MEDDOCS Open Access Publisher

Journal of Nanomedicine

Open Access | Review Article

Amino acid-based liposomal assemblies: Intracellular plasmid DNA delivery nanoparticles

Satya Ranjan Sarker[1,2]; Shinji Takeoka[1*]

[1]Department of Life Science and Medical Bioscience, Waseda University, Japan

[2]Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Bangladesh

***Corresponding Author (s): Shinji Takeoka,**

Department of Life Science and Medical Bioscience, Waseda University, Japan

Tel: +81-3-5369-7324, Fax: +81-3-5369-7324

Email: takeoka@waseda.jp

Received: Mar 01, 2018

Accepted: Apr 26, 2018

Published Online: May 07, 2018

Journal: Journal of Nanomedicine

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Abstract

Due to the limitations of viral vectors with regard to the cytotoxicity and immunogenicity, nonviral vectors, especially cationic liposomes, have been thoroughly investigated for gene therapy both *in vitro* and *in vivo*. In this review, the influence of structure-activity relationship of various amino acid-based cationic lipids with respect to *in vitro* gene delivery efficiencies has been discussed. Cationic lipids have been extensively investigated to explore the parameters responsible for influencing the gene delivery efficiencies so that lipid structures for efficient gene delivery can be designed for both *in vitro* and *in vivo* applications. The morphology and the physicochemical properties of the cationic lipid assemblies are influenced by different parts of the cationic lipids such as the ionization states and the structure of the hydrophilic head group, nature and length of the spacers between the hydrophilic head group and the hydrophobic moiety, and type and length of the hydrophobic alkyl chains.

Introduction

Intracellular delivery of genetic materials (*i.e.,* plasmid DNA, siRNA or oligonucleotides) and anticancer drugs (e.g., doxorubicin) has become one of the most pursued areas to treat various complex diseases that have been considered incurable until now. Gene therapy could be performed by supplementing the aberrant gene with a functional one [1] or by the delivery of a suicide gene [2] or via transfer of genes for the synthesis of new therapeutic proteins [3]. For example, small interfering RNAs (siRNAs) have the potential to use as therapeutic agents for the treatment of various diseases including cancer, genetic disorders and viral infections [4,5]. They are usually 19 to 21 nucleotides in length, negatively charged, and therefore, they can't pass through the cytoplasmic membrane through simple diffusion [6]. Furthermore, siRNAs are susceptible to RNase degradation in the systemic circulation as well as in the interstitial spaces [7]. PEGylation of siRNA/cationic liposome complexes increase the stability of the complexes in the systemic circulation through the reduced adsorption of opsonins and increase the amount of delivered siRNA in the target tissue [8]. Although cationic liposomes form aggregates with nonspecific proteins present in the serum, many research groups have used various cationic lipids such as DOTAP, DOTMA, DDAB, and molecularly targeted cationic lipids (MTCL) to prepare siRNA/cationic liposome complexes through electrostatic interactions and have successfully delivered siRNA *in vitro* as well as *in vivo* [8,9]. A commercially available cationic lipid formulation (i.e., Lipofectamine) is usually used for the delivery of siRNA [10,11].

Over the last few decades, many research groups have exploited different strategies to deliver both the genetic materials and anticancer drugs *in vitro* as well as *in vivo*. Intracellular delivery vehicles can be broadly classified into two groups such as viral and nonviral vectors. Among the viral vectors, Adenovirus, Adeno-associated Virus (AAV) and retrovirus are the most common viruses used to deliver genetic materials to different type of cancer cells. Viral vectors have several limitations like exces-

Cite this article: Sarker SR, Takeoka S. Amino acid-based liposomal assemblies: Intracellular plasmid DNA delivery nanoparticles. J Nanomed. 2018; 2: 1008.

sive immune response, insertional mutagenesis [12], limited loading capacity [13], broad tropism [14], germ cell altercations [15], inherent difficulties in pharmaceutical processing and scale-up, and the possibility of the reversion of an engineered virus to the wild type [16]. Viral vectors, therefore, are not safe in clinical application, despite their easy entry into susceptible cells due to the presence of a protein envelope on their surface that helps them to specifically bind to the surface polysaccharides (e.g., heparin sulfate) and receptor proteins of the target cells. In contrast, nonviral vectors are less toxic, cost effective and can be used for targeted delivery to specific cells [14]. Furthermore, nonviral vectors have several other advantages such as lack of specific immune responses, ability to deliver large size DNA, ease of handling and preparation techniques, and well defined physical and chemical compositions [17-20], and high purities. Important nonviral vectors such as liposomes [21-25], PEGylated cationic cerosomes [26], micelles [27,28], dendrimers [29,30], cationic polymers [31-33], polypeptides [34], gold nanoparticles [35], iron oxide nanoparticles [36], modified silica nanoparticles [12,37,38] as well as physical methods such as electroporation have been exploited to deliver genetic materials to various cells *in vitro*. However, the Achilles' heel of these nonviral vectors is their relatively low transfection efficiency and short duration of gene expression [17,39]. Therefore, researchers have been focusing on the design and synthesis of new nanoparticles with high transfection efficiency that can be comparable with the viral vectors. Among all the nonviral vectors, cationic liposomes prepared upon hydration of cationic lipids that are not biological lipid analogs, have the potential to be used as an intracellular delivery vehicle with biodegradability and associated low cytotoxicity.

In this review, the authors explained the chemical composition of different cationic lipids, physicochemical properties of the cationic liposomes that influence the efficiency of *in vitro* gene delivery, and cytotoxicity associated with the cationic lipids mediated gene delivery.

Cationic lipids

In 1987, Felgner *et al*. first introduced cationic lipids as the gene delivery vehicle [21]. Lipids are amphiphilic in nature and contain a non-polar (*i.e.,* hydrophobic) part and a polar (*i.e.,* hydrophilic) head group. Furthermore, the cationic lipids used as intracellular delivery vehicle also contain a linker to tether the hydrophobic and hydrophilic moieties [40-42] and a spacer between the hydrophilic and hydrophobic moieties [43,44] (Figure 1).

The cationic head group interacts with the negatively charged biomacromolecules such as DNA, RNA, proteins and ultimately with the negatively charged cell membranes. The hydrophobic tails influence the assembling state and stability as well as the release profile of the entrapped molecules from the liposome. The spacer modulates the hydrophilic/hydrophobic balance of the assemblies. The linker acts as a scaffold part in cationic lipid and tether the hydrophilic head group and the multiple hydrophobic tails. In aqueous systems, lipid molecules assemble to form a closed bilayer structure (Figure 1) to protect their nonpolar groups from the aqueous environment while keeping contact with the aqueous phase through the polar head group [45].

The overall molecular structure of cationic lipids is important to determine the assembling morphology upon hydration and controls their complexation ability with genetic materials and transfection efficiency. Until now, many cationic lipids (Figure 2) have been studied as cationic liposomes for gene delivery vehicle such as DOTMA [21], DOTAP [46], DOSPA or DOGS [47], DC-Chol [48], SAINT-2 [49], DORIE [50], DMRIE [51], and α-tocopherol [52].

Many cationic lipids don't attain stable assembling states upon hydration. Therefore, several helper lipids such as DOPE, DOPC, DPPC, and cholesterol have been widely mixed with cationic lipids in order to make stable assemblies. This review mainly focuses on the structure-activity relationship of cationic lipids and does not comment on any roles of helper lipids in the gene delivery efficiency.

Hydrophobic moiety

Hydrophobic (nonpolar) parts of amphiphiles play a crucial role for the formation of bilayer vesicles (liposomes) that eventually interact with the host cell membranes and have been investigated quite extensively. The structural variations of the hydrophobic domain such as length, specificity of chemical bonds, the orientation of the hydrocarbon chains [52], as well as the asymmetry and type of the hydrophobic domain can influence the transfection efficiency. Different alkyl chain lengths (*i.e.,* carbon number 12 to 18), and steroid derivatives have been used as the hydrophobic part of the liposome-forming cationic lipids.

Cationic lipids with two alkyl chains in the hydrophobic moiety have been the most efficient structure for plasmid DNA delivery. However, cationic lipids with one, three or four (in case of cationic gemini lipids) alkyl chains have also been reported [53]. Pinnaduwage *et al*. [54] found that the transfection efficiency of a cationic lipid CTAB (cetyltrimethylammoniumbromide) with single alkyl chain was lesser than that of DOTMA, a double tailed cationic lipid. It was also reported that the transfection efficiency of the lipid with one alkyl chain was inferior to that of the di- or tri alkyl chains counterparts [55]. Furthermore, the cationic lipids with three alkyl chains usually show the lower transfection efficiency than lipids having two alkyl chains [55]. The reasons for the lower transfection efficiency of the lipids with one and three alkyl chains are their tendency to form micelles and different molecular assembly, respectively, in aqueous medium and therefore known as surfactants and have higher toxicity.

The saturation level and the length of the alkyl chain also play an important role in determining the physicochemical properties of liposomes and their transfection efficiency. Obika *et al*. reported that the transfection activity was increased, when an unsaturated bond was introduced in a long alkyl chain [56]. The presence of the unsaturated bond in the hydrophobic chain of lipid provides the larger aliphatic cross-sectional area due to cis-bonding, the increased fluidity of bilayer membrane and thereby stimulates the fusogenic ability of cationic liposomes with the cell membranes [22] that in turn increases the transfection efficiency. On the contrary, alkyl chain saturation results in the increased stiffness of phospholipid bilayers [57,58]. Fletcher *et al*. [59] demonstrated that the replacement of a double bond with a triple bond in the alkyl chains of DOTAP influenced both the structural and functional properties (*i.e.,* transfection efficiency) of liposomes and lipoplexes. They also reported that shifting of the triple bond location towards the end of the alkyl chains increased the stability of the lipoplexes at physiological temperatures. It was anticipated that alkyl chains with triple bonds exhibit reduced kinks compared to cis-double bonds and

provide more rigid liposomal bilayers. Hoekstra *et al*. [60,61] revealed that the unsaturated alkyl chains enhanced the transfection properties of the pyridinium-based amphiphiles due to the more dynamic packing features in terms of chain orientation in a bilayer. It is also possible that the alkyl chains having double bonds facilitated the permeation of the cellular membranes, an usual property of amphiphiles that are able to perform intracellular gene delivery. The transfection efficiency is also influenced by the length of the alkyl chains. Obata *et al*. [62] demonstrated that the transfection efficiency of amino acid-based cationic assemblies was influenced by the length of alkyl chains. The degree of plasmid DNA delivery efficiency with regard to the length of alkyl chains was dimyristyl (di-C14:0) > di-palmityl (di-C16:0) > disteryl (di-C18:0). Felgner *et al*. also reported the synthesis and evaluation of a novel homologous series of hydroxyethyl quaternary ammonium derivatives with different alkyl chain substitutions. The order of transfection efficiency was dimyristyl (di-C14:0) > dioleoyl (di-C18:1) > di-palmityl (di-C16:0) > disteryl (di-C18:0). Similarly, *Folch et al*. [63] found that the transfection efficiency of a series of phospholipids depend on the length of hydrophobic alkyl chains and followed the order: C14 (myristyl) > C16 (palmityl) > C18 (stearyl). Furthermore, cationic lipids with C12 alkyl chains are also highly efficient in *in vitro* plasmid DNA delivery. However, these liposomes are highly unstable and more amount of lipid is required to form lipoplexes. Hence, cationic lipid polymorphism and liposomal membrane integrity affect the DNA binding affinity of cationic liposomes [64]. The superior transfection efficiency of lipids with shorter alkyl chains is due to the reduced rigidity and increased fluidity of the membrane bilayer that resulted in the lower phase transition temperature when compared to their longer alkyl chains counterparts. Thus, they bring about a greater transfer of lipids between the lipid membranes and their mixing. This results in the disruption of endosomes followed by the release of DNA into the cytosol [65].

The *in vitro* activities of asymmetric lipids are usually superior to their symmetric analogues. The intermembrane mixing is enhanced by the asymmetry of the hydrophobic moiety of a lipid and the rate of the plasmid DNA association is low with the symmetry which results in the greater DNA delivery efficiencies of asymmetric lipids.

Among the steroid compounds used as the hydrophobic moiety for cationic lipids include cholesterol [48], vitamin D [66], bile acids [67], antibiotic [68], cholestane and litocholic acid [69]. In 1991, Huang *et al*. [48] pioneered the synthesis and evaluation of the cholesterol-based cationic lipid, 3-β-[N-(N',N'-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol) in A431 human epidermoid carcinoma cells, YPT minipig primary endothelial cells, L929 mouse fibroblast cells, and A549 human lung carcinoma cells.The low cytotoxicity of DC-cholesterol made it the first cationic lipid subjected to clinical trials [45]. Kedika *et al*. [70] reported the synthesis and evaluation of a series of serum compatible novel tocopherol based monocationic lipids. They demonstrated that these tocopherol based lipids had 4-fold higher transfection efficiency than that of the commercial formulation 'Lipofect' and can be targeted to liver.

Linker

The linker acts as a scaffold in the cationic lipids and tethers the hydrophobic moieties and the hydrophilic head group. The most frequently used linkers are ether or ester bonds, carbamates, amides, glycerol, and amino acids (e.g., aspartate and glutamate). Many lipids have been nomenclated according to the linker present in a particular lipid. For example, lipids having glycerol as the linker are known as glycerol-based lipids and the lipids having amino acids (e.g., aspartate and glutamate) as the linker are known as amino acid-based lipids. Type, length and orientation are the important properties of the linker that influence both the physicochemical properties and the gene delivery efficiency of cationic liposomes. Felgner *et al*. [22] revealed that the ester linkage of DOTAP was easily metabolizable and that might be responsible for the lower cytotoxicity when compared to its lipid counterpart DOTMA having ether bond. Ghosh *et al*. [40,41] reported that the nature of linkage between the cholesteryl backbone and the cationic head group controlled the gene transfection efficiency. They demonstrated that the presence of an ether linkage showed the superior gene transfection efficiency than that of the ester and urethane linkages. Because the presence of ether linkage makes these compounds hydrolytically stable and their aqueous suspensions are known to have long shelf lives. However, many reports including ours [43,44,62,64,71,72] have been published demonstrating the excellent transfection efficiency and very low cytotoxicity using cationic lipids comprised of alkyl chains that are tethered to different amino acids (e.g., lysine and arginine) as the cationic head group through ester linkage. The advantages of cationic lipids bearing ester linkage are their sensitivity to endosomal acidic pH and hydrolysis by the cellular esterase. Kim *et al*. [71] reported the synthesis of two different cationic lipids comprised of a core of lysine, two C-14 hydrocarbon chains and either aspartate or glutamate as the linker that held the two alkyl chains through ester bond formation. They demonstrated that the cationic lipid with glutamate as the linker showed better gene delivery efficiency both *in vitro* and *in vivo* when compared to their aspartate linker containing counterpart. The presence of one more carbon in the core backbone structure of the glutamate containing lipid may influence the structure/conformation of the lipoplexes and brings about the increased exposure of the lipoplexes' positively charged groups. Hence, the interaction between the resulting positively charged and stable lipoplexes and the negatively charged cell surface would be more effective and will bring about a better intracellular uptake of plasmid DNA.

In the same line, Obika *et al*. [56] explained that the quaternary ammonium group of the cationic triglycerides having a short linker was embedded in the bilayer of liposomes. Consequently, the interaction between the positively charged head group of the liposomes and the negatively charged plasmid DNA and/or the cell membrane would be interrupted. Therefore, cationic lipids with a longer linker are recommended for better efficiency of plasmid delivery. Apart from the type and length, the orientation of the linker influences the physicochemical properties as well as the gene delivery efficiency of cationic lipids. Rajesh *et al*. [73] reported that a minor structural variation such as any changes in the orientation of the linker profoundly influenced DNA binding ability, membrane rigidity, membrane fusibility, cellular uptake, and gene delivery efficiency of cationic liposomes.

Spacer

Bhattacharya *et al*. [74-77] are the pioneer in introducing spacers of different nature and length to the cationic gemini lipids that typically possess two cationic lipid monomers comprised of two polar head groups and four long aliphatic chains, linked by either a rigid or flexible spacer between the cationic head groups (Figure 3).

Furthermore, each cationic gemini lipid monomer can also have cholesterol or thiocholesterol moieties as the hydrophobic tails attached to the hydrophilic cationic head group either via ether or disulfide linkage [78,79]. The nature of the spacer could be hydrophobic flexible (e.g., -$(CH_2)_5$-), hydrophobic rigid (e.g., -C_6H_4-) or hydrophilic flexible (e.g., -CH_2CH_2-O-CH_2CH_2-) (79). The nature and length of the spacer and their hydrophobicity influence the assembling structure and transfection properties of gemini amphiphiles [79]. They also demonstrated that (i) membrane properties of the gemini amphiphiles can be modulated by the spacer chain length of the ion-paired amphiphiles [76], (ii) the size and morphology of vesicles, and the degree of hydration of the gemini amphiphiles depend strongly on the length of the spacer [80], (iii) the length and conformation of the spacer influence the thermal properties such as phase transition temperature (T_c), enthalpy (ΔH), and entropy (ΔS) of the cationic gemini amphiphiles [76,80], and (iv) the transfection efficiencies of the cationic gemini amphiphiles are serum compatible [78,81]. Luciani *et al*. [82] reported that gemini amphiphiles with long spacers get more hydrated which influence the organization of the corresponding liposomes. They also found that a small variation of the lipid hydration level influences plasmid DNA conformation in the lipoplexes. Obata *et al.* [44] for the first time demonstrated that the spacers of amino acid-based cationic lipid influence the physicochemical properties of cationic liposomes and evaluated the gene delivery efficiency of the pDNA-encapsulating liposomes in the COS-1 cell line. They synthesized a series of amino acid-based cationic lipids with lysine as the head group and 1,5-dihexadecyl-L-glutamate (*i.e.,* Glu_2C_{16}) as the hydrophobic moieties. A hydrocarbon chain composed of 0, 3, 5, 7, or 11 carbons was used as a hydrophobic spacer or an oxyethylene chain (10 carbons and 3 oxygen molecules) was investigated as a hydrophilic spacer. They concluded that the cationic lipids with hydrophobic spacers showed the higher fusogenic potential and, in turn, the higher transfection efficiency in general when compared to their hydrophilic spacer counterparts and the cationic lipid with a 7-hydrocarbon chain spacer was the best in particular. The superior transfection efficiency of the liposomes with hydrophobic spacers is due to the exposure of the hydrophobic spacer unit to the hydrophilic moiety of the lipids and thereby enhances the fusogenic potential to a biomembrane through hydrophobic interaction [83]. Therefore, the cationic lipids with a large hydrophobic spacer showed a higher fusogenic potential. On the contrary, the hydrophilic nature of the oxyethylene spacer brought about a lower fusogenic potential due to hydrophilic repulsion with the endosomal membrane [44]. They have also reported the synthesis and characterization of a series of amino acid-based cationic lipids with lysine as the cationic head group, different hydrocarbon chain spacers (*i.e.,* 0, 3, 5, or 7), and 1,5-ditetradecyl-L-glutamate (*i.e.,* Glu_2C_{14}) as the hydrophobic moieties. They also evaluated the transfection efficiencies of the cationic assemblies in the COS-7 cell line. The length of the hydrocarbon chain spacer influences the size and the morphology of the lipid assemblies. The cationic lipid with no hydrocarbon chain spacer formed highly unstable cationic assemblies and the size of the cationic assemblies increased as the spacer length increased from 3 to 5. The cationic lipid with a 3-hydrocarbon chain spacer showed the best transfection efficiency to COS-7 cells. Furthermore, the cationic lipid with a 7-hydrocarbon chain spacer form either tube-like or rod-like structures rather than the usual vesicular structure [43]. Indeed, other reports also explained that the cationic lipid assemblies prepared from amino acid based cationic lipids formed vesicles, fibers, or ribbons, depending on small structural variations [62,84,85]. There is no significant influence of the spacer length on the phase transition temperature of the lipid assemblies [43]. However, some previous reports demonstrated that the spacer of cationic gemini amphiphiles significantly influence the phase transition temperature of their assemblies [76,80]. It is possible because of the orientation of the spacer in the lipid molecule. If a spacer is added between the hydrophilic head group and the hydrophobic moiety of a lipid, the orientation of the spacer would be vertical to the membrane. Therefore, the spacer's orientation significantly influences the location of the hydrophilic head group. However, if a spacer acts as a cross linker between the cationic head groups of the lipid monomers, it will influence the lateral molecular packing of the membrane structure rather than the location of the hydrophilic head group. Consequently, the spacer's role in molecular packing of the cationic assemblies would be different because of the structural differences of the lipids.

Hydrophilic moiety

Hydrophilic (*i.e.,*polar) moiety of the lipid amphiphiles determines the surface charge of the resultant liposomes. For example, lipids with phosphatidyl ethanolamine (*i.e.,* DOPE) and phosphatidyl choline (*i.e.,* DOPC and DPPC) have a zwitter ionic head group are neutral lipids and the lipids with cationic head groups (e.g., amino acids, and ammonium groups) are known as cationic lipids. In case of amino acid based cationic lipids, the hydrophilic amino acids (e.g., lysine, arginine etc.) as well as their counterions (i.e., HCl- or TFA-counterion) influence the zeta potential of the cationic lipids. It has been reported that cationic lipids with TFA-counterion in the cationic head group (i.e., either lysine or arginine) shows higher zeta potential when compared to the presence of HCl-counterion [43,86]. Furthermore, the hydrophilic moiety (*i.e.,* head group) of lipid is influenced by the aqueous environment and the positively charged head group interacts directly with the negatively charged plasmid DNA through electrostatic interaction. Lipids possessing a quaternary ammonium group [*i.e.,* -$N^+(CH_3)_3$] such as DOTAP and DOTMA showed the relatively lower transfection efficiency due to the steric hindrance at the nitrogen atom [45]. Steric hindrance deters plasmid DNA from the usual electrostatic interaction with the positively charged quaternary ammonium group to form the lipoplexes and thereby result in the lower transfection efficiency.

The trimethyl ammonium group of DOTAP and DOTMA has been modified by substituting one of its methyl group with a ß-hydroxyethyl group to synthesize 1,2-dioleoyloxypropyl-3-*N*,*N'*-dimethyl-*N'*-hydroxyethylammonium bromide (DORIE) and 1,2-dimyristoyloxypropyl-3-*N*,*N'*-dimethyl-*N'*-hydroxyethylammonium bromide (DMRIE), respectively [22]. Behr *et al*. [47] synthesized a series of lipospermines such as DOGS and DPPES having multiple positive charges in the head group and evaluated their gene delivery efficiency. They successfully delivered plasmid DNA to various cell lines with transient and stable expression and low cytotoxicity.

The hydroxyl group enhances the electrostatic interaction of plasmid DNA with lipids and consequently improves the affinity of the resulting lipoplexes with the cellular membranes. This leads to their superior transfection efficiency over DOTAP and DOTMA both *in vitro* and *in vivo* [22]. Banerjee *et al*. [87] synthesized a series of non-glycerol-based cationic lipids with one or two hydroxyethyl groups directly linked to the quaternary ammonium head group. They reported that the lipid with two hydroxyethyl groups had better transfection efficiency than that

of the lipid with one hydroxyethyl group. The enhanced transfection efficiencies are due to the improved interactions of the functionalized cationic lipids or the lipoplexes with the cell surface of biological membranes via hydrogen bonding [22,87,88].

Majeti *et al*. [89] delineated the superior lung transfection efficiency of the cyclic head group containing cationic lipid when compared to their open head analogs. The superiority of the cyclic head lipids may be related to the faster rate of subsequent serum-induced disintegration. Furthermore, the covalent grafting of the Tris-base component of Tris-buffer in the head group region of cationic lipid was shown to impart excellent serum compatibility in the presence of up to 90 % serum and high transfection efficiency [90]. The high transfection efficiency might be due to the favorable interactions between the three hydroxyl groups of the Tris-lipid and cell surface components. Bajaj *et al*. [91] reported that the gene transfer efficiencies depend on the nature of the head group and cholesterol based cationic lipid bearing 4-*N*,*N´*- Dimethylaminopyridine (DMAP) showed the highest transfection efficiency in the presence of serum.

A novel series of nonglycerol based cationic lipids with increasing hydrophobic tails and different amino acids such as serine, alanine and ß-alanine as the head group functionalities have been synthesized and evaluated in terms of gene delivery efficiency to different cell lines *in vitro* [92]. It was found that the plasmid delivery efficiency of cationic lipids can be modulated significantly through little structural variations in both the polar head group and nonpolar tail moieties of the lipid. The synthesis and relative *in vitro* transfection efficiencies of a series of mono-, di-, and tri-lysinated cationic lipids with different alkyl chains have also been reported [93]. Liposome formulations prepared from the lipid bearing myristyl tail and single lysine as the head group and DOPE as colipid showed higher gene delivery efficiency when compared to other lipid formulations such as Lipofectamine. The presence of multiple positive charges in the head group functionalities didn't influence the compactness and hydrodynamic size of the lipoplexes, the transfection efficiency and the cytotoxicity of the cationic amphiphiles.

The gene delivery efficiency of the amino acid-based cationic liposomes with arginine as the cationic head group have better transfection efficiency than that of their lysine head group counterparts to neuronal cells such as SH-SY5Y and PC-12 cells as well as HeLa cells [86]. Arginine head group containing cationic liposomes have also higher plasmid DNA delivery efficiency to primary cultured neurons when compared to that of the lysine head group containing cationic liposomes as well as Lipofectamine™2000 [94]. The superior transfection efficiency of arginine-based liposomes is due to the guanidino group of arginine that provides strong capability to condense negatively charged DNA. The pK_a value of the guanidine group and the side chain of lysine is 12.48 and 10.53, respectively. Therefore, cationic liposomes with arginine as the cationic head group possess stronger positive charges when compared to their lysine head group counterpart at physiological pH 7.4. Moreover, the guanidinium group remains protonated over a wide range of pH, forms hydrogen bonds with purine and pyrimidine nucleobases, and also has a crucial role in various DNA binding proteins like histones and protamines. It is also capable of forming zwitterionic hydrogen bonds with phosphate ions [28,95]. Furthermore,the formation of hydrogen bonds between the guanidino moiety of arginine and the phosphates, sulphates, carboxylate groups of the cell membrane components might be influenced synergistically [96]. The transfection biology of novel non-cholesterol based cationic lipids with a single guanidinium head group and C14 to C18 aliphatic hydrocarbon as the tail have also been reported [96]. Vigneron*et al* [28] reported the synthesis of Bis-Guanidinium-Tren-Cholesterol (BGTC), a cholesterol derivative bearing two guanidinium groups. They demonstrated that BGTC can be used as an efficient transfection reagent into a variety of mammalian cell lines when used as a micellar solution as well as liposomal formulation in combination with the helper lipid DOPE.

The conjugation of an endosome-disrupting histidine moiety in the hydrophilic region of cationic amphiphiles increases their plasmid DNA delivery efficiency [97]. Later on it has been reported that the correlation between the *in vitro* gene delivery efficiency and the presence of the number of histidine moieties in the hydrophilic regions of histidinylated cationic amphiphiles is not linear [98]. The medium used in the formation of lipoplexes between the histidinylated cationic amphiphiles and the plasmid DNA significantly influences the transfection efficiencies as well as the serum compatibilities. However, amino acid-based cationic lipids bearing histidine as the head group functionalities formed tube-like structures albeit lysine and arginine as the head groups formed unilamellar vesicles [62]. The order of transfection efficiency was as follows: lysine > arginine > histidine.Heyes *et al*. [99] also reported the synthesis of cationic lipids with lysine, arginine, histidine and tryptophan as the head group. The transfection efficiency was the highest for lysine/arginine derivatives. Recently, cholesterol based cationic lipids bearing amino acids (e.g., lysine or histidine) as the cationic head groups have been used as the efficient gene delivery vehicles [100]. Furthermore, amino acid based cationic lipids with α-tocopherol as the hydrophobic tail and glycine, histidine-glycine, or lysine-glycine as the cationic head group were used as gene delivery vehicles [52]. It has also been reported that amino acid based cationic lipids with dipeptide (*i.e.,* lysine-glycine) as the hydrophilic head group perform better as a gene delivery agent when compared to that of only amino acids such as glycine [52].

Aberle *et al*. [101] reported the influence of counterions on the gene delivery efficiency of quaternary trimethylammonium cytofectin, DOTAP and the lipids with iodine as the counterion showed the best transfection efficiency among the halogens. Sarker *et al.* [43] reported the design, synthesis and characterization of a series of lysine-based cationic lipids with 1,5-ditetradecyl-L-glutamate as the hydrophobic moiety, 3-hydrocarbon chain as the spacer, and lysine as the hydrophilic moiety with different ionization states such as NH_2 (a), $-NH_3^+Cl^-$(b),or $-NH_3^+TFA^-$(c). They demonstrated that the ionization states of the lysine head group influenced the size and morphology, zeta potential, phase transition temperature as well as the transfection efficiency of the amino acid-based cationic assemblies [43]. However, cationic lipid **b** formed a micellar structure and didn't show any distinct phase transition temperature. This is presumably due to the presence of highly hydrophilic $-NH_3^+Cl^-$ in the lysine head group that results in the bigger head group volume and formation of a micellar structure. By contrast, **a** and **c** formed multilamellar vesicular structures and **c** had the highest zeta potential. Furthermore, the ionization state of cationic head group influenced the phase transition temperature (T_c), ΔH, and ΔS of the cationic assemblies. Thus, difference in the ionization states in the hydrophilic head group of amino acid-based cationic assemblies influences their molecular assembly as well as their packing states.

We have summarized the hydrophobic moieties and the amino acids used as the hydrophilic moiety to prepare amino acid based cationic lipids in Table 1.

Intracellular delivery of plasmid DNA

The intracellular delivery of plasmid DNA can be performed through either simple complex formation between plasmid DNA and cationic liposomes or encapsulation of plasmid DNA within the hydrophilic core of the liposomes comprised of mixed lipid formulations [43,44,108,109]. Caracciolo *et al.* [110] reported the distinct advantages of the encapsulation strategy such as: (i) encapsulation requires a lower amount of cationic lipid to condense plasmid DNA and the lesser amount of cationic lipid contributes to lower cytotoxicity; (ii) particles for encapsulation have the higher fusogenic potential when compared to the lipoplexes. This phenomenon could be due to the greater interaction between the cationic liposomal lipids and the anionic lipids of cell membranes because of the absence of DNA in the liposomal membrane and to the less number of lipid membranes to be removed. Hence, the encapsulation of plasmid DNA in the lipid envelope has a distinct advantage for releasing DNA in the cytosol; (iii) the encapsulation of plasmid DNA with lipid membrane or insertion in the lipid bilayer membranes help to block the usual interaction between the negatively charged plasmid DNA and the positively charged serum proteins during the *in vivo* applications. Furthermore, encapsulation strategy helps to protect plasmid DNA from the attack of endogenous nucleases [44,110]. Recently, Kobuta *et al.* have reported that encapsulated siRNA within lipid nanoparticles (LNPs) also showed higher stability and cell uptake efficiency as well as low cytotoxicity including reduced release of cytokines (i.e., TNF-α and IL-1β) [111]. However, the encapsulation of plasmid DNA in the aqueous core of liposome is a very tedious process and the encapsulation efficiency is also very low. On the other hand, the lipoplexes are formed by very simple strategy such as electrostatic interaction between the positively charged cationic liposomes and the negatively charged plasmid DNA added to the liposome dispersion. In the absence of serum, the positively charged lipoplexes bind electrostatically with the negatively charged sulphated groups of mammalian cell surface proteoglycans [110]. Then the lipoplexes can be taken up inside the cell either via an endocytosis process or a membrane fusion process (Figure 5).

Lipoplexes can also interact with a specific cell surface receptor provided the surface of liposomes contains any ligands and go through the receptor-mediated endocytosis. Lipoplexes usually follow either clathrin-mediated or caveolin-mediated endocytic pathways. The postulated events of the dissociation of plasmid DNA from lipoplexes can be described as follows: (i) the dissociation of DNA from the lipoplexes through the neutralization of the positively charged cationic lipids with the anionic lipids of cell membranes; (ii) phase change/evolution of the lipoplex lipids (conversion from lamellar to hexagonal phases) upon interaction with the cellular lipids is decisive for transfection process; (iii) fusion of lipoplexes with endosomal membranes facilitates DNA release from endosomes into cytoplasm, and promotes DNA expression. It was reported that the superior transfection efficiency of liposomes strictly correlated with their ability to escape from endosomes and the endosomal rupture resulted in the extraordinary homogeneous distribution of unbound plasmid DNA throughout the cytoplasm and in the nucleus [112]. Xu *et al.* [65] also proposed that the cationic lipoplexes are taken up by the cells via endocytosis and bring about the destabilization of the endosomal membrane which stimulates flip-flop of the anionic lipids (*i.e.,* phosphatidyl serine (PS)) of the cytoplasm facing monolayer. Then the unstable endosomal membrane diffuses laterally with the lipoplexes and forms a neutral ion pair between PS and the cationic lipids. This results in the dissociation of plasmid DNA from the lipoplexes and the release of DNA into the cell cytoplasm. This mechanism is supported by the superior transfection efficiency of the cationic liposomes containing DOPE as the helper lipid. The increased transfection efficiency is attributed to DOPE's potential to promote the formation of an inverted hexagonal phase (H_{II}) than lamellar phase (Lα) [113,114]. It was also reported that plasmid DNA delivered to neuroblastoma cells (*i.e.,* SH-SY5Y cells) by the amino acid-based cationic liposomes having arginine as the head group was released from the early endosomes [115]. Furthermore, the cationic liposomes had the higher ability to destabilize the endosomal membrane when compared to cationic polymers (e.g., PEI) to release plasmid DNA prior to fuse with the endosome/lysosome. Thus, the plasmid DNA escapes the lysosomal degradation and bring about the increased transfection efficiency [116]. Finally, the released plasmid DNA got transported to the cell nucleus through nuclear pore complex (NPC) followed by transcription and gene expression.

In the case of simple membrane fusion, lipoplexes fuse to the random sites in the plasma membrane and release plasmid DNA to cytosol, that eventually reach the nucleus through previously mentioned mechanism. However, in the presence of serum, serum proteins interact with the positively charged lipoplexes. To this end, two phenomena are possible: first, serum proteins attach on the positively charged lipoplexes and result in the aggregation of the lipoplex particles; second, serum proteins bind to the lipoplexes and bring about the negative zeta potential and stabilize particle size due to the electrostatic repulsion among the lipoplex particles. In the latter case, the lipoplex particles are highly stable. However, the cellular uptake mechanism of these negatively charged lipoplexes in the presence of serum is still unknown. We assume that the exposed positive charges of the negatively charged lipoplexes would interact with the negatively charged cell membrane, albeit the net zeta potential of the lipoplexes is negative. The zeta potential of the lipoplexes has been reported as a critical factor for their stability, interaction with the cell surface, prevention of interaction with the cell surface as well as the protection of plasmid DNA from nuclease degradation *in vivo* [117]. Lipoplexes with positive charge show elevated gene delivery efficiency because of the electrostatic interaction with the sulphated membrane-associated proteoglycans [118]. Sulphated proteoglycans are highly negatively charged components of cell membrane and composed of a group of proteins covalently linked to one or more polysulfated glycosaminoglycan (GAGs) polysaccharides [119]. By contrast, lipoplexes with negative potential show lower transfection efficiency due to weak interaction with the cell surface GAGs, since very few positive charges are exposed on the lipoplexes even though the net zeta potential is negative [86]. It has also been reported that cellular uptake rate and amount are positively correlated with the surface charge of the nanoparticles [120].

The intracellular delivery of plasmid DNA is presumably influenced by the size and zeta potential of lipoplexes [117,121,122]. In fact, lipoplexes enter into cells by endocytic pathways such as clathrin- and caveolae-mediated endocytosis and they are dependent on the particle size [123,124]. The lipoplexes of 250 nm in size were taken up via clathrin-mediated endocytosis, whereas the lipoplexes of 500 nm were taken up via caveolae-

mediated endocytosis [116]. Albanese *et al*. [125] reported that the cell and mediator types may play a significant role in the cellular uptake efficiency. For example, the cellular uptake efficiency of aggregated nanoparticles for HeLa and A549 cells was reduced by 25% when compared to the single and monodisperse nanoparticles. However, the uptake of large aggregates was 2-fold more for MDA-MB 435 cells.

The gene delivery efficiency depends on the morphology of the cationic assemblies such as vesicular, micellar, rod/tube-like structures [43,62]. It was also reported that rod/tube shaped liposomes did not show any transfection efficiency. When lipoplexes formed from the cationic liposomes are exposed to acidic conditions, they disassemble significantly and release DNA that result in the higher transfection efficiency. On the contrary, the morphology of micellar aggregates is unresponsive to pH change that result in the lower conversion of lamellar to hexagonal phase and the inefficient release of the plasmid DNA from the early endosomes, thereby low transfection efficiency [126].

If the lipoplexes go through the lysosomal pathway, plasmid DNA may get degraded while reaching the lysosomes by nucleases. Hence, for efficient transfection, plasmid DNA needs to be released to the cytosol from the early endosomes [127]. The pH of early endosomes is slightly acidic (*i.e.,* pH 6.0-6.5) that results in the release of plasmid DNA from the endosomes. The endosomal release of plasmid DNA may be due to: i) the fusion of lipoplexes with the endosomal membrane, ii) flip-flop movements of the negatively charged phospholipid from the cytoplasmic-facing monolayer of the endosomal membrane to the inner monolayer induce the formation of a neutral charge ion pair with a positively charged group of the cationic lipid, iii) osmotic rupture of the endosomes [128]. The ability of transfection reagents to switch over to the inverted hexagonal phase from the lamellar phase in the acidic endosomal environment facilitated the endosomal release and escape of DNA. Lipids in the vesicular structure have preference for hexagonal phase than micellar structure.

Cytotoxicity

The cytotoxicity of cationic liposomes is significantly lower than that of viral vectors. However, cationic liposomes have some cytotoxicity both *in vitro* and *in vivo*. For example, cationic liposomes after a certain concentration range bring about various changes of the exposed cells such as cell shrinking, reduced number of mitoses and vacuolization of the cytoplasm [126]. Important proteins like protein kinase C may also be inhibited by cationic liposomes [129]. Other toxic effects include induction of hemolysis [130], induction of fusion between erythrocytes [131], enhanced superoxide production by neutrophils [132], decreased production of IgG and IgM by human peripheral blood mononuclear cells [133], and down regulation of nitric oxide and tumor necrosis factor α synthesis [134]. Positively charged liposomes have also been noted to cause complement activation via the alternative pathway [135] and induction of acute systemic inflammatory reactions. The cytotoxicity of cationic lipid assemblies could be attributed to the hydrophilic head groups, nature of the linker, and the hydrophobic tails. The quaternary amine head group is more toxic than their tertiary amine counterparts [129] because of the steric hindrance. The replacement of the linear amine head group with a heterocyclic ring such as pyridinium, guanidine or imidazole spread the positive charge of the cationic head and thereby reduces toxicity significantly. We have demonstrated that amino acid-based cationic liposomes with primary amine group containing cationic head group have very low cytotoxicity [43,62]. Such low cytotoxicity of amine derivatives might be due to the absence of steric hindrance that gives plasmid DNA an easy access to the cationic head group.

Ester bond is less toxic because of their easy hydrolysis by esterase that results in the higher biodegradability. However, ether bond is too stable to be biodegraded in the physiological condition and thus cause toxicity, albeit their excellent transfection efficiency [136]. Lipids with carbamate bonds also have low cytotoxicity [137,138]. Because they are stable in the physiological pH and get degraded in the endosomal pH that is acidic and 1-2 lower than the physiological pH. The length of the linker is also known to influence the cytotoxicity and the increased length of the linker decreases cytotoxicity [139]. Composition of the hydrophobic moiety influences the toxicity of the cationic liposomes. Cationic lipids with steroid back bones such as derivatives of cholesterol as the hydrophobic moiety inhibit Protein Kinase C (PKC) and therefore more toxic when compared to their alkyl chains counterparts [129]. Micellar structures are more toxic than the liposomal structures. Micellar structures have higher membrane curvature that is responsible for the lower fusion with the endosomal membrane and result in the lower transfection efficiency. Therefore, efficiency of the fusion with the endosomal membrane followed by the release of plasmid DNA from the endosomes is the key step for the elevated transfection efficiency as well as low cytotoxicity. However, the incorporation of DOPE with the cationic lipids increases toxicity to immune effector cells such as macrophages albeit its endosomal membrane destabilization ability to release nucleic acids into the cytoplasm [134]. The replacement of DOPE by Dipalmitoylphosphatidylcholine (DPPC) significantly reduces liposome toxicity towards macrophages. Cationic liposomes inhibit Protein Kinase C (PKC), an important enzyme that is responsible for the synthesis of Nitric Oxide (NO) and Tumor Necrosis Factor-α (TNF-α). At higher concentrations, cationic liposomes are toxic because of their higher affinity to interact with the cell membrane as well as the endosomal membrane when compared to the lipoplexes.

Conclusions and future perspectives

Cationic liposomes are the most potential alternative non-viral vectors for the intracellular delivery of plasmid DNA or oligonucleotides. The morphology and the transfection efficiencies of cationic assemblies are influenced by the different parts of the cationic lipids such as hydrophobic moieties, hydrophilic head groups, ionization state of the head groups, type and length of the spacers and linker molecules. Until now, many research groups have been trying to design and synthesize cationic lipids that can be used to deliver plasmid DNA both *in vitro* and *in vivo* with low cytotoxicity. One typical example is a series of amino acid-based cationic assemblies with lysine or arginine as the hydrophilic head group having different ionization states, glutamate as the backbone to hold two alkyl chains as the hydrophobic moieties, the hydrocarbon chain spacers between the hydrophilic head group and the hydrophobic part showed highly efficient gene delivering efficiency with low cytotoxicity. Therefore, it would be worth to explore further on the cellular uptake mechanism of the amino acid based cationic assemblies and their drug (*i.e.,* plasmid DNA, oligonucleotides and proteins) delivery efficiency *in vivo*.

Figures

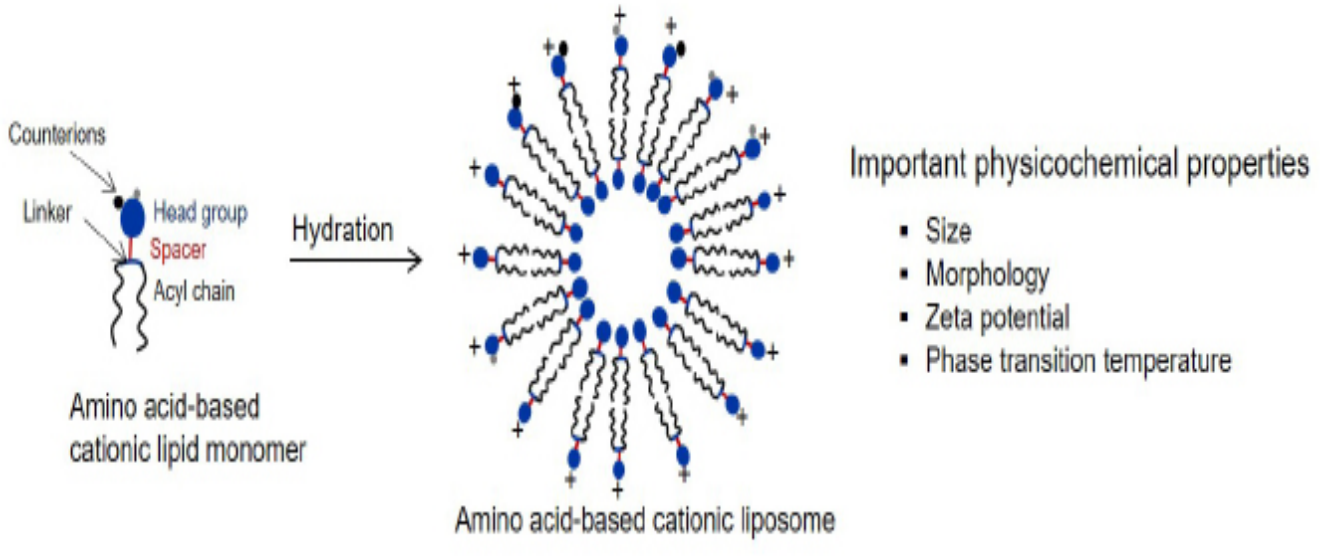

Figure 1: Schematic representation of amino acid-based cationic lipid monomer, cationic liposome and important physicochemical properties.

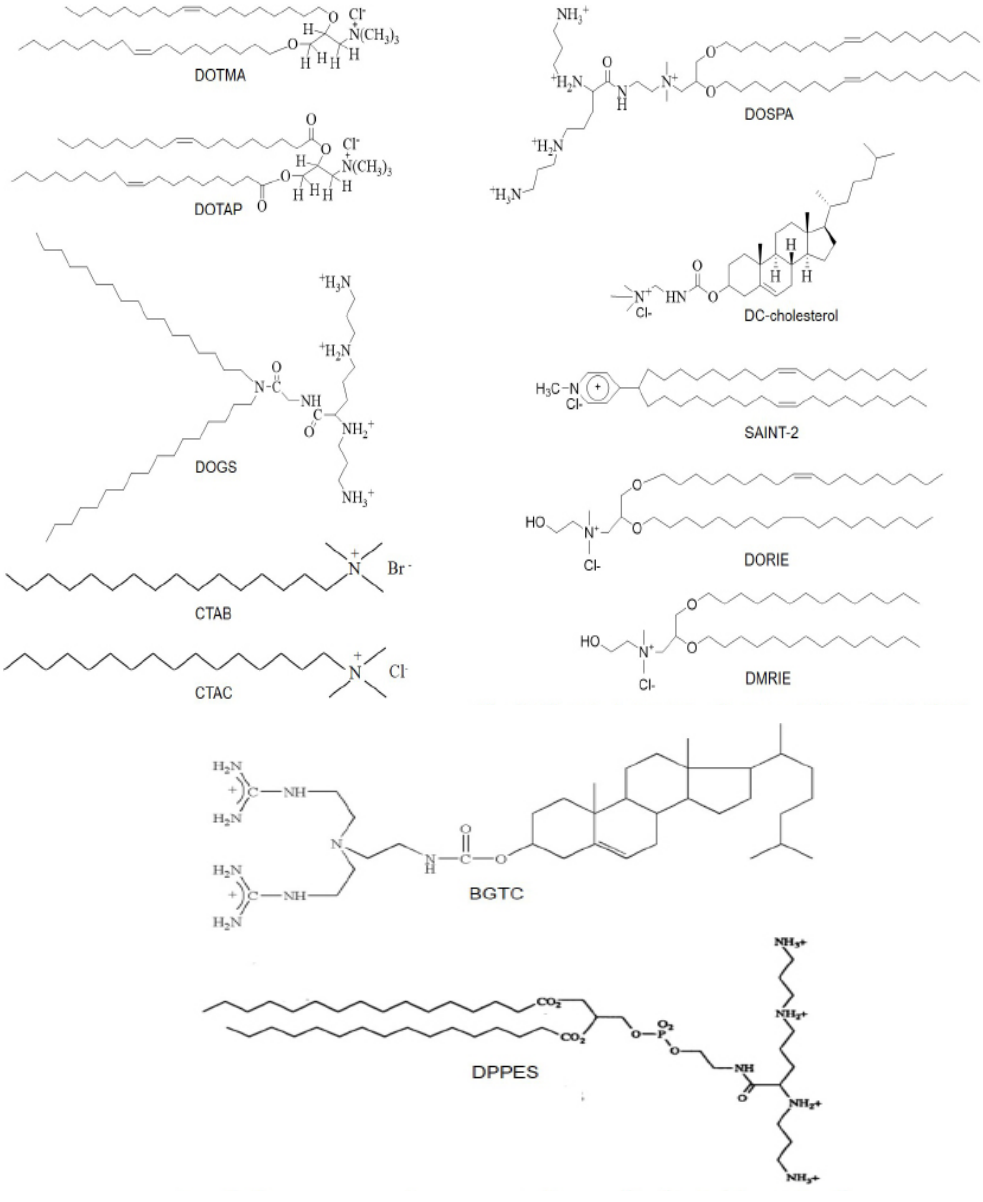

Figure 2: Chemical structure of representative cationic lipids.

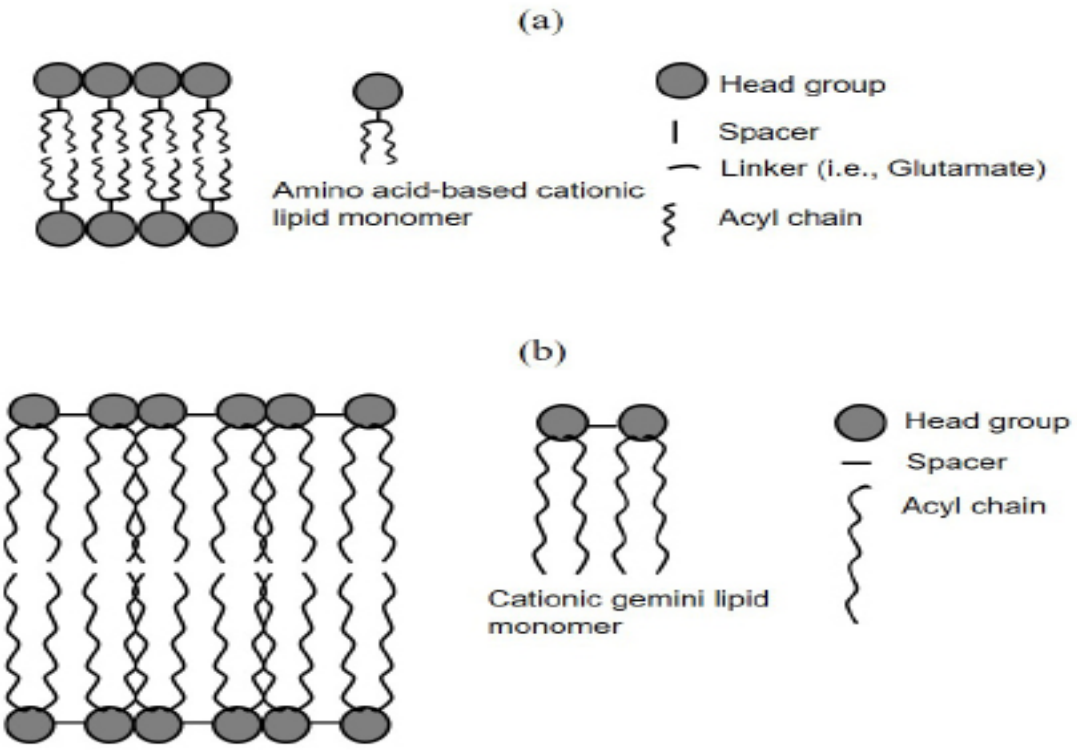

Figure 3: Structural representation of amino acid-based cationic lipid (a), and cationic gemini amphiphiles (b).

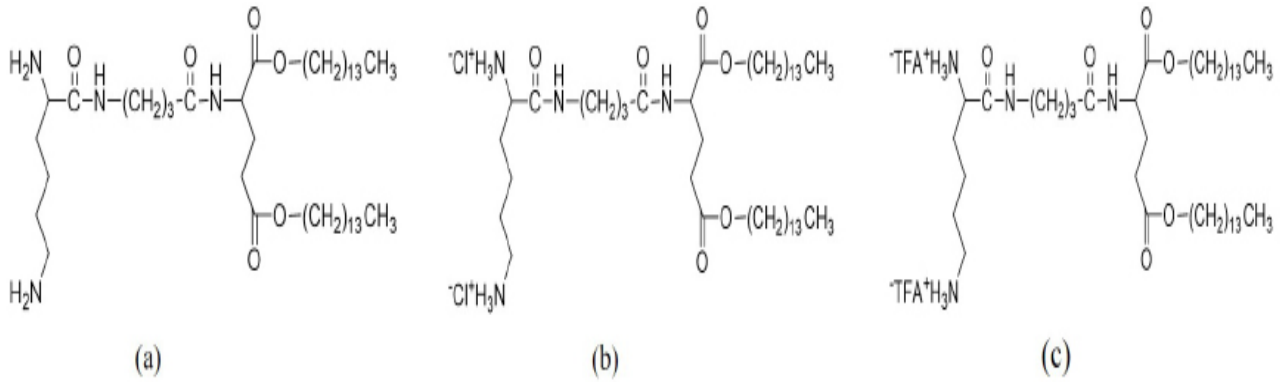

Figure 4: Structure of amino acid-based cationic lipid 1,5-ditetradecyl-N-lysyl-L-glutamate with different ionization states in the lysine head group (a) NH_2 (b)-$NH_3^+Cl^-$,and (c) -$NH_3^+TFA^-$.

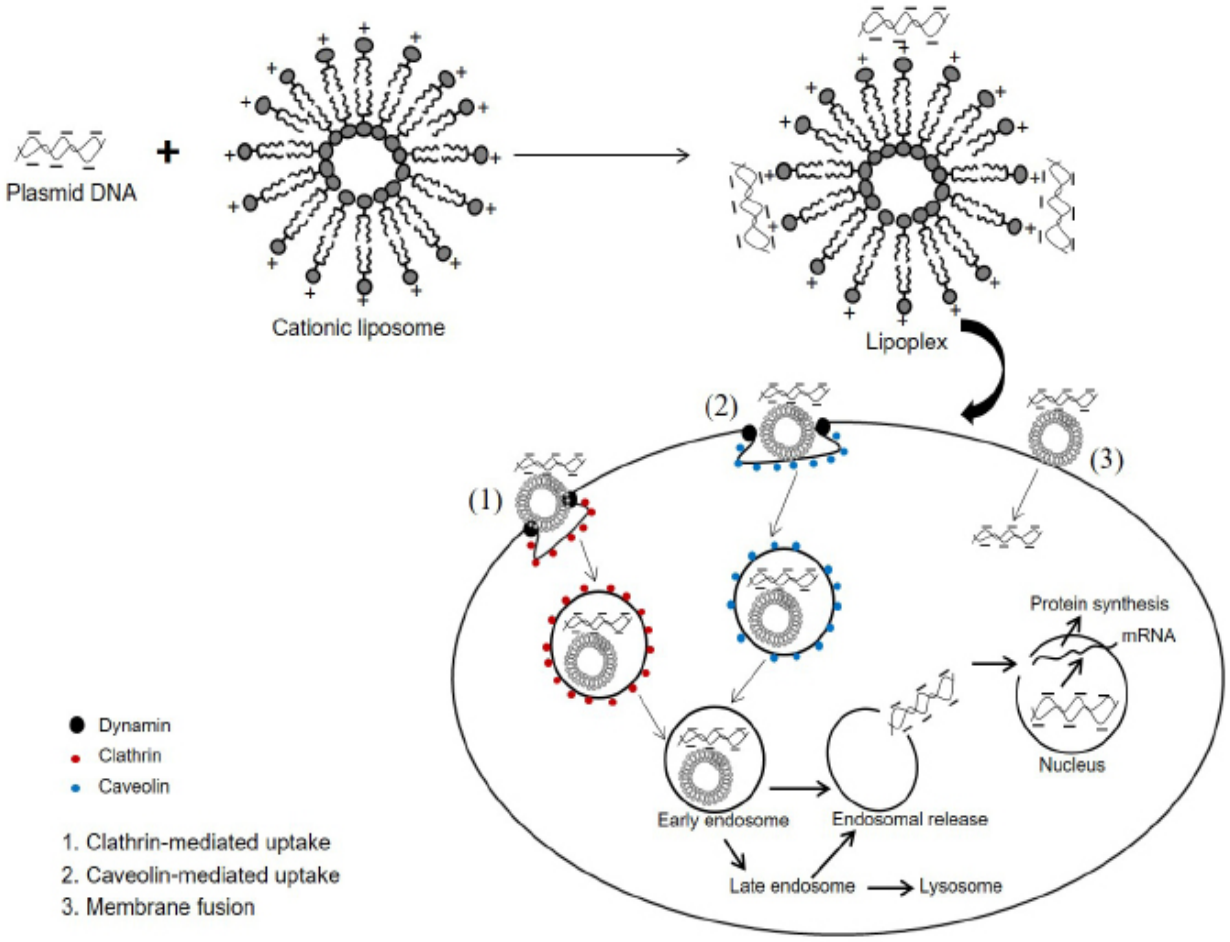

Figure 5: Postulated mechanism of intracellular plasmid DNA delivery through cationic liposomes.

Tables

Table 1: Hydrophobic moieties and amino acids present in the amino acid based cationic lipids.

Hydrophobic moieties	Amino Acids	Refs.
n-lauroyl amide	Arginine	[102,103]
n-tetradecyl alcohol	Lysine, Arginine, Histidine	[62]
n-hexadecyl alcohol	Lysine, Arginine, Histidine	[62]
n-octadecyl alcohol	Lysine, Arginine, Histidine	[62]
n-tetradecyl alcohol	Lysine-HCl counterion or TFA-counterion	[43]
n-tetradecyl alcohol	Arginine-HCl counterion or TFA-counterion	[43]
α-tocopherol	Glycine, Histidine-glycine, Lysine-glycine	[52]
Oleylamine	Glutamic acid	[104]
Myristyl alcohol	Mono-, di-, tri- lysine	[93]
n-hexadecylamine	Histidine	[97,98]
Cholesterol	Lysine, histidine, arginine	[105]
Alkyl chains such as C12, C14, C16, C18 and Oleyl	Arginine, histidine, lysine, tryptophan	[106]
Dialkyl-β-alanine, dialkylglycine	Lysine	[107]

References

1. Miller AD. Human gene therapy comes of age. Nature. 1992; 357: 455-460.
2. Springer CJ. Introduction to vectors for suicide gene therapy. Methods in molecular medicine. 2004; 90: 29-45.
3. Luo D, Saltzman WM. Synthetic DNA delivery systems. Nature biotechnology. 2000; 18: 33-37.
4. Akhtar S, Benter IF. Nonviral delivery of synthetic siRNAs in vivo. Journal of Clinical Investigation. 2007; 117: 3623-3632.
5. de Fougerolles A, Vornlocher H-P, Maraganore J, Lieberman J. Interfering with disease: a progress report on siRNA-based therapeutics. Nature Reviews Drug Discovery. 2007; 6: 443-453.
6. Lu JJ, Langer R, Chen J. A Novel Mechanism Is Involved in Cationic Lipid-Mediated Functional siRNA Delivery. Molecular pharmaceutics. 2009; 6: 763-771.
7. Larson SD, Jackson LN, Chen LA, Rychahou PG, Evers BM. Effectiveness of siRNA uptake in target tissues by various delivery methods. Surgery. 2007; 142: 262-269.
8. Kim HK, Davaa E, Myung CS, Park JS. Enhanced siRNA delivery using cationic liposomes with new polyarginine-conjugated PEG-lipid. International journal of pharmaceutics. 2010; 392: 141-147.
9. Bathula SR, Sharma K, Singh DK, Reddy MP, Sajja PR, Deshmukh AL, et al. siRNA Delivery Using a Cationic-Lipid-Based Highly Selective Human DNA Ligase I Inhibitor. ACS Applied Materials & Interfaces. 2018; 10: 1616-1622.
10. Dalby B. Advanced transfection with Lipofectamine 2000 reagent: primary neurons, siRNA, and high-throughput applications. Methods. 2004; 33: 95-103.
11. Tseng YC, Mozumdar S, Huang L. Lipid-based systemic delivery of siRNA. Advanced drug delivery reviews. 2009; 61: 721-731.
12. Bharali DJ, Klejbor I, Stachowiak EK, Dutta P, Roy I, Kaur N, et al. Organically modified silica nanoparticles: a nonviral vector for in vivo gene delivery and expression in the brain. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102: 11539-11544.
13. Xu Z, Yue Y, Lai Y, Ye C, Qiu J, Pintel DJ, et al. Trans-Splicing Adeno-Associated Viral Vector-Mediated Gene Therapy Is Limited by the Accumulation of Spliced mRNA but Not by Dual Vector Coinfection Efficiency. Human Gene Therapy. 2004; 15: 896-905.
14. Bergen JM, Park IK, Horner PJ, Pun SH. Nonviral Approaches for Neuronal Delivery of Nucleic Acids. Pharmaceutical Research. 2007; 25: 983-998.
15. Boyce N. Trial halted after gene shows up in semen. Nature. 2001; 414: 677.
16. Davis SS. Biomédical applications of nanotechnology-implications for drug targeting and gene therapy. Trends in Biotechnology. 1997; 15: 217-224.
17. Li S, Huang L. Nonviral gene therapy: promises and challenges. Gene Therapy. 2000; 7: 31-34.
18. Li SD, Huang L. Gene therapy progress and prospects: non-viral gene therapy by systemic delivery. Gene Therapy. 2006; 13: 1313-139.
19. Ma B, Zhang S, Jiang H, Zhao B, Lv H. Lipoplex morphologies and their influences on transfection efficiency in gene delivery. Journal of Controlled Release. 2007; 123: 184-194.
20. Mintzer MA, Simanek EE. Nonviral Vectors for Gene Delivery. Chemical Reviews. 2009; 109: 259-302.
21. Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, et al. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proceedings of the National Academy of Sciences of the United States of America. 1987; 84: 7413-7417.
22. Felgner JH, Kumar R, Sridhar CN, Wheeler CJ, Tsai YJ, Border R, et al. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. The Journal of biological chemistry. 1994; 269: 2550-2561.
23. Shi N, Pardridge WM. Noninvasive gene targeting to the brain. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97: 7567-7572.
24. Govindarajan S, Kitaura K, Takafuji M, Ihara H, Varadarajan KS, Patel AB, et al. Gene delivery into human cancer cells by cationic lipid-mediated magnetofection. International journal of pharmaceutics. 2013; 446: 87-99.
25. Sarker SR, Hokama R, Takeoka S. Intracellular delivery of universal proteins using a lysine headgroup containing cationic liposomes: deciphering the uptake mechanism. Molecular pharmaceutics. 2014; 11: 164-174.
26. Li Y, Zheng S, Liang X, Jin Y, Wu Y, Bai H, et al. Doping hydroxylated cationic lipid into PEGylated cerasome boosts in vivo siRNA transfection efficacy. Bioconjugate chemistry. 2014; 25: 2055-2066.
27. Itaka K, Yamauchi K, Harada A, Nakamura K, Kawaguchi H, Kataoka K. Polyion complex micelles from plasmid DNA and poly(ethylene glycol)-poly(L-lysine) block copolymer as serum-tolerable polyplex system: physicochemical properties of micelles relevant to gene transfection efficiency. Biomaterials. 2003; 24: 4495-4506.
28. Vigneron JP, Oudrhiri N, Fauquet M, Vergely L, Bradley JC, Basseville M, et al. Guanidinium-cholesterol cationic lipids: efficient vectors for the transfection of eukaryotic cells. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93: 9682-9686.
29. Haensler J, Szoka FC. Polyamidoamine cascade polymers mediate efficient transfection of cells in culture. Bioconjugate chemistry. 2002; 4: 372-379.
30. Kukowska-Latallo JF, Bielinska AU, Johnson J, Spindler R, Tomalia DA, Baker JR, Jr. Efficient transfer of genetic material into mammalian cells using Starburst polyamidoamine dendrimers. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93: 4897-4902.
31. Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, et al. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. Proceedings of the National Academy of Sciences of the United States of America. 1995; 92: 7297-7301.
32. Wagner E, Zenke M, Cotten M, Beug H, Birnstiel ML. Transferrin-polycation conjugates as carriers for DNA uptake into cells. Proceedings of the National Academy of Sciences of the United States of America. 1990; 87: 3410-3414.
33. Shi B, Zheng M, Tao W, Chung R, Jin D, Ghaffari D, et al. Challenges in DNA Delivery and Recent Advances in Multifunctional Polymeric DNA Delivery Systems. Biomacromolecules. 2017; 18: 2231-2246.
34. Barati S, Chegini F, Hurtado P, Rush RA. Hybrid tetanus toxin C fragment-diphtheria toxin translocation domain allows specific gene transfer into PC12 cells. Experimental neurology. 2002; 177: 75-87.

35. Usachev YM, Khammanivong A, Campbell C, Thayer SA. Particle-mediated gene transfer to rat neurons in primary culture. Pflugers Archiv : European journal of physiology. 2000; 439: 730-738.

36. Xiang JJ, Tang JQ, Zhu SG, Nie XM, Lu HB, Shen SR, et al. IONP-PLL: a novel non-viral vector for efficient gene delivery. The journal of gene medicine. 2003; 5: 803-817.

37. Song H, Yu M, Lu Y, Gu Z, Yang Y, Zhang M, et al. Plasmid DNA Delivery: Nanotopography Matters. Journal of the American Chemical Society. 2017; 139: 18247-18254.

38. Ji Q, Yamazaki T, Sun J, Górecka Ż, Huang N-C, Hsu S-h, et al. Spongelike Porous Silica Nanosheets: From "Soft" Molecular Trapping to DNA Delivery. ACS Applied Materials & Interfaces. 2017; 9: 4509-4518.

39. Miller AD. Cationic liposomes for gene therapy. Angewandte Chemie International Edition. 1998; 37: 1768-1785.

40. Ghosh YK, Visweswariah SS, Bhattacharya S. Advantage of the Ether Linkage between the Positive Charge and the Cholesteryl Skeleton in Cholesterol-Based Amphiphiles as Vectors for Gene Delivery. Bioconjugate chemistry. 2002; 13: 378-384.

41. Ghosh YK, Visweswariah SS, Bhattacharya S. Nature of linkage between the cationic headgroup and cholesteryl skeleton controls gene transfection efficiency. FEBS letters. 2000; 473: 341-344.

42. Banerjee R, Mahidhar YV, Chaudhuri A, Gopal V, Rao NM. Design, synthesis, and transfection biology of novel cationic glycolipids for use in liposomal gene delivery. Journal of medicinal chemistry. 2001; 44: 4176-4185.

43. Sarker SR, Arai S, Murate M, Takahashi H, Takata M, Kobayashi T, et al. Evaluation of the influence of ionization states and spacers in the thermotropic phase behaviour of amino acid-based cationic lipids and the transfection efficiency of their assemblies. International journal of pharmaceutics. 2012; 422: 364-373.

44. Obata Y, Saito S, Takeda N, Takeoka S. Plasmid DNA-encapsulating liposomes: effect of a spacer between the cationic head group and hydrophobic moieties of the lipids on gene expression efficiency. Biochimica et biophysica acta. 2009; 1788: 1148-1158.

45. Bhattacharya S, Bajaj A. Advances in gene delivery through molecular design of cationic lipids. Chemical Communications. 2009: 4632.

46. Leventis R, Silvius JR. Interactions of mammalian cells with lipid dispersions containing novel metabolizable cationic amphiphiles. Biochimica et biophysica acta. 1990; 1023: 124-132.

47. Behr JP, Demeneix B, Loeffler JP, Perez-Mutul J. Efficient gene transfer into mammalian primary endocrine cells with lipopolyamine-coated DNA. Proceedings of the National Academy of Sciences of the United States of America. 1989; 86: 6982-6986.

48. Gao X, Huang L. A novel cationic liposome reagent for efficient transfection of mammalian cells. Biochemical and biophysical research communications. 1991; 179: 280-285.

49. Rejman J, Wagenaar A, Engberts JB, Hoekstra D. Characterization and transfection properties of lipoplexes stabilized with novel exchangeable polyethylene glycol-lipid conjugates. Biochimica et biophysica acta. 2004; 1660: 41-52.

50. Hurley CA, Wong JB, Hailes HC, Tabor AB. Asymmetric synthesis of dialkyloxy-3-alkylammonium cationic lipids. The Journal of organic chemistry. 2004; 69: 980-983.

51. Stephan DJ, Yang ZY, San H, Simari RD, Wheeler CJ, Felgner PL, et al. A new cationic liposome DNA complex enhances the efficiency of arterial gene transfer in vivo. Hum Gene Ther. 1996; 7: 1803-1812.

52. Yi WJ, Zheng LT, Su RC, Liu Q, Zhao ZG. Amino acid-based cationic lipids with alpha-tocopherol hydrophobic tail for efficient gene delivery. Chemical biology & drug design. 2015; 86: 1192-1202.

53. Zhi D, Zhang S, Wang B, Zhao Y, Yang B, Yu S. Transfection efficiency of cationic lipids with different hydrophobic domains in gene delivery. Bioconjugate chemistry. 2010; 21: 563-577.

54. Pinnaduwage P, Schmitt L, Huang L. Use of a quaternary ammonium detergent in liposome mediated DNA transfection of mouse L-cells. Biochimica et biophysica acta. 1989; 985: 33-37.

55. Cameron FH, Moghaddam MJ, Bender VJ, Whittaker RG, Mott M, Lockett TJ. A transfection compound series based on a versatile Tris linkage. Biochimica et biophysica acta. 1999; 1417: 37-50.

56. Obika S, Yu W, Shimoyama A, Uneda T, Miyashita K, Doi T, et al. Symmetrical cationic triglycerides: an efficient synthesis and application to gene transfer. Bioorganic & medicinal chemistry. 2001; 9: 245-254.

57. Lichtenberg D, Freire E, Schmidt CF, Barenholz Y, Felgner PL, Thompson TE. Effect of surface curvature on stability, thermodynamic behavior, and osmotic activity of dipalmitoylphosphatidylcholine single lamellar vesicles. Biochemistry. 1981; 20: 3462-3467.

58. Lichtenberg D, Felgner P, Thompson T. Transition of a liquid crystalline phosphatidylcholine bilayer to the gel phase in a vesicle reduces the internal aqueous volume. Biochimica et Biophysica Acta (BBA)-Biomembranes. 1982; 684: 277-281.

59. Fletcher S, Ahmad A, Perouzel E, Heron A, Miller AD, Jorgensen MR. In vivo studies of dialkynoyl analogues of DOTAP demonstrate improved gene transfer efficiency of cationic liposomes in mouse lung. Journal of medicinal chemistry. 2006; 49: 349-357.

60. van der Woude I, Wagenaar A, Meekel AA, ter Beest MB, Ruiters MH, Engberts JB, et al. Novel pyridinium surfactants for efficient, nontoxic in vitro gene delivery. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94: 1160-1165.

61. Zuhorn IS, Oberle V, Visser WH, Engberts JB, Bakowsky U, Polushkin E, et al. Phase behavior of cationic amphiphiles and their mixtures with helper lipid influences lipoplex shape, DNA translocation, and transfection efficiency. Biophysical journal. 2002; 83: 2096-2108.

62. Obata Y, Suzuki D, Takeoka S. Evaluation of cationic assemblies constructed with amino acid based lipids for plasmid DNA delivery. Bioconjugate chemistry. 2008; 19: 1055-1063.

63. Floch V, Le Bolc'h G, Audrezet MP, Yaouanc JJ, Clement JC, des Abbayes H, et al. Cationic phosphonolipids as non viral vectors for DNA transfection in hematopoietic cell lines and CD34+ cells. Blood cells, molecules & diseases. 1997; 23: 69-87.

64. Kim HS, Moon J, Kim KS, Choi MM, Lee JE, Heo Y, et al. Gene-transferring efficiencies of novel diamino cationic lipids with varied hydrocarbon chains. Bioconjugate chemistry. 2004; 15: 1095-1101.

65. Xu Y, Szoka FC, Jr. Mechanism of DNA release from cationic liposome/DNA complexes used in cell transfection. Biochemistry. 1996; 35: 5616-5623.

66. Ren T, Zhang G, Liu F, Liu D. Synthesis and evaluation of vitamin D-based cationic lipids for gene delivery in vitro. Bioorganic & medicinal chemistry letters. 2000; 10: 891-894.

67. Walker S, Sofia MJ, Kakarla R, Kogan NA, Wierichs L, Longley CB, et al. Cationic facial amphiphiles: a promising class of transfection agents. Proceedings of the National Academy of Sciences. 1996; 93: 1585-1590.

68. Kichler A, Leborgne C, Savage PB, Danos O. Cationic steroid antibiotics demonstrate DNA delivery properties. Journal of controlled release: official journal of the Controlled Release Society. 2005; 107: 174-182.

69. Fujiwara T, Hasegawa S, Hirashima N, Nakanishi M, Ohwada T. Gene transfection activities of amphiphilic steroid-polyamine conjugates. Biochimica et biophysica acta. 2000; 1468: 396-402.

70. Kedika B, Patri SV. Design, synthesis, and in vitro transfection biology of novel tocopherol based monocationic lipids: a structure-activity investigation. Journal of medicinal chemistry. 2011; 54: 548-561.

71. Kim HS, Song IH, Kim JC, Kim EJ, Jang DO, Park YS. In vitro and in vivo gene-transferring characteristics of novel cationic lipids, DMKD (O,O'-dimyristyl-N-lysyl aspartate) and DMKE (O,O'-dimyristyl-N-lysyl glutamate). Journal of controlled release: official journal of the Controlled Release Society. 2006; 115: 234-241.

72. Obata Y, Tajima S, Takeoka S. Evaluation of pH-responsive liposomes containing amino acid-based zwitterionic lipids for improving intracellular drug delivery in vitro and in vivo. Journal of controlled release: official journal of the Controlled Release Society. 2010; 142: 267-276.

73. Rajesh M, Sen J, Srujan M, Mukherjee K, Sreedhar B, Chaudhuri A. Dramatic influence of the orientation of linker between hydrophilic and hydrophobic lipid moiety in liposomal gene delivery. Journal of the American Chemical Society. 2007; 129: 11408-11420.

74. Bhattacharya S, De S. Vesicle formation from dimeric surfactants through ion-pairing. Adjustment of polar headgroup separation leads to control over vesicular thermotropic properties. Journal of the Chemical Society, Chemical Communications. 1995: 651.

75. Bhattacharya S, De S, George SK. Synthesis and vesicle formation from novel pseudoglyceryl dimeric lipids. Evidence of formation of widely different membrane organizations with exceptional thermotropic properties. Chemical Communications. 1997: 2287-2288.

76. Bhattacharya S, De S. Vesicle Formation from Dimeric Ion-Paired Amphiphiles. Control over Vesicular Thermotropic and Ion-Transport Properties as a Function of Intra-amphiphilic Headgroup Separation†. Langmuir. 1999; 15: 3400-3410.

77. Bhattacharya S, De S. Synthesis and Vesicle Formation from Dimeric Pseudoglyceryl Lipids with (CH2) m Spacers: Pronounced m-Value Dependence of Thermal Properties, Vesicle Fusion, and Cholesterol Complexation. Chemistry-a European Journal. 1999; 5: 2335-2347.

78. Bajaj A, Kondiah P, Bhattacharya S. Design, Synthesis, and in Vitro Gene Delivery Efficacies of Novel Cholesterol-Based Gemini Cationic Lipids and Their Serum Compatibility: A Structure−Activity Investigation. Journal of medicinal chemistry. 2007; 50: 2432-2442.

79. Bajaj A, Kondaiah P, Bhattacharya S. Effect of the Nature of the Spacer on Gene Transfer Efficacies of Novel Thiocholesterol Derived Gemini Lipids in Different Cell Lines: A Structure–Activity Investigation. Journal of medicinal chemistry. 2008; 51: 2533-2540.

80. Bhattacharya S, Bajaj A. Membrane-Forming Properties of Gemini Lipids Possessing Aromatic Backbone between the Hydrocarbon Chains and the Cationic Headgroup. The Journal of Physical Chemistry B. 2007; 111: 13511-13519.

81. Bajaj A, Kondaiah P, Bhattacharya S. Gene Transfection Efficacies of Novel Cationic Gemini Lipids Possessing Aromatic Backbone and Oxyethylene Spacers. Biomacromolecules. 2008; 9: 991-999.

82. Luciani P, Bombelli C, Colone M, Giansanti L, Ryhänen SJ, Säily VMJ, et al. Influence of the Spacer of Cationic Gemini Amphiphiles on the Hydration of Lipoplexes. Biomacromolecules. 2007; 8: 1999-2003.

83. Sakaguchi N, Kojima C, Harada A, Kono K. Preparation of pH-Sensitive Poly(glycidol) Derivatives with Varying Hydrophobicities: Their Ability to Sensitize Stable Liposomes to pH. Bioconjugate chemistry. 2008; 19: 1040-1048.

84. Yamada N, Ariga K, Naito M, Matsubara K, Koyama E. Regulation of β-Sheet Structures within Amyloid-Like β-Sheet Assemblage from Tripeptide Derivatives. Journal of the American Chemical Society. 1998; 120: 12192-12199.

85. Shimizu T. Bottom-Up Synthesis and Morphological Control of High-Axial-Ratio Nanostructures through Molecular Self-Assembly. Polymer Journal. 2003; 35: 1-22.

86. Sarker SR, Aoshima Y, Hokama R, Inoue T, Sou K, Takeoka S. Arginine-based cationic liposomes for efficient in vitro plasmid DNA delivery with low cytotoxicity. International journal of nanomedicine. 2013; 8: 1361-1375.

87. Banerjee R, Das PK, Srilakshmi GV, Chaudhuri A, Rao NM. Novel series of non-glycerol-based cationic transfection lipids for use in liposomal gene delivery. Journal of medicinal chemistry. 1999; 42: 4292-4299.

88. Bennett MJ, Aberle AM, Balasubramaniam RP, Malone JG, Malone RW, Nantz MH. Cationic lipid-mediated gene delivery to murine lung: correlation of lipid hydration with in vivo transfection activity. Journal of medicinal chemistry. 1997; 40: 4069-4078.

89. Majeti BK, Singh RS, Yadav SK, Bathula SR, Ramakrishna S, Diwan PV, et al. Enhanced intravenous transgene expression in mouse lung using cyclic-head cationic lipids. Chemistry & biology. 2004; 11: 427-437.

90. Mukherjee K, Bhattacharyya J, Sen J, Sistla R, Chaudhuri A. Covalent grafting of common trihydroxymethylaminomethane in the headgroup region imparts high serum compatibility and mouse lung transfection property to cationic amphiphile. Journal of medicinal chemistry. 2008; 51: 1967-1971.

91. Bajaj A, Mishra SK, Kondaiah P, Bhattacharya S. Effect of the headgroup variation on the gene transfer properties of cholesterol based cationic lipids possessing ether linkage. Biochimica et biophysica acta. 2008; 1778: 1222-1236.

92. Sen J, Chaudhuri A. Gene transfer efficacies of novel cationic amphiphiles with alanine, beta-alanine, and serine headgroups: a structure-activity investigation. Bioconjugate chemistry. 2005; 16: 903-912.

93. Karmali PP, Kumar VV, Chaudhuri A. Design, syntheses and in vitro gene delivery efficacies of novel mono-, di- and trilysinated cationic lipids: a structure-activity investigation. Journal of medicinal chemistry. 2004; 47: 2123-2132.

94. Aoshima Y, Hokama R, Sou K, Sarker SR, Iida K, Nakamura H, et al. Cationic amino acid based lipids as effective nonviral gene delivery vectors for primary cultured neurons. ACS chemical neuroscience. 2013; 4: 1514-1519.

95. Sen J, Chaudhuri A. Design, syntheses, and transfection biology of novel non-cholesterol-based guanidinylated cationic lipids. Journal of medicinal chemistry. 2005; 48: 812-820.

96. Nakase I, Takeuchi T, Tanaka G, Futaki S. Methodological and cellular aspects that govern the internalization mechanisms of arginine-rich cell-penetrating peptides. Advanced drug delivery reviews. 2008; 60: 598-607.

97. Kumar VV, Pichon C, Refregiers M, Guerin B, Midoux P, Chaudhuri A. Single histidine residue in head-group region is sufficient to impart remarkable gene transfection properties to cationic lipids: evidence for histidine-mediated membrane fusion at acidic pH. Gene Ther. 2003; 10: 1206-1215.

98. Karmali PP, Majeti BK, Sreedhar B, Chaudhuri A. In vitro gene transfer efficacies and serum compatibility profiles of novel mono-, di-, and tri-histidinylated cationic transfection lipids: a structure-activity investigation. Bioconjugate chemistry. 2006; 17: 159-171.

99. Heyes JA, Niculescu-Duvaz D, Cooper RG, Springer CJ. Synthesis of novel cationic lipids: effect of structural modification on the efficiency of gene transfer. Journal of medicinal chemistry. 2002; 45: 99-114.

100. Ju J, Huan ML, Wan N, Qiu H, Zhou SY, Zhang BL. Novel cholesterol-based cationic lipids as transfecting agents of DNA for efficient gene delivery. International journal of molecular sciences. 2015; 16: 5666-5681.

101. Aberle AM, Bennett MJ, Malone RW, Nantz MH. The counterion influence on cationic lipid-mediated transfection of plasmid DNA. Biochimica et biophysica acta. 1996; 1299: 281-283.

102. Lundberg D, Faneca H, Morán MDC, Pedroso De Lima MC, Miguel MDG, Lindman B. Inclusion of a single-tail amino acid-based amphiphile in a lipoplex formulation: Effects on transfection efficiency and physicochemical properties. Molecular membrane biology. 2011; 28: 42-53.

103. Rosa M, Rosa M, Penacho N, Simöes S, Lima MCP, Lindman B, et al. DNA pre-condensation with an amino acid-based cationic amphiphile. A viable approach for liposome-based gene delivery. Molecular membrane biology. 2009; 25: 23-34.

104. Suh MS, Shim G, Lee HY, Han S-E, Yu Y-H, Choi Y, et al. Anionic amino acid-derived cationic lipid for siRNA delivery. Journal of Controlled Release. 2009; 140: 268-276.

105. Li L, Nie Y, Zhu R, Shi S, Luo K, He B, et al. Preparation and gene delivery of alkaline amino acids-based cationic liposomes. Archives of Pharmacal Research. 2008; 31: 924-931.

106. Heyes JA, Niculescu-Duvaz D, Cooper RG, Springer CJ. Synthesis of Novel Cationic Lipids: Effect of Structural Modification on the Efficiency of Gene Transfer. Journal of medicinal chemistry. 2002; 45: 99-114.

107. Walsh CL, Nguyen J, Tiffany MR, Szoka FC. Synthesis, Characterization, and Evaluation of Ionizable Lysine-Based Lipids for siRNA Delivery. Bioconjugate chemistry. 2012; 24: 36-43.

108. Meyer O, Kirpotin D, Hong K, Sternberg B, Park JW, Woodle MC, et al. Cationic liposomes coated with polyethylene glycol as carriers for oligonucleotides. The Journal of biological chemistry. 1998; 273: 15621-15627.

109. Zhang HW, Zhang L, Sun X, Zhang ZR. Successful transfection of hepatoma cells after encapsulation of plasmid DNA into negatively charged liposomes. Biotechnology and bioengineering. 2007; 96: 118-124.

110. Caracciolo G, Pozzi D, Capriotti AL, Marianecci C, Carafa M, Marchini C, et al. Factors determining the superior performance of lipid/DNA/protammine nanoparticles over lipoplexes. Journal of medicinal chemistry. 2011; 54: 4160-4171.

111. Kubota K, Onishi K, Sawaki K, Li T, Mitsuoka K, Sato T, et al. Effect of the nanoformulation of siRNA-lipid assemblies on their cellular uptake and immune stimulation. International journal of nanomedicine. 2017; 12: 5121-5133.

112. Caracciolo G, Caminiti R, Digman MA, Gratton E, Sanchez S. Efficient escape from endosomes determines the superior efficiency of multicomponent lipoplexes. The journal of physical chemistry B. 2009; 113: 4995-4997.

113. Koltover I, Salditt T, Radler JO, Safinya CR. An inverted hexagonal phase of cationic liposome-DNA complexes related to DNA release and delivery. Science. 1998; 281: 78-81.

114. Smisterova J, Wagenaar A, Stuart MC, Polushkin E, ten Brinke G, Hulst R, et al. Molecular shape of the cationic lipid controls the structure of cationic lipid/dioleylphosphatidylethanolamine-DNA complexes and the efficiency of gene delivery. The Journal of biological chemistry. 2001; 276: 47615-47622.

115. Obata Y, Ciofani G, Raffa V, Cuschieri A, Menciassi A, Dario P, et al. Evaluation of cationic liposomes composed of an amino acid-based lipid for neuronal transfection. Nanomedicine : nanotechnology, biology, and medicine. 2010; 6: 70-77.

116. Rejman J, Bragonzi A, Conese M. Role of clathrin- and caveolae-mediated endocytosis in gene transfer mediated by lipo- and polyplexes. Molecular therapy : the journal of the American Society of Gene Therapy. 2005; 12: 468-474.

117. Simoes S, Filipe A, Faneca H, Mano M, Penacho N, Duzgunes N, et al. Cationic liposomes for gene delivery. Expert opinion on drug delivery. 2005; 2: 237-254.

118. Mislick KA, Baldeschwieler JD. Evidence for the role of proteoglycans in cation-mediated gene transfer. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93: 12349-12354.

119. Kjellen L, Lindahl U. Proteoglycans: structures and interactions. Annual review of biochemistry. 1991; 60: 443-475.

120. Yue ZG, Wei W, Lv PP, Yue H, Wang LY, Su ZG, et al. Surface charge affects cellular uptake and intracellular trafficking of chitosan-based nanoparticles. Biomacromolecules. 2011; 12: 2440-2446.

121. Cardoso AM, Faneca H, Almeida JA, Pais AA, Marques EF, de Lima MC, et al. Gemini surfactant dimethylene-1,2-bis(tetradecyldimethylammonium bromide)-based gene vectors: a biophysical approach to transfection efficiency. Biochimica et biophysica acta. 2011; 1808: 341-351.

122. Almofti MR, Harashima H, Shinohara Y, Almofti A, Li W, Kiwada H. Lipoplex size determines lipofection efficiency with or without serum. Molecular membrane biology. 2003; 20: 35-43.

123. Hoekstra D, Rejman J, Wasungu L, Shi F, Zuhorn I. Gene delivery by cationic lipids: in and out of an endosome. Biochemical Society transactions. 2007; 35: 68-71.

124. Zuhorn IS, Engberts JB, Hoekstra D. Gene delivery by cationic lipid vectors: overcoming cellular barriers. European biophysics journal: EBJ. 2007; 36: 349-362.

125. Albanese A, Chan WC. Effect of gold nanoparticle aggregation on cell uptake and toxicity. ACS nano. 2011; 5: 5478-5489.

126. Lappalainen K, Jaaskelainen I, Syrjanen K, Urtti A, Syrjanen S. Comparison of cell proliferation and toxicity assays using two cationic liposomes. Pharm Res. 1994; 11: 1127-1131.

127. Wasungu L, Hoekstra D. Cationic lipids, lipoplexes and intracellular delivery of genes. Journal of controlled release : official

journal of the Controlled Release Society. 2006; 116: 255-264.

128. Elouahabi A, Ruysschaert JM. Formation and intracellular trafficking of lipoplexes and polyplexes. Molecular therapy : the journal of the American Society of Gene Therapy. 2005; 11: 336-347.

129. Bottega R, Epand RM. Inhibition of protein kinase C by cationic amphiphiles. Biochemistry. 1992; 31: 9025-9030.

130. Senior JH, Trimble KR, Maskiewicz R. Interaction of positively-charged liposomes with blood: implications for their application in vivo. Biochimica et biophysica acta. 1991; 1070: 173-179.

131. Sakurai F, Nishioka T, Saito H, Baba T, Okuda A, Matsumoto O, et al. Interaction between DNA-cationic liposome complexes and erythrocytes is an important factor in systemic gene transfer via the intravenous route in mice: the role of the neutral helper lipid. Gene Ther. 2001; 8: 677-686.

132. Ferencik M, Lacko I, Devinsky F. Immunomodulatory activity of some amphiphilic compounds. Die Pharmazie. 1990; 45: 695-696.

133. Jahnova E, Ferencik M, Nyulassy S, Devinsky F, Lacko I. Amphiphilic detergents inhibit production of IgG and IgM by human peripheral blood mononuclear cells. Immunology letters. 1993; 39: 71-75.

134. Filion MC, Phillips NC. Toxicity and immunomodulatory activity of liposomal vectors formulated with cationic lipids toward immune effector cells. Biochimica et biophysica acta. 1997; 1329: 345-356.

135. Chonn A, Cullis PR, Devine DV. The role of surface charge in the activation of the classical and alternative pathways of complement by liposomes. Journal of immunology. 1991; 146: 4234-4241.

136. Lv H, Zhang S, Wang B, Cui S, Yan J. Toxicity of cationic lipids and cationic polymers in gene delivery. Journal of controlled release: official journal of the Controlled Release Society. 2006; 114: 100-109.

137. Liu D, Hu J, Qiao W, Li Z, Zhan S, Cheng L. Synthesis and characterization of a series of carbamate-linked cationic lipids for gene delivery. Lipids. 2005; 40: 839-848.

138. Liu D, Hu J, Qiao W, Li Z, Zhang S, Cheng L. Synthesis of carbamate-linked lipids for gene delivery. Bioorganic & medicinal chemistry letters. 2005; 15: 3147-3150.

139. Floch V, Loisel S, Guenin E, Herve AC, Clement JC, Yaouanc JJ, et al. Cation substitution in cationic phosphonolipids: a new concept to improve transfection activity and decrease cellular toxicity. Journal of medicinal chemistry. 2000; 43: 4617-4628.