid is a relatively small molecule (MW 441 Da), therefore, it has the advantages of being stable and nonimmunogenic compared to monoclonal antibodies (Kane et al., 1986), and still having a relatively high receptor affinity. In a study, the folate moiety was attached to a lipid membrane anchor via a cysteinyI-PEG₃₄₀₀ spacer, which greatly increased specific cellular uptake to FR overexpressing cancer cells in comparison with unmodified cationic liposome and can significantly improve the transfection efficiency of a cationic liposomal formulation (Reddy et al., 2002). Recently, Yoshizawa et al. (Yoshizawa et al., 2008) developed a folate-linked nanoparticle (NP-F), which was composed of cholesteryl-3β-carboxyamidoethylene-N-hydroxyethylamine, Tween 80 and folate-poly(ethylene glycol)-distearoyl-phosphatidylethanolamine conjugate (f-PEG2000-DSPE), and was delivered synthetic siRNA with high transfection efficiency and selectivity into nasopharyngeal tumor KB cells.

The asialoglycoprotein receptor (ASPG), present at the surface of hepatocytes, could recognize and bind to β-D-galactoside terminated glycoproteins for the targeting of cationic vector-based gene delivery systems (Ashwell & Harford, 1982). Kawakami et al. (Kawakami et al., 1998) have studied liposomal with asialoglycoprotein receptor gene carrier systems for gene delivery to hepatocytes, which was a novel galactosylated cholesterol derivatives, cholesten-5-yloxy-N-4-((1-imino-2-β-D-thiogalactosylthethyl)amino)alkyl) formamide. In human hepatoma cells (HepG2), the liposomes containing this galactolipid showed higher transfection activities than DC-Chol liposomes based on a receptor-mediated mechanism.
Later, they used galactosylated cationic liposomes to target liver cell asialoglycoprotein receptors in vivo (Kawakami et al., 2000a). Many groups also reported a few other glycosylated cationic bolaamphiphiles similar to the compound (Letrou-Bonneval et al., 2008). Brunelle et al. (Brunelle et al., 2009) synthesized a new series of dissymmetric hemifluorocarbon bolaamphiphiles (Fig. 10.), and the dissymmetric functionalization of diiodoperfluorooctane led to bolaamphiphile molecules composed of a partially fluorocarbon core end-capped with a glycoside and an ammonium salt. They found that the incorporation of two fluorinated segments in the molecular structure of the bolaamphiphiles is detrimental for an efficient DNA condensation.

Fig. 10. A set of dissymmetric hemifluorocarbon bolaamphiphiles.

The mannose receptor (MR) is found on the surface of macrophages and dendritic cells can recognize complex carbohydrates that are located on glycoproteins that are a part of many different biological processes, and bind terminal mannosates found on vector. Kawakami et al. (Kawakami et al., 2000b) have developed a novel mannosylated cholesterol derivative, Man-C4-Chol, consisting of modified cationic liposomes with mannose moieties for NPC-selective gene delivery via mannose receptors on NPC. The mannosylated cationic
liposomes can deliver pDNA to liver nonparenchymal cells (Kawakami et al., 2000b) and splenic DCs and improve immune activation in DNA vaccines (Hattori et al., 2004). Since their early use for the targeting to erythroblasts (Zhang et al., 2010), targeting proteins such as antibodies or transferrin have become one of the most widely used ligands for targeting of synthetic vectors, and been used in conjunction with cationic lipoplexes (Rao, 2010) to mediate uptake of plasmid DNA and antisense oligonucleotides. Another important class of cell proteins with promise for targeted gene delivery is growth factor receptor, which is a most commonly single pass transmembrane protein with an extracellular ligand binding domain and an intracellular region with enzymatic activity, usually a tyrosine kinase domain, which transmits a growth factor signal from the cell’s environment to its interior. It has been used to target pol lysine complexes (Cristiano & Roth, 1996), liposomes (Kikuchi, 1996), PEI polyplexes (Cristiano & Roth, 1996), and adenovirus-derived peptides (Medina-Kauwe et al., 2001) to receptor-positive cells.

Targeting provides a generic strategy to improve the specificity of a pharmaceutical formulation independently of the specificity of the drug or gene itself, primarily through a modulation of the carriers’ biodistribution, so that a dose differential is created between healthy and diseased tissue.

5. Conclusion

Cationic liposomes-mediated gene transfer has shown to be a safe and effective way to transfer genes for gene therapy, and will gain clinical application in the near future. Some traditional cationic lipids have been used in gene transfer for a long time; many of them have trademarks such as lipofectin, lipofectamine and transfectam. These cationic lipids, however, may not be enough for the application in clinical trials; on the other hand, they have some shortcomings for a special application field. Therefore, many novel cationic lipids in chemical structure nature have been researching to meet the requirements with respect to gene therapy. Besides the lipid structure the next level should be the formulations of cationic liposomes, as in order to increase transfection efficiency and to decrease cytotoxicity other ingredients such as hyper lipids could be chosen. Different formulations may cause various morphologies of lipoplexes formed by the combination between cationic liposomes and genes. It shows that the purposeful design of morphologies could increase transfection efficiency. The hybrid utilization of non-viral vectors based on cationic liposomes provides another solution to gene delivery. Much research has shown the promising combination between cationic liposomes and polymers, the favorable conjugation between lipids and peptides and between targeting moieties and lipids.

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7. References

Ahmad, A.; Evans, H.M.; Ewert, K.; George, C.X.; Samuel, C.E. & Safinya1, C.R. (2005). New Multivalent Cationic Lipids Reveal Bell Curve for Transfection Efficiency Versus
Cationic Liposomes in Different Structural Levels for Gene Delivery

Membrane Charge Density: Lipid–DNA Complexes for Gene Delivery. *J. Gene. Med.*, Vol.7, No.6, pp. 739-748, ISSN 1099-498X

Ahn, C.H.; Chae, S.Y.; Bae, Y.H. & Kim, S.W. (2004). Synthesis of Biodegradable Multi-block Copolymers of Poly(L-lysine) and Poly(ethylene glycol) As a Non-viral Gene Carrier. *J. Controlled Release*, Vol.97, No.3, pp. 567-574, ISSN 0168-3659

Amenitsch, H.; Caracciolo, G.; Foglia, P.; Fuscoletti, V.; Giansanti, P.; Marianecchi, C.; Pozzi, D. & Laganà, A. (2011). Existence of Hybrid Structures in Cationic Liposome/DNA Complexes Revealed by Their Interaction with Plasma Proteins. *Colloids Surf., B*, Vol.82, No.1, pp. 141-146, ISSN 0927-7765

Ashwell, G. & Harford, J. (1982). Carbohydrate-specific Receptors of the Liver. *Annu. ReV. Biochem.*, Vol.51, pp. 531-554, ISSN 0006-4154

Behr, J.P.; Demeneix, B.; Loeffler, J.P. & Perez-Mutul, J. (1989). Efficient Gene-transfer into Mammalian Primary Endocrine-cells with Lipopolyamine-coated DNA. *Proc. Natl. Acad. Sci. U.S.A.*, Vol.86, No.18, pp. 6982-6986, ISSN 0027-8424

Bennett, M.J.; Aberle, A.M.; Balasubramaniam, R.P.; Malone, J.G.; Malone, R.W. & Nantz, M.H. (1997). Cationic Lipid-mediated Gene Delivery to Murine Lung: Correlation of Lipid Hydration with in Vivo Transfection Activity. *J. Med. Chem.*, Vol.40, No.25, pp. 4069-4078, ISSN 0022-2623

Bottega, R. & Epand, R.M. (1992). Inhibition of Protein Kinase C by Cationic Amphiphiles. *Biochemistry*, Vol.31, No.37, pp. 9025-9030, ISSN 0006-2960

Brunelle, M.; Polidori, A.; Denoyelle, S.; Fabiano, A.S.; Vuillaume, P.Y.; Laurent-Lewandowski, S. & Pucci, B. (2009). A Structure-activity Investigation of Hemifluorinated Bifunctional Bolaamphiphiles Designed for Gene Delivery. *C. R. Chimie*, Vol.12, No.1-2, pp. 188-208, ISSN 1631-0748

Byk, G.; Dubertret, C.; Escriou, V.; Frederic, M.; Jaslin, G.; Rangara, R.; Pitard, B.; Crouzet, J.; Wils, P.; Schwartz, B. & Scherman, D. (1998). Synthesis, Activity, and Structure-Activity Relationship Studies of Novel Cationic Lipids for DNA Transfer. *J. Med. Chem.*, Vol.41, No.2, pp. 224-235, ISSN 0022-2623

Byk, G.; Wetzer, B.; Frederic, M.; Dubertret, C.; Pitard, B.; Jaslin, G. & Scherman, D. (2000). Reduction-sensitive Lipopolyamines as a Novel Nonviral Gene Delivery System for Modulated Release of DNA with Improved Transgene Expression. *J. Med. Chem.*, Vol.43, No.23, pp. 4377-4387, ISSN 0022-2623

Carriere, M.; Tranchant, I.; Niore, P.A.; Byk, G.; Mignet, N.; Escriou, V.; Scherman, D. & Herscovici, J. (2002). Optimization of Cationic Lipid Mediated Gene Transfer: Structure-function, Physico-chemical, and Cellular Studies. *J. Liposome. Res.*, Vol.12, No.1-2, pp. 95-106, ISSN 0898-2104

Chen, Y.C.; Sen, J.; Bathula, S.R.; Yang, Q.; Fittipaldi, R. & Huang, L. (2009). Novel Cationic Lipid That Delivers siRNA and Enhances Therapeutic Effect in Lung Cancer Cells. *Mol. Pharm.*, Vol.6, No.3, pp. 696-705, ISSN 1543-8384

Cheng, H.; Zhu, J.L.; Zeng, X.; Jing, Y.; Zhang, X.Z. & Zhuo, R.X. (2009). Targeted Gene Delivery Dedicated by Folate-polyethyleneimine-block-poly(ethylene glycol) with Receptor Selectivity. *Bioconjugate Chem.*, Vol.20, No.3, pp. 481-487, ISSN 1043-1082

Choi, J.S.; Lee, E.J.; Jang, H.S. & Park, J.S. (2001). New Cationic Liposomes for Gene Transfer into Mammalian Cells with High Efficiency and Low Toxicity. *Bioconjugate Chem.*, Vol.12, No.1, pp. 108-113, ISSN 1043-1082
Coles, D.J.; Esposito, A.; Chuah, H.T. & Toth, I. (2010). The Synthesis and Characterization of Lipophilic Peptide-based Carriers for Gene Delivery. *Tetrahedron*, Vol.66, No.29, pp. 5435-5441, ISSN 0040-4020

Cristiano, R.J. & Roth, J.A. (1996). Epidermal Growth Factor Mediated DNA Delivery into Lung Cancer Cells via the Epidermal Growth Factor Receptor. *Cancer Gene Ther.*, Vol.3, No.1, pp. 4-10, ISSN 0929-1903

Dauty, E.; Remy, J. S.; Zuber, G. & Behr, J.-P. (2002). Intracellular Delivery of Nanometric DNA Particles via the Folate Receptor. *Bioconjugate Chem.*, Vol.13, No.4, pp. 831-839, ISSN 1043-1082

Donkuru, M.D.; Badea, I.; Wettig, S.; Verrall, R.; Elsabahy, M. & Foldvari, M. (2010). Advancing Nonviral Gene Delivery: Lipid- and Surfactant-based Nanoparticle Design Strategies. *Tetrahedron, Nanomedicine (Lond)*, Vol.5, No.7, pp. 1103-1127, ISSN 1743-5889

Duan, Y.J.; Zheng, J.N.; Han, S.F.; Wu, Y.; Wang, Y.M.; Li, D.J.; Kong, D.L. & Yu, Y.T. (2008). A Tumor Targeted Gene Vector Modified with G250 Monoclonal Antibody for Gene Therapy. *J. Controlled Release*, Vol.127, No.2, pp. 173-179, ISSN 0168-3659

El-Anned, A. (2004). An Overview of Current Delivery Systems in Cancer Gene Therapy. *J. Controlled Release*, Vol.94, No.1, pp. 1-14, ISSN 0168-3659

Elouahabi, A. & Ruyschaert, J.M. (2005). Formation and Intracellular Trafficking of Lipoplexes and Polyplexes. *Mol. Ther.*, Vol.11, No.3, pp. 336-347, ISSN 1525-0016

Escriou, V.; Ciolina, C.; Lacroix, F.; Byk, G.; Scherman, D. & Wils, P. (1998). Cationic Lipid-mediated Gene Transfer: Effect of Serum on Cellular Uptake and Intracellular Fate of Lipopolyamine/DNA Complexes. *Biochim. Biophys. Acta.*, Vol.1368, No.2, pp. 276-288, ISSN 0005-2736

Ewert, K.; Ahmad, A.; Evans, H.M. & Safinya, C.R. (2005a). Cationic Lipid-DNA Complexes for Non-viral Gene Therapy: Relating Supramolecular Structures to Cellular Pathways. *Expert Opin. Biol. Ther.*, Vol.5, No.1, pp. 33-53, ISSN 1471-2598

Ewert, K.; Ahmad, A.; Evans, H.M.; Schmidt, H.W. & Safinya, C.R. (2002). Efficient Synthesis and Cell-transfection Properties of a New Multivalent Cationic Lipid for Nonviral Gene Delivery. *J. Med. Chem.*, Vol.45, No.23, pp. 5023-5029, ISSN 0022-2623

Ewert, K.; Evans, H.M.; Ahmad, A.N.; Slack, L.; Lin, A.J.; Martin-Herranz, A. & Safinya, C.R. (2005b). Lipoplex Structures and Their Distinct Cellular Pathways. *Adv. Genet.*, Vol.53, No.8, pp. 119-155, ISBN 13:978-0-12-374621-4

Ewert, K.; Evans, H.M.; Zidovska, A.; Bouxsein, N.F.; Ahmad, A. & Safinya, C.R. (2006). A Columnar Phase of Dendritic Lipid-based Cationic Liposome-DNA Complexes for Gene Delivery: Hexagonally Ordered Cylindrical Micelles Embedded in a DNA Honeycomb Lattice. *J. Am. Chem. Soc.*, Vol.128, No.12, pp. 3998-4006, ISSN 0002-7863

Ewert, K.; Slack, N.L.; Ahmad, A.; Evans, H.M.; Lin, A.J.; Samuel, C.E. & Safinya, C.R. (2004). Cationic Lipid-DNA Complexes for Gene Therapy: Understanding the Relationship Between Complex Structure and Gene Delivery Pathways at The Molecular Level. *Curr. Med. Chem.*, Vol.11, No.2, pp. 133-149, ISSN 1568-0118

Fabio, K.; Gaucheron, J.; Giorgio, C.D. & Vierling P. (2003). Novel Galactosylated Polyamine Bolaamphiphiles for Gene Delivery. *Bioconjugate Chem.*, Vol.14, No.2, pp. 358-367, ISSN 1043-1082
Fanning, G.; Amado, R. & Symonds, G.J. (2003). Gene Therapy for HIV/AIDS: The Potential for a New Therapeutic Regimen. *J Gene Med*, Vol.5, No.8, pp. 645-653, ISSN 1521-2254

Farago, O.; Gronbech-Jensen, N. & Pincus, P. (2006). Mesoscale Computer Modeling of Lipid-DNA Complexes for Gene Therapy. *Phys. Rev. Lett.*, Vol.96, No.4, pp. 018102-018106, ISSN 0031-9007

Farhood, H.; Serbina, N. & Huang, L. (1995). The Role of Dioleoyl Phosphatidylethanolamine in Cationic Liposome Mediated Gene Transfer. *Biochim. Biophys. Acta*, Vol.1235, No.2, pp. 289-295, ISSN 0005-2736

Felgner, J.H.; Kumar, R.; Sridhar, C.N.; Wheeler, C.J.; Tsai, Y.J.; Border, R.; Ramsey, P.; Martin, M. & Felgner, P.L. (1994). Enhanced Gene Delivery and Mechanism Studies with a Novel Series of Cationic Lipid Formulations. *J. Biol. Chem.*, Vol.269, No.4, pp. 2550-2561, ISSN 0021-9258

Felgner, P.L.; Gadek, T.R.; Holm, M.; Roman, R.; Chan, H.S.; Wenz, M.; Northrop, J.P.; Ringgold, G.M. & Danielson, M. (1987). Lipofection: A Highly Efficient, Lipid-Mediated DNA-transfection Procedure. *Proc. Natl. Acad. Sci. U.S.A.*, Vol.84, No.21, pp. 7413-7417, ISSN 0027-8424

Felgner, P.L. & Ringold, G.M. (1989). Cationic Liposome-mediated Transfection. *Nature*, Vol.11, No.2, pp. 387-388, ISSN 0028-0836

Ferrari, M.E.; Nguyen, C.M.; Zelphati, O.; Tsai, Y. & Felgner, P.L. (1998). Analytical Methods for the Characterization of Cationic Lipid Nucleic Acid Complexes. *Hum. Gene Ther.*, Vol.9, No.3, pp. 341-351, ISSN 1043-0342

Ferrari, M.E.; Rusalov, D.; Enas, J. & Wheeler, C.J. (2002). Synergy between Cationic Lipid and Co-lipid Determines the Macroscopic Structure and Transfection Activity of Lipoplexes. *Nucleic Acids Res.*, Vol.30, No.8, pp. 1808-1816, ISSN 0005-1048

Fletcher, S.; Ahmad, A.; Perouzel, E.; Heron, A.; Miller, A.D. & Jorgensen, M.R. (2006). In Vivo Studies of Dialkynoyl Analogues of DOTAP Demonstrate Improved Gene Transfer Efficiency of Cationic Liposomes in Mouse Lung. *J. Med. Chem.*, Vol.49, No.1, pp. 349-357, ISSN 0022-2623

Floch, V.; Loisel, S.; Guénin, E.; Hervé, A.C.; Yaouanc, J.J.; Clément, J.C.; Férec, C. & Des Abbayes, H. (2000). Cation Substitution in Cationic Phosphonolipids: A New Concept to Improve Transfection Activity and Decrease Cellular Toxicity. *J. Med. Chem.*, Vol.43, No.23, pp. 4617-4628, ISSN 0022-2623

Folda, T.; Gros, L. & Ringsdorf, H. (1982). Polyreactions in Oriented Systems, 29a). Formation of Oriented Polypeptides and Polyamides in Monolayers and Liposomes. *Makromol Chem Rapid Commun*, Vol.3, No.3, pp. 167-174, ISSN 0173-2803

Frederic, M.; Scherman, D. & Byk, G. (2000). Introduction of Cyclic Guanidines into Cationic Lipids for Non-viral Gene Delivery. *Tetrahedron Lett.*, Vol.41, No.5, pp. 675-679, ISSN 0040-4039

Fujiwara, T.; Hasegawa, S.; Hirashima, N.; Nakanishi, M. & Ohwada, T. (2000). Gene Transfection Activities of Amphiphilic Steroid-polyamine Conjugates. *Biochim. Biophys. Acta, Biomembr.*, Vol.1468, No.1-2, pp. 396-402, ISSN 0005-2736

Funhoff, A.M.; van Nostrum, C.F.; Lok, M.C.; Kruijtzer, J.A.; Crommelin, D.J. & Hennink, W.E. (2005). Cationic Polymethacrylates with Covalently Linked Membrane Destabilizing Peptides as Gene Delivery Vectors. *J. Controlled Release*, Vol.101, No.1-3, pp. 233-246, ISSN 0168-3659
Gao, H. & Hui, K.M. (2001). Synthesis of a Novel Series of Cationic Lipids that Can Act As Efficient Gene Delivery Vehicles Through Systematic Heterocyclic Substitution of Cholesterol Derivatives. *Gene Ther*, Vol.8, No.11, pp. 855-863, ISSN 0969-7128

Gao, K. & Huang, L. (2009). Nonviral Methods for siRNA Delivery. *Mol. Pharm.*, Vol.6, No.3, pp. 651-658, ISSN 1543-8384

Gao, X. & Huang, L. (1991). A Novel Cationic Liposome Reagent for Efficient Transfection of Mammalian Cells. *Biochem. Biophys. Res. Commun.*, Vol.179, No.1, pp. 280-285, ISSN 0006-291X

Gao, X. & Huang, L. (1995). Cationic Liposome-mediated Gene Transfer. *Gene Ther*, Vol.2, No.10, pp. 710-722, ISSN 0969-7128

Gao, X. & Huang, L. (1996). Potentiation of Cationic Liposome-mediated Gene Delivery by Polycations. *Biochemistry*, Vol.35, No.3, pp. 1027-1036, ISSN 0006-2960

Gaucheron, J.; Boulanger, C.; Santaella, C.; Sbirrazzuoli, N.; Boussif, O. & Vierling, P. (2001a). In Vitro Cationic Lipid-mediated Gene Delivery with Fluorinated Glycerophosphoethanolamine Helper Lipids. *Bioconjugate Chem.*, Vol.12, No.6, pp. 949-963, ISSN 1043-1082

Gaucheron, J.; Santaella, C. & Vierling, P. (2001b). Highly Fluorinated Lipospermines for Gene Transfer: Synthesis and Evaluation of Their in Vitro Transfection Efficiency. *Bioconjugate Chem.*, Vol.12, No.1, pp. 114-128, ISSN 1043-1082

Gaucheron, J.; Santaella, C. & Vierling, P. (2001c). In Vitro Gene Transfer with a Novel Galactosylated Spermine Bolaamphiphile. *Bioconjugate Chem.*, Vol.12, No.4, pp. 569-575, ISSN 1043-1082

Gaucheron, J.; Santaella, C. & Vierling, P. (2001d). Improved in Vitro Gene Transfer Mediated by Fluorinated Lipoplexes in the Presence of a Bile Salt Surfactant. *J. Med. Chem.*, Vol.3, No.4, pp. 338-344, ISSN 0022-2623

Gaucheron, J.; Wong, T.; Wong, K.F.; Maurer, N. & Cullis, P.R. (2002). Synthesis and Properties of Novel Tetraalkyl Cationic Lipids. *Bioconjugate Chem.*, Vol.13, No.3, pp. 671-675, ISSN 1043-1082

Gershon, H.; Ghirlando, R.; Guttman, S.B. & Minsky, A. (1993). Mode of Formation and Structural Features of DNA-Cationic Liposome Complexes Used for Transfection. *Biochemistry*, Vol.32, No.28, pp. 7143-7151, ISSN 0006-2960

Hattori, Y.; Kawakami, S.; Suzuki, S.; Yamashita, F. & Hashida, M. (2004). Enhancement of Immune Responses by DNA Vaccination Through Targeted Gene Delivery Using Mannosylated Cationic Liposome Formulations Following Intravenous Administration in Mice. *Biochem. Biophys. Res. Commun.*, Vol.317, No.4, pp. 992-999, ISSN 0006-291X

Heinze, M.; Brezesinski, G. & Dobner, B. (2010). Langner A: Novel Cationic Lipids Based on Malonic Acid Amides Backbone: Transfection Efficacy and Cell Toxicity Properties. *Bioconjugate Chem.*, Vol.21, No. 4, pp. 696-708, ISSN 1043-1802

Heyes, J.A.; Niculescu-Duvaz, D.; Cooper, R.G. & Springer, C.J. (2002). Synthesis of Novel Cationic Lipids: Effect of Structural Modification on the Efficiency of Gene Transfer. *J. Med. Chem.*, Vol.45, No.1, pp. 99-114, ISSN 0022-2623

Huang, C.Y.; Uno, T.; Murphy, J.E.; Lee, S.; Hamer, J.D.; Cesobeda, J.A.; Cohen, F.E.; Radakrishnan, R.; Dwarki, V. & Zuckermann R.N. (1998). Lipitoids-novel Cationic Lipids for Cellular Delivery of Plasmid DNA in Vitro. *Chem Biol.*, Vol.5, No.6, pp. 345-354, ISSN 1074-5521
Legendre, J.Y.; Trzeciak, A.; Bohrmann, B.; Deuschle, U.; Kitas, E. & Supersaxo, A. (1997). Dioleoylmeitlin as a Novel Serum-Insensitive Reagent for Efficient Transfection of Mammalian Cells. *Bioconjugate Chem.*, Vol.8, No.1, pp. 57-63, ISSN 1043-1082

Ilies, M.A.; Seitz, W.A.; Caprioiu, M.T.; Wentz, M.; Garfield, R.E. & Balaban, A.T. (2003). Pyridinium-based Cationic Lipids As Gene-transfer Agents. *Eur. J. Org. Chem.*, Vol.2003, No.14, pp. 2645-2655, ISSN 1099-0690

Jacopin, C.; Hofland, H.; Scherman, D. & Herscovici, J. (2001). Synthesis and Transfecting Properties of a Glycosylated Polycationic DNA Vector. *Bioorg. Med. Chem. Lett.*, Vol.11, No.3, pp. 419-422, ISSN 0960-894X

Kane, M.A.; Elwood, P.C.; Portillo, R.M.; Antony, A.C. & Kolhouse, J.F. (1986). The Interrelationship of the Soluble and Membrane-associated Folate-binding Proteins in Human KB cells. *J. Biol. Chem.*, Vol.261, No.33, pp. 15625-15631, ISSN 0021-9258

Katas, H. & Alpar, H.O. (2006). Development and Characterisation of Chitosan Nanoparticles for siRNA Delivery. *J. Controlled Release*, Vol.115, No.2, pp. 216-225, ISSN 0168-3659

Kawakami, S.; Fumoto, S.; Nishikawa, M.; Yamashita, F. & Hashida, M. (2000a). In Vivo Gene Delivery to the Liver Using Novel Galactosylated Cationic Liposomes. *Pharm. Res.*, Vol.17, No.3, pp. 306-313, ISSN 0724-8741

Kawakami, S.; Sato, A.; Nishikawa, M.; Yamashita, F. & Hashida, M. (2000b). Mannose Receptor-mediated Gene Transfer into Macrophages Using Novel Mannosylated Cationic Liposomes. *Gene Ther*, Vol.7, No.4, pp. 292-299, ISSN 0969-7128

Kawakami, S.; Yamashita, F.; Nishikawa, M.; Takakura, Y. & Hashida, M. (1998). Asialoglycoprotein Receptor-mediated Gene Transfer Using Novel Galactosylated Cationic Liposomes. *Biochem. Biophys. Res. Commun.*, Vol.252, No.1, pp. 78-83, ISSN 0006-291X

Kedika, B. & Patri, S.V. (2011). Design, Synthesis, and in Vitro Transfection Biology of Novel Tocopherol Based Monocationic Lipids: A Structure-Activity Investigation. *J. Med. Chem.*, Vol.54, No.2, pp. 548-561, ISSN 0022-2623

Kennedy, M.T.; Pozharski, E.V.; Rakhananova, V.A. & MacDonald, R.C. (2000). Factors Governing the Assembly of Cationic Phospholipid-DNA Complexes. *Biophys. J.*, Vol.78, No.3, pp. 1620-1633, ISSN 0006-3495

Kichler, A.; Leborgne, C.; Savage, P.B. & Danos, O. (2005). Cationic Steroid Antibiotics Demonstrate DNA Delivery Properties. *J. Controlled Release*, Vol.107, No.1, pp. 174-182, ISSN 0168-3659

Kikuchi, A.; Sugaya, S.; Ueda, H.; Tanaka, K.; Aramaki, Y.; Hara, T.; Arima, H.; Tsuchiya, S. & Fuwa, T. (1996). Efficient Gene Transfer to EGF Receptor Overexpressing Cancer Cells by Means of EGF-labeled Cationic Liposomes. *Biochem. Biophys. Res. Commun.*, Vol.227, No.3, pp. 666-671, ISSN 0006-291X

Kneuer, C.; Ehrhardt, C.; Bakowsky, H.; Kumar, M.N.; Oberle, V.; Lehr, C.M.; Hoekstra, D. & Bakowsky, U. (2006). The Influence of Physicochemical Parameters on the Efficacy of Non-viral DNA Transfection Complexes: a Comparative Study. *J. Nanosci Nanotechnol.*, Vol.6, No.9-10, pp. 2776-2782, ISSN 1550-7033
Koltover, I.; Salditt, T.; Radler, J.O. & Safinya, C.R. (1998). An Inverted Hexagonal Phase of Cationic Liposome-DNA Complexes Related to DNA Release and Delivery. *Science, Vol.* 281, No. 5373, pp. 78-81, ISSN 0036-8075

Koyanova, R. & Tenchov, B. (2010). Cationic Lipids: Molecular Structure/Transfection Activity Relationships and Interactions with Biomembranes. *Top Curr Chem, Vol.* 296, No. 2010, pp. 51-93, ISSN 0340-1022

Lampela, P.; Elomaa, M.; Ruponen, M.; Urtti, A.; Mannisto, P.T. & Raasmaja, A. (2003). Different Synergistic Roles of Small Polyethylenimine and Dospers in Gene Delivery. *J. Controlled Release, Vol.* 88, No. 1, pp. 173-183, ISSN 0168-3659

Lasic, D.D. (1997). Recent Developments in Medical Applications of Liposomes: Sterically Stabilized Liposomes in Cancer Therapy and Gene Delivery in Vivo. *J. Controlled Release, Vol.* 48, No. 2-3, pp. 203-222, ISSN 0168-3659

Lee, E.R.; Marshall, J.; Siegel, C.S.; Jiang, C.; Yew, N.S.; Nichols, M.R.; Nietupski, J.B.; Ziegler, R.J.; Lane, M.B.; Wang, K.X.; Wan, N.C.; Scheule, R.K.; Harris, D.J.; Smith, A.E. & Cheng, S.H. (1996). Detailed Analysis of Structures and Formulations of Cationic Lipids for Efficient Gene Transfer to the Lung. *Hum. Gene Ther., Vol.* 7, No. 14, pp. 1701-1717, ISSN 1043-0342

Lee, L.K.; Siapati, E.K.; Jenkins, R.G.; McAnulty, R.J.; Hart, S.L. & Shamloo, P.A. (2003). Biophysical Characterization of an Integrin-targeted Non-viral Vector. *Med. Sci. Monit., Vol.* 9, No. 1, pp. 54-61, ISSN 1234-1010

Lee, R.J. & Huang, L. (1996). Folate-targeted, Anionic Liposome-entrapped Polylysine-condensed DNA for Tumor Cell-specific Gene Transfer. *J. Biol. Chem., Vol.* 271, No. 14, pp. 8481-8487, ISSN 0021-9258

Lentriv-Bonneval, E.; Chèvre, R.; Lambert, O.; Costet, P.; André, C.; Tellier, C. & Pitard, B. (2008). Galactosylated Multimodular Lipoplexes for Specific Gene Transfer into Primary Hepatocytes. *J Gene Med, Vol.* 10, No. 11, pp. 1198-1209, ISSN 1521-2254

Lensink, M.F.; Lonez, C.; Ruyschaert, J.M. & Vandenbranden, M. (2009). Characterization of the Cationic DiC14-amidine Bilayer by Mixed DMPC/DiC14-amidine Molecular Dynamics Simulations Shows an Interdigitated Nonlamellar Bilayer Phase. *Langmuir, Vol.* 25, No. 9, pp. 5230-5238, ISSN 0743-7463

Leventis, R. & Silvius, J.R. (1990). Interactions of Mammalian Cells with Lipid Dispersions Containing Novel Metabolizable Cationic Amphiphiles. *Biochim. Biophys. Acta, Biomembr., Vol.* 1023, No. 1, pp. 124-132, ISSN 0005-2736

Lian, T. & Ho, R.J. (2003). Design and Characterization of a Novel Lipid-DNA Complex That Resists serum-induced Destabilization. *J. Pharm. Sci., Vol.* 92, No. 12, pp. 2373-2385, ISSN 0022-3549

Lin, A.J.; Slack, N.L.; Ahmad, A.C.; George, X.; Samuel, C.E. & Safinya, C.R. (2003). Three-dimensional Imaging of Lipid Gene-carriers: Membrane Charge Density Controls
Universal Transfection Behavior in Lamellar Cationic Liposome-DNA Complexes. 

Litzinger, D. & Huang, L. (1992). Phosphatidylethanolamine Liposomes: Drug Delivery, Gene Transfer and Immunodiagnostic Applications. *Biochim.Biophys.Act.*, Vol.1113, No.2, pp. 201-227, ISSN 0005-2736

Liu, D.L.; Hu, J.J.; Qiao, W.H.; Li, Z.S.; Zhang, S.B.; Cheng, L.B. (2005a). Synthesis of Carbamate-linked Lipids for Gene Delivery. *Bioorg. Med. Chem. Lett.*, Vol.15, No.12, pp. 3147–3150, ISSN 0960-894X

Liu, D.L.; Hu, J.J.; Qiao, W.H.; Li, Z.S.; Zhang, S.B.; Cheng, L.B. (2005b). Synthesis and Characterization of a Series of Carbamate-linked Cationic Lipids for Gene Delivery. *Lipids*, Vol.40, No.8, pp. 839–848, ISSN 0024-4201

Liu, D.L.; Qiao, W.H.; Li, Z.S.; Chen, Y.; Cui, X.; Li, K.; Yu, L.; Yan, K.; Zhu, L.; Guo, Y. & Cheng, L.B. (2008). Structure–function Relationship Research of Glycerol Backbone-based Cationic Lipids for Gene Delivery. *Chem. Biol. Drug Des.*, Vol.71, No.4, pp. 336–344, ISSN 1747-0277

Liu, F. & Huang, L. (2002). Development of Non-viral Vectors for Systemic Gene Delivery. *J. Controlled Release*, Vol.78, No.1-3, pp. 259-266, ISSN 0168-3659

Li, S. & Huang, L. (1997). In Vivo Gene Transfer via Intravenous Administration of Cationic Lipid-protamine-DNA (LPD) Complexes. *Gene Ther*, Vol.4, No.9, pp. 891-900, ISSN 0969-7128

Li, S.D. & Huang, L. (2006). Targeted Delivery of Antisense Oligodeoxynucleotide and Small Interference RNA into Lung Cancer Cells. *Mol. Pharm.*, Vol.3, No.5, pp. 579-588, ISSN 1543-8384

Love, K.T.; Mahon, K.P.; Levins, C.G.; Whitehead, K.A.; Querbes, W.; Dorkin, J.R.; Qin, J. & Anderson, D.G. (2010). Lipid-like Materials for Low-dose, in Vivo Gene Silencing. *Proc. Natl. Acad. Sci. U.S.A.*, Vol.107, No.5, pp. 1864–1869, ISSN 0027-8424

Mao, S.; Sun, W. & Kissel, T. (2010). Chitosan-based Formulations for Delivery of DNA and siRNA. *Adv Drug Deliv Rev.*, Vol.62, No.1, pp. 12-27, ISSN 0169-409X

Marchini, C.; Pozzi, D.; Montani, M.; Alfonsi, C.; Amici, A.; Amenitsch, H.; Sanscitis, S.C.D. & Caracciolo, G. (2010). Tailoring Lipoplex Composition to the Lipid Composition of Plasma Membrane: A Trojan Horse for Cell Entry. *Langmuir*, Vol.26, No.17, pp. 13867–13873, ISSN 0743-7463

Martin-Rendon, E. & Blake, D.J. (2003). Protein Glycosylation in Disease: New Insights into the Congenital Muscular Dystrophies. *Trends Pharmacol Sci*, Vol.24, No.4, pp. 178-183, ISSN 0165-6147

McGregor, C.; Perrin, C.; Monck, M.; Camilleri, P. & Kirby, A.J. (2001). Rational Approaches to the Design of Cationic Gemini Surfactants for Gene Delivery. *J. Am. Chem. Soc.*, Vol.123, No.26, pp. 6215-6220, ISSN 0002-7863

Medina-Kauwe, L.K.; Maguire, M.; Kasahara, N. & Kedes, L. (2001). Nonviral Gene Delivery to Human Breast Cancer Cells by Targeted Ad5 Penton Proteins. *Gene Ther*, Vol.8, No.23, pp. 1753–1761, ISSN 0969-7128

Medvedeva, D.A.; Maslov, M.A.; Serikov, R.N.; Morozova, N.G.; Serebrenikova, G.A.; Sheglov, D.V.; Latyshev, A.V.; Vlassov, V.V.; Zenkova, M.A. (2009). Novel Cholesterol-Based Cationic Lipids for Gene Delivery. *J. Med. Chem.*, Vol.52, No.21, pp. 6558-6568, ISSN 0022-2623
Niculescu-Duvaz, D.; Heyes, J. & Springer, C. J. (2003). Structure-activity Relationship in Cationic Lipid Mediated Gene Transfection. *Curr. Med. Chem.*, Vol.10, No.14, pp. 1233-1261, ISSN 0929-8673

Obata, Y.; Ciofani, G.; Raffa, V.; Cuschieri, A. & Takeoka. S. (2010). Evaluation of Cationic Liposomes Composed of an Amino Acid-based Lipid for Neuronal Transfection. *Nanomed: Nanotech, Biol. Med.*, Vol.6, No.1, pp. 70-77, ISSN 1549-9634

Obata, Y.; Saito, S.; Takeda, N. & Takeoka, S. (2009). Plasmid DNA-encapsulating Liposomes: Effect of a Spacer between the Cationic Head Group and Hydrophobic Moieties of the Lipids on Gene Expression Efficiency. *Biochim. Biophys. Acta, Biomembr.*, Vol.1788, No.5, pp. 1148-1158, ISSN 0005-2736

Obata, Y.; Suzuki, D. & Takeoka, S. (2008). Evaluation of Cationic Assemblies Constructed with Amino Acid Based Lipids for Plasmid DNA Delivery. *Bioconjugate Chem.*, Vol.19, No.5, pp. 1055–1063, ISSN 0943-1082

O'Hern, C.S. & Lubensky, T.C. (1998). Sliding Columnar Phase of DNA-Lipid Complexes. *Phys. Rev. Lett.*, Vol.80, No.19, pp. 4345-4348, ISSN 0031-9007

Randazzo, R.A.S.; Bucki , R.; Janney, P.A. & Diamond, S.L. (2009). A Series of Cationic Sterol Lipids with Gene Transfer and Bactericidal Activity. *Bioorg Med Chem.*, Vol.17, No.9, pp. 3257-3265, ISSN 0968-0896

Rao, N.M. (2010). Cationic Lipid-mediated Nucleic Acid Delivery: Beyond Being Cationic. *Chem Phys Lipids*, Vol.163, No.3, pp. 245-252, ISSN 0009-3084

Reddy, J.A.; Abburi, C.; Hofland, H.; Howard, S.J.; Vlahov, I.; Wils, P. & Leamon C.P. (2002). Folate-targeted, Cationic Liposome-mediated Gene Transfer into Disseminated Peritoneal Tumors. *Gene Ther*, Vol.9, No.22, pp. 1542–1550, ISSN 0969-7128

Pedroso de Lima, M.C.; Neves, S.; Filipe, A; Düzgünes, N. & Simoes, S. (2003). Cationic Liposomes for Gene Delivery: From Biophysics to Biological Applications. *Curr. Med. Chem.*, Vol.10, No.14, pp. 1221-1231, ISSN 0929-8673

Pitard, B.; Aguerre, O.; Airiau, M.; Lachagès, A.M.; Boukhnikachvili, T.; Byk, G.; Dubertret, C.; Herviou, C.; Scherman, D.; Mayaux, J.F. & Crouzet, J. (1997). Virus-sized Self-assembling Lamellar Complexes between Plasmid DNA and Cationic Micelles Promote Gene Transfer. *Proc. Natl. Acad. Sci. U. S. A.*, Vol.94, No.26, pp. 14412-14417, ISSN 0027-8424

Rejman, J.; Oberle, V.; & Zuhorn, I.S. (2004). Size-dependent Internalization of Particles via the Pathways of Clathrin- and Caveolae-mediated Endocytosis. *Biochem. J.*, Vol.377, No.1, pp. 159-169, ISSN 0024-6021

Ren, T.; Zhang, G.; Liu, F. & Liu, D. (2000). Synthesis and Evaluation of Vitamin D-based Cationic Lipids for Gene Delivery in Vitro. *Bioorg. Med. Chem. Lett.*, Vol.10, No.9, pp. 891-894, ISSN 0960-894X

Ren, T.; Zhang, G.S. & Liu, D.X. (2001). Synthesis of Bifunctional Cationic Compound for Gene Delivery. *Tetrahedron Lett.*, Vol.42, No.6, pp. 1007–1010, ISSN 0040-4039

Ross, P.C. & Hui, S.W. (1999). Lipoplex Size Is a Major Determinant of in Vitro Lipofection Efficiency. *Gene. Ther.*, Vol.6, No.4, pp. 651-659, ISSN 0969-7128

Ruponen, M.; Ylä-Herttuala, S. & Urtti, A. (1999). Interactions of Polymeric and Liposomal Gene Delivery Systems with Extracellular Glycosaminoglycans: Physicochemical and Transfection Studies. *Biochim. Biophys. Acta, Biomembr.*, Vol.1415, No.2, pp. 331-341, ISSN 0005-2736
Safinya, C.R. (2001). Structures of Lipid–DNA Complexes: Supramolecular Assembly and Gene Delivery. *Curr. Opin. Struct. Biol.* Vol.11, No.4, pp. 440-448 ISSN 0959-440X

Sakaguchi, N.; Kojima, C.; Harada, A.; Koizumi, K.; Emi, N. & Kono, K. (2008). Generation of Highly Potent Nonviral Gene Vectors by Complexation of Lipoplexes and Transferrin-bearing Fusogenic Polymer-modified Liposomes in Aqueous Glucose Solution. *Biomaterials*, Vol.29, No.9, pp. 1262-1272, ISSN 0142-9612

Scarzello, M.J.; Smisterova, A.; Wagenaar, M.C.; Stuart, D.; Hoekstra, J.B. & Engberts, R. (2005). Sunfish Cationic Amphiphiles: Toward an Adaptative Lipoplex Morphology. *J. Am. Chem. Soc.* Vol.127, No.29, pp. 10420-10429, ISSN 0002-7863

Seol, J.G., Heo, D.S., Kim, H.K., Yoon, J.-H., Choi, B.I., Lee, H.-S., Kim, N.K. & Kim, C.Y. (2000). Selective Gene Expression in Hepatic Tumor with Trans-arterial Delivery of DNA/Liposome/Transferrin Complex. *In Vivo*, Vol.14, No.3, pp. 513–518, ISSN 0258-851X

Sochanik, A.; Kaida, I.; Mitrus, I.; Rajca, A. & Szala, S. (2000). A New Cholesterol Derivative Suitable for Transfecting Certain Type of Cells in the Presence of 10% Serum. *Cancer Gene Ther.*, Vol.7, No.4, pp. 513–520, ISSN 0929-1903

Sternberg, B.; Sorgib, F.L. & Huang, L. (1994). New Structures in Complex Formation between DNA and Cationic Liposomes Visualized by Freeze-fracture Electron Microscopy. *FEBS. Lett.*, Vol.356, No.2-3, pp. 361-366, ISSN 0014-5793

Sternberg, B.; Hong, K.; Zheng, W. & Papahadjopoulos, D. (1998). Ultrastructural Characterization of Cationic Liposome-DNA Complexes Showing Enhanced Stability in Serum and High Transfection Activity in Vivo. *Biochim. Biophys. Act.*, Vol.1375, No.1-2, pp. 23-35, ISSN 0005-2736

Tang, F. & Hughes, J.A. (1999a). Synthesis of a Single-tailed Cationic Lipid and Investigation of Its Transfection. *J. Controlled Release*, Vol.62, No.3, pp. 345-358, ISSN 0168-3659

Tang, F. & Hughes, J.A. (1999b). Use of Dithiodiglycolic Acid As a Tether for Cationic Lipids Decreases the Cytotoxicity and Increases Transgene Expression of Plasmid DNA in Vitro. *Bioconjugate Chem.*, Vol.10, No.5, pp. 791–796, ISSN 1043-1082

Turek, J.; Dubertret, C.; Jaslin, G.; Antonakis, K.; Scherman, D. & Pitard, B. (2000). Formulations Which Increase the Size of Lipoplexes Prevent Serum-associated Inhibition of Transfection. *J. Gene. Med.*, Vol.2, No.1, pp. 32-40, ISSN 1099-498X

Wasungu, L. & Hoekstra, D. (2006). Cationic Lipids, Lipoplexes and Intracellular Delivery of Genes. *J. Controlled Release*, Vol.116, No.2, pp. 255-264, ISSN 0168-3659

Williams, P.D.; Ranjzad, P.; Kakar, S.J. & Kingston, P.A. (2010). Development of Viral Vectors for Use in Cardiovascular Gene Therapy. *Viruses*, Vol.2, No.2, pp. 334-371, ISSN 1999-4915

Xu, Y.; Hui, S.W.; Frederik, P. & Szoka, F.C.J. (1999). Physicochemical Characterization and Purification of Cationic Lipoplexes. *Biophys. J.*, Vol.77, No.1, pp. 341-353, ISSN 0006-3495

Yingyongnarongkul, B.E.; Howarth, M.; Elliott, T. & Bradley, M. (2004). Solid Phase Synthesis of 89 Polyamine-based Cationic Lipids for DNA Delivery to Mammalian Cells. *Chem. Eur. J.*, Vol.10, No.2, pp. 463-473, ISSN 1521-3765

Yoshizawa, T.; Hattori, Y.; Hakoshima, M.; Koga, K. & Maitani, Y. (2008). Folate-linked Lipid-based Nanoparticles for Synthetic siRNA Delivery in KB Tumor xenografts. *Eur J Pharm Biopharm.*, Vol.70, No.3, pp. 718-725, ISSN 0939-6411
Zuhorn, I.S.; Bakowsky, U.; Polushkin, E.; Visser, W.H.; Stuart, M.C.; Engberts, J.B. & Hoekstra, D. (2005). Nonbilayer Phase of Lipoplex-membrane Mixture Determines Endosomal Escape of Genetic Cargo and Transfection Efficiency. *Mol. Ther.*, Vol.11, No.5, pp. 801-810, ISSN 1525-0016

Zhang, Z.; Yang, C.; Duan, Y.; Wang, Y.; Liu, J.; Wang, L. & Kong, D. (2010). Poly(ethylene glycol) Analogs Grafted with Low Molecular Weight Poly(ethylene imine) As Non-viral Gene Vectors. *Acta Biomater.*, Vol.6, No.7, pp. 2650-2657, ISSN 1742-7061

Zhang, S.B.; Zhao, B.D.; Jiang, H.M.; Wang, B. & Ma, B.C. (2007). Cationic Lipids and Polymers Mediated Vectors for Delivery of siRNA. *J. Controlled Release*, Vol.123, No.1, pp. 1-10, ISSN 0168-3659

Zhang, S.B.; Zhao, Y.N.; Zhao, B.D. & Wang, B. (2010). Hybrids of Nonviral Vectors for Gene Delivery. *Bioconjugate Chem.*, Vol.21, No.6, pp. 1003-1009, ISSN 1043-1082

Zhdanov, R.I.; Podobed, O.V. & Vlassov, V.V. (2002). Cationic Lipid-DNA Complexes-lipoplexes-for Gene Transfer and Therapy. *Bioelectrochemistry*. Vol.58, No.1, pp. 53-64, ISSN 1567-5394

Zhi, D.F.; Zhang, S.B.; Wang, B., Zhao, Y.N., Yang B.L. & Yu S.J. (2010). Transfection Efficiency of Cationic Lipids with Different Hydrophobic Domains in Gene Delivery. *Bioconjugate Chem.*, Vol.21, No.4, pp. 563-577, ISSN 1043-1082
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