Nonviral gene therapy

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Abstract: The development of gene medicine based on the concept of molecular therapy has opened new medical horizons. Gene design and delivery are especially significant in clinical applications. This Review is covering the most common non-viral gene-delivery techniques, a broad spectrum of disciplines that include chemistry, molecular biology, cell biology, and pharmacokinetics. Major sections introduce molecules for gene delivery and their properties; technologies of controlled gene delivery *in vitro* and *in vivo*; therapeutic genes and the status of clinical applications shown to be key factors in gene medicine.
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1. Introduction

Gene therapy is the treatment or prevention of disease by transfer of genetic materials, either RNA or DNA, into specific human tissues or cells, to replace defective genes, substitute missing genes, silence unwanted gene expression, or introduce new cellular biofunctions (Bhavsar and Amiji, 2007).

During the last decades the advanced molecular biology and biotechnology techniques have greatly enhanced the understanding of the genetics of pathogenesis and led to identification of numerous disease-causing genes.Exploration of gene therapy has extended drastically beyond hereditary conditions, as hemophilia (Walsh, 2003), human β _thalassaemia (Cavazzana-Calvo *et al.,* 2010), and cystic fibrosis (Mitomo *et al.,* 2010) to a broad spectrum of acquired diseases, including cancer (Kerr, 2003; McNeish *et al.,* 2004), neurodegenerative disorders (Tuszynski, 2002 and Burton *et al.,* 2003), cardiovascular conditions (Nabel, 1999), immunodeficiencies (Kohn, 2010).

The first step of gene therapy involves gene delivery—that is, the presentation of the therapeutic genetic material in the interior of a living cell and its subsequent expression. However, the complexities of thebiological system present numerous obstacles to successful gene delivery. In many cases, systemic administration of gene medicines is needed as many disease sites are not easy to access otherwise. Under these circumstances, gene medicines had to penetrate through a series of extracellular barriers (opsonins, phagocytes, extracellular matrices, and degradative enzymes) and intracellular barriers (lack of proper recognition characteristics necessary to direct intracellular transport, degradation within lysosomal compartments, release from transport vesicles and nuclear uptake) which can prevent gene delivery, transcription, and translation (Kabanov, 1999, Nishikawa and Huang, 2001,Wong, 2007). Therefore the gene therapy is onlypossible with an efficient carrier for protection and transportation (Liu *et al.,* 2010).

Many practices involved direct injection of naked DNA, through the use of electrical impulses (electroporation), or bombardment with gold particles (gene gun),to force them across cellular membranes (Nishikawa and Huang, 2001). These direct methods has been proofed to be very inefficient and severely limited to tissues that are easily accessible to direct injection such as eye, muscle, skin and tumors (Shi *et al.,* 2002 and Walther *et al.,* 2003).

Development of a stable and efficient delivery system has been considered as a major challenge for gene therapy. Ideally the optimal delivery strategy aims to improve the stability of genes after their administration into the body, improve gene pharmacokinetics and biodistribution, deliver genes specifically to the desired tissue site, reduce off-target effects, facilitate the cellular uptake of genes within target cells, and promote efficient intracellular trafficking (Zhu *et al.,* 1993). The ultimate vector for systemic gene therapy must be safe and well tolerated upon systemic administration, must have appropriate pharmacokinetic attributes to ensure delivery to disease sites, must deliver intact DNA to target tissue and mediate transfection of that tissue, It must be non-immunogenic and stable upon manufacture so that large batches can be prepared with uniform reproducible specifications (Medina-Kauwe *et al.,* 2003). In the next section we will be discussing some of the most used gene delivery vectors.

Two major systems of gene delivery have been developed, viral and non-viral.

Viral vectors are known as biological systems derived from naturally evolved viruses capable of transferring their genetic materials into the host cells and have been proofed as very effective in achieving high efficiency for both gene delivery and expression.They mediate efficient gene transfer through their favorable cell uptake and intracellular trafficking machineries.

Viruses can be transformed into gene delivery vehicles by removing part of the virus genome and replacing it with a therapeuticgene, hence eliminate their toxicity and maintain their high capacity for gene transfer (Liu, 2010).

High transduction efficiency and DNA packaging capability has been always considered as critically important advantages of viral vectors. This exclusive level of control over DNA packaging process does not usually exist for non-viral vectors which rely on an uncontrolled vector/DNA self-assembly process to package DNA into condensed particles. While viruses reproducibly package a single DNA chain at their core, non-viral vectors tend to form particles with multiple chains of DNA in each particle (Brenda, 2010) .

Retrovirus, adenovirus, herpes simplex virus and vaccinia virus are the most commonly used types in gene therapy (Young *et al.,* 2006). Various factors has been reported to control the choice of the virus which used as a gene therapy carrier these factors include; thetarget cells, disease conditions, and for successful expression, sufficient amounts of the gene should be delivered without producing any substantial toxicity (Kay *et al.,* 2001). Different viruses have also been reported to represent differential activity based on cell division, Some viruses can infect both dividing and non-dividing cells (e.g., neurons), whereas others are effective only in dividing cells.

For decades viral vectors have been considered as the most effective, easiest and highly successful methods for transfer of a gene of interest into the cells (Tomanin and Scarpa, 2004). Interestingly severe fatal adverse events including acute immune response and insertion mutagenesis have occurred during gene therapy clinical trials raising serious safety concerns about the use of viral vectors (Thomas, 2003 and Woods *et al.,* 2006). Moreover, the viral vector have limited size capacity, cause weakness of cell targeting, and have high cost which limited their clinical application and boosted efforts to search for non-viral options (Mansouri *et al.,* 2004).

Due to the peeved problems encountered with the viral vectors the research focus has shifted towards the development of safer non-viral gene delivery vectors. In most cases, these vectors are relatively simple to synthesize and are to a great extent devoid of the health risks that are associated with their viral counterparts. The advantages associated with these kinds of vectors include theuse of synthetic or highly purified components,their large-scale manufacture, their low immunogenic response, well defined physicochemicalproperties with high degree of molecular diversity, versatility formanipulating their physicochemical properties, and the capacity to carry large inserts. However, non-viral vectors for clinicalapplications, has suffered from problems including toxicity, low gene transfer efficiency, and instability (Al-Dosariand Gao, 2009, Mao *et al.,* 2010,Tros de Ilarduya *et al.,* 2010).

The non-viral transfection systems are mainly of a cationic nature; bind electrostatically to DNA and condensing the genetic material into nanometer-scale complexes (a few tens to several hundred nanometers in diameter) whichprotectthe genes and allow them to enter cells.Theyareusually composed of cationic peptides, cationic polymers,cationic lipids, or combination of some of them (Tokunaga *et al.,* 2004; del Pozo-Rodriguez *et al.,* 2009). Because of their permanent cationic charge, these non viral victors interact with negatively charged DNA through electrostatic interactions leading to polyplexes or lipoplexes.

Cationic polymers condense DNA into compact structures by electrostatic interactions between anionic phosphate groups of nucleic acids and cationic groups of polycations under physiologic conditions(at physiological pH). The resulting cationic polymer/DNA complexes, or polyplexes, capable of gene transfer into the targeted cells (Zhanga *et al.,* 2003).Cationic polymer are completely soluble in water (Elouahabi and Ruysschaert, 2005) they have the obvious advantage of compressing DNA molecules to a relatively small size, which can be crucial for gene transfer, as small particle size may be favorable for improving transfection efficiency (Gershon *et al.,* 1993; Ruponen *et al.,* 1999). These polyplexes condensed DNA can be taken up by cells through adsorptive endocytosis due to their positive charged character; leading to considerable expression of exogenous genes (Persiani and Shen, 1989; Weiss *et al.,* 2006). Modifications to these polymers such as molecular weight, geometry (linear vs. branched) and the possibility of covalent binding of targeting moieties for gene expression mediated through specific receptors (Gao and Huang, 1996, Somia and Verma, 2000, Liu and Yao, 2002) opens the way to extensive structure/function relationship studies.

The efficacy of cationic polymers for gene delivery is considered less than ideal, because of the low transfection efficiency and undesirable toxicity.

Various modifications have been explored to improve the gene delivery efficacy (Park *et al.,* 2006; Jeong *et al.,* 2007). Among these modifications, the conjugation of hydrophobic segments to the polycations which displayed promising results. The hydrophobic moieties could enhance the complex–plasma membrane interactions, and facilitate the endocytosis as the polyplexes must traverse through a hydrophobic lipid-based plasma membrane (Alshamsan *et al.,* 2009). Hydrophobic interactions play an important role in the gene delivery process, and the introduction of hydrophobic chains can affect not only the interaction with the plasma membrane, but interactions at most steps during the whole gene delivery process (Liua *et al.,* 2010). The addition of polycations, i.e. polyl-lysine and protamine, as co-polymer is reported to markedlyenhance the transfection efficiency of several types ofcationic liposome by 2–28-fold in a number of celllines *in vitro* (Gao and Huang, 1996) and *in vivo* (Li and Huang, 1997).

The major problems with these polymers are their toxicity due to cationic charges and nonbiodegradable properties, or immunogenicity as polymers based on amino acids such as polyl-lysines (Vanderkerken *et al.,* 2000; Gao *et al.,* 2009;).

The toxicity of cationic polymers is a result of the quantity of polymer required to achieve the optimal +/− charge ratio for the polyplex. In many instances, such as with polyethylenimine, the charge ratio that provides optimal efficiency is very near that which induces severe toxicity. Also the high ionic strength of many cationic polymers that condense DNA cannot withstand the stringent conditions of delivery, therefore it is essential to use polymers that bind tightly, but reversibly, to the DNA (Chollet *et al.,* 2004). Polymer length/molecular weight has also been found to influence the toxicity of the polyplexes (Ahn, 2004; Kramer, 2004).

Common polycations include polylysine, polyamines such as polyethylenimine, histone proteins, polyarginine and cationic dendrimers.

Polyethylenimine (PEI), is a cationic polyamine first introduced by Boussif *et al.*(1999) and shown to have one of the highest transfection efficiencies of all cationic polymers based on favorable characteristics of DNA protection, cell binding and uptake, ability to act as a “proton sponge”, which promotes release from endosomal compartments and release from the carrier (Zhang *et al.,* 2004).

Polyethyleneimine (PEI), formed either as a linear or branched polymer and the transfection efficiency is influenced by its geometry (Godbey *et al.,* 1999). Linear PEI (22 kDa) has a higher transfection efficiency than branched PEI of a similar MW, and has increased efficacy in non-dividing cells (Wightman *et al.,* 2001; Wiseman *et al.,* 2003; Itaka *et al.,* 2004). However, the transfection efficiency of branched PEI can be increased at low MWby decreasing the degree of branching Figure 1(Fischer *et al.,* 1999). The transfection efficiency of polyplexes formed with both PEI and PLL increases with increasingmolecular weight (MW), but high MW also increases toxicity, limiting effectiveness both in vitro and in vivo (Godbey *et al.,* 1999).

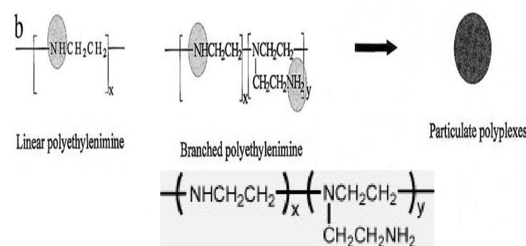

Figure 1: Polyethylenimine structure

Poly L-lysine (PLL):8

PLL polymers are one of the first cationic polymers employed for gene transfer (Wu and Wu, 1997). The poly(l-lysine) is synthesized by polymerization of the N-carboxy-anhydride of lysine, fractionated andcharacterized in terms of the average degree ofpolymerization and average molecular weight. As a linear polypeptides with repeating lysine unit; they possess a biodegradable nature which is very useful for *in vivo* applications.However,when PLL polyplexes entered into the circulatory system, they were rapidly bound to plasma proteins and cleared from the circulation, which cause lower transfection efficiency (Ward *et al.,* 2001).The inadequate escape of the complexes from endosomes or the inefficient release of DNA from the complexes results also in low transfection.

PLL has poor transfection ability when applied alone or without modifications (Pouton *et al.,* 1998; Brown *et al.,* 2000). One popular modification that can increase both the transfection ability and the circulation half-life of these vectors is coating with PEG. Another efficient strategy involves the addition of both PEG and a targeting ligand to PLL to optimize transfection (Brown *et al.,* 2000). Interducinghistidine residues to the backbone of PLL was found to improve the endosome escape property (Midoux and Monsigny,1999).Conjugating PLL with chitosan or lipids such as palmitic, myristicand stearic acids have also been reported to be able to increase the transfection efficiency of PLL (Yu *et al.,* 2007; Abbasi *et al.,* 2008).

Cationic polysaccharides are considered to be the most attractivecandidates for gene delivery. They are natural, non-toxic, biocompatible, andbiodegradable

materials and can be modified easily to improve physicochemical properties (Schepetkin & Quinn, 2006; Li, Ma, & Liu, 2007; Dergunova *et al.,* 2009). The two typical classes are chitosan derivatives and cationic polymers based on dextran-spermine (D-SPM).

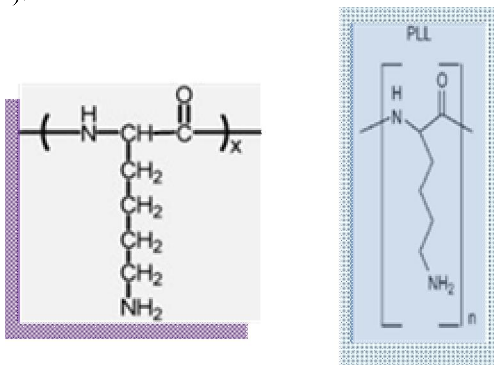

Figure 2: Poly L-lysine Structure.

Chitosan is a linear amino-polysaccharide composed of 1-4 linked N-acetyl-D-glucosamine and D-glucosamine subunits (Fig. 2), obtained by deacetylation of chitin (a polysaccharide found in the exoskeleton of crustaceans and insects (Romoren *et al.,* 2002; Hejazi *et al.,*2003). Mumper *et al.,*1995 was the first to propose the using of chitosan as a vector. The cationic polyelectrolyte nature of chitosan provides a strong electrostatic interaction with negatively charged DNA forming stable, small (20–500 nm) particles, depending on the molecularweightand the degree of deacetylation, Fang et al., 2001 and protects the DNA from nuclease degradation (Cui and Mumper, 2001).

Chitosan is potentially safe and non-toxic; biodegradable and biocompatible both in experimental animals (Rao and Sharma, 1997) and humans (Aspden *et al.,*1997). Chitosancan be degraded into H2O and CO2 in the body, which ensures its biosafety. It is biocompatibil to the human body and does not elicit stimulation of the mucosa and the derma,Themucoadhesive property of chitosan potentially permits a sustained interaction between the macromolecule being “delivered” and the membrane epithelia,promoting more efficient uptake (Takeuchi *et al.,*1996). It has also the ability to open intercellular tight junctions and facilitatingits transport into the cells (Illum, 2001).

Chitosan/DNA complexes appeared to have ability to overcome three major obstacles for transfection, i.e. cell uptake, endosomal release and nuclear localization (Ishii *et al.,* 2001).

The role of chitosan charge density is well established. It is accepted that the high charge density of chitosan at pHs below its pKa results beneficial for polyplex preparation, and also that its low charge density at pH 7.4 contributes to a low polyplex cytotoxicity and facilitates the intracellular release of DNA from the complex after its endocytotic cellular uptake (Strand *et al.,* 2010). By contrast, the role of chitosan valence on transfection efficiency is contradictory, several studies promote the use of high molecular weight chitosans (Huang *et al.,* 2005), some other publications report that lower molecularweightchitosans are superior for gene transfer (Koping-Hoggard *et al.,* 2003; Lavertu *et al.,*2006).

However, chitosan shows two major disadvantages: one is poor solubility because the amino groups on chitosan are only partially protonized at physiological pH 7.4. The other is low transfection efficiency. Many techniques have been tried to overcome thesedisadvantages including quaternization of NH2 groups (Thanou *et al.,*2002), linking or conjugating cell-specific ligands as PEG, deoxycholic acid or urocanic acid (Park *et al.,* 2001; Kim *et al.,*2001; Kim *et al.,*2003; Kimet *et al.,*2004) to the polysaccharide backbone through NH2 groups or hydroxyl groups.

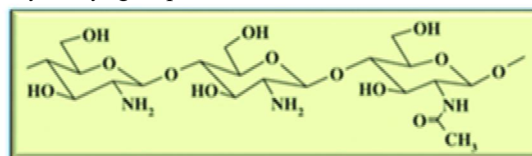

Figure 3:Chemical Structure of chitosan

Dendrimers are synthetic macromolecules with a tree-like structure firstintroduced by Tomalia *et al.* (1985) and Newkome *et al*., 1985. They are spherical, highly branched polymershaving a hierarchical, three-dimensional structure. Dendrimersposses three distinguishable architectural components; an interior core, a central point from which monomers will ramify, interior layer (generations)) composed ofrepeating units radially attached to the interior core, and exterior(terminal functionality) attached to outermost interior generation. The higher generation dendrimers, due to their globularstructure, occupy a smaller hydrodynamic volume compared tothe corresponding linear polymers (Jain and Gupta, 2008). The currently used dendrimers are polyamines, polyamides or polyesters, but the most commonly encountered is polyamidoamine (PAMAM) because of its high transfection efficiency.

Dendrimers bear primary amine groups on their surface and tertiary amine groups inside. The primary amine groups participate in DNA binding, compact it into nanoscale particles and promote its cellular uptake, while the buried tertiary amino groups act as a proto-sponge inendosomes and enhance the release of DNA into the cytoplasm. The most important advantages of dendrimers are conjugation of a large

number of different molecules on the dendrimer surface (Ward and Baker, 2008).

Thesize and diameter of dendrimers have an influence on their transfectionefficiency. The transfection efficiency obtained with high generation dendrimers is clearly superior to low generation dendrimers (Kukowska-Latallo *et al.*, 1996). Partially degraded PAMAM dendrimers are reported to have more flexible structures than intact dendrimers and therefore to interact more efficiently with DNA (Tang *et al.*, 1996). A fragmentation step consisting of hydrolytic cleavage of the amine bonds is needed to enhance the transfection efficiency (Tang *et al.*, 1996; Tang and Szoka, 1997; Dennig and Duncan, 2002; Ding *et al.*, 2002).

The toxicity of dendrimersisthe major concern for their medical use.Generally, in vivo dendrimerstoxicity isrelated to various factors including the chemical structure, surface charge, generation and dose of dendrimers (Aillobn *et al.*, 2009). Surface modification with PEG or replacement with low generationdendrimers have been reported to be able to improve the biocompatibilityof these biomaterials (Jevprasesphant *et al.,* 2003; Russ *et al.*, 2008).

Polyamidoamine (PAMAM) dendrimers are a form of symmetric hyper branched polymer applied to polyplex formation (Haensler and Szoka, 2003; Fu *et al.*, 2007). The size and charge of PAMAM is dependent on the generation created. Generation six has been reported as the most efficient at transfection (Felgner *et al.*, 1987). PAMAM has relatively low toxicity to cells when compared to other compounds as PLL (Felgner *et al.*, 1987). No cytotoxicty effects was reported when PAMAM polyplexes where used to transfect cells on a PLGA film. PAMAM dendrimer can be manufactured in a partially degraded form and are reported to be 50-fold more efficient at gene transfer than normal non-degraded PAMAM (Felgner *et al.*, 1987).

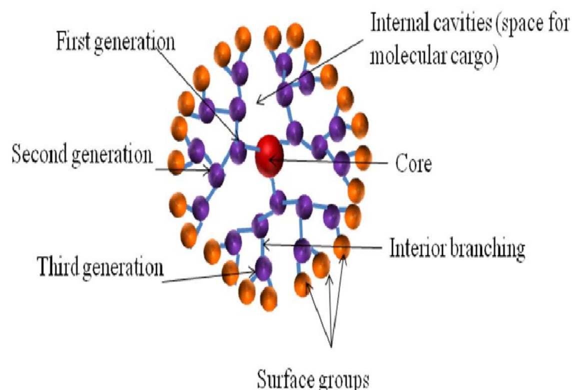

Figure 4: General structure of dendrimers

The first non-viral vectors as gene carriers was the use of lipid - based vectors byFelgner et al. in the late 1980s and since then these vesicles have been considered one of the most promising methods for non - viral gene delivery. Cationic liposome-DNA complexes (lipoplexes) form spontaneously because of electrostatic interactions between thepositively charged liposomes and the negatively charged DNA, producing physically stableformulations suitable to transfect relatively high amounts of plasmid DNA to cells in culture (Felgner *et al.*, 1987).

Cationic lipids used for gene therapy are composed of three basic domains: a positive charged head group; for the binding of nucleic acid phosphate groups, a hydrophobic chain, and a linker which joins the polar and non-polar regions.

The polar and hydrophobic domains of cationic lipids can effect both transfection and toxicity levels. (Gascn and Pedraz, 2008).The most common types of chain lengths are C8:0 to C18:1 as shown figure 5. The use of mono-unsaturated fatty acid chains have resulted in higher transfection, possibly because of theirinfluence on enhancing membrane fluidity (Floch *et al.*, 2000).

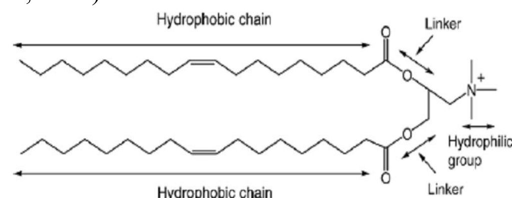

Figure 5: Schematic representation of DOTAP, a commonly used cationic lipid forgene delivery.

The size and charge of the cationic headgroup are generally more important for transfection efficiency than those of the alkyl chains (Horobin and Weissig, 2005). The different types of headgroups can be categorized as; quaternary ammonium, primary, secondary or tertiary amines, guanidinium, heterocyclics, amino acids and peptides. The head group of cationic lipids is generally associated with amine groups with different degrees of substitution, but others like amidine, guanidinium or pyridinium groups havebeen also reported (Vigneron *et al.*, 1996; Zhang *et al.*, 1997; Elouahabi *et al.*, 1997; van der Woude *et al.,* 1997).

Many varieties of synthetic cationic lipids have been developed and are commercially available. They can be classified into various subgroups according to their basic structural characteristics; monovalent aliphatic lipids characterized by a single amine function in their head group, including DOTAP and DC- Chol, a multivalent headgroup whose polar head groups contain several amine functions such as the

spermine group, e.g. dioctadecylamidoglycylspermine (DOGS) and cationic cholesterol derivatives, e.gbis-guanidium-tren-cholesterol (BGTC).

Four main conformations of lipoplexeshas been reported; a multilamellarstructure(Fig.6), with DNA monolayers sandwiched between cationicmembranes, (Lasic *et al.,* 1997; Radler *et al.,* 1997; Salditt *et al.,* 1997; Battersby *et al.,* 1998; Dias *et al.,* 2002), an inverted hexagonalstructure (the inverted “honeycomb” phase) with DNA encapsulated within cationic lipidmonolayer tubes (Koltover *et al.,* 1998), The ‘‘beads on a string’’ model where positively charged vesicles attach to the extended DNA molecule (Felgner *et al.,* 1987; Gershon *et al.,* 1993; Ruozi *et al.,* 2007; Sternberg *et al.,* 1994), and a final one where DNA is collapse and attach in the form of a globule into the outer surface of positively charged vesicles (Miguel *et al.,* 2003).

Some studies have suggested that there is direct correlation between structure and transfection efficiency. Zuhorn *et al.* (2005) have shown that formation of the hexagonal phase in lipoplexes following interaction with anionic lipids is important for the translocation of nucleic acids across endosomes into the cytoplasm. However Caracciolo *et al.* (2003) have demonstrated thatDOTAP/DOPE/cholesterol liposomes with a multilayer structure facilitate a more than 4-fold increase in transgene expression in OVCAR-3 and SK-OV-3 cells, compared with DC-Chol/DOPE liposomes (3:2 molar ratio), that are known to self-assemble into a columnar inverted hexagonal phase (HII). By contrast, DC-Chol/DOPE complexes transfect human tracheal epithelial cells more efficiently than other cationic lipid–DNA complexes (Colosimo *et al.,* 1999). It should be noted that the interaction of the lipoplexes with cellular membrane lipids may result in a different structural organization than the original structure. Thus, lipoplex-mediated gene transfer depends not only on the formulations of the cationic liposome and their original structure, but also on their interaction with cells and the resulting structures.

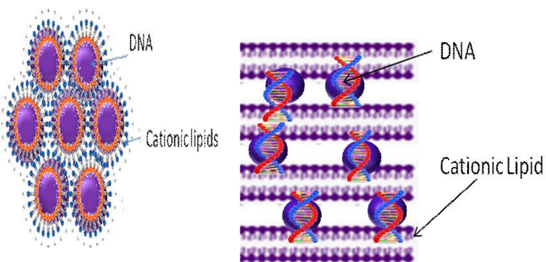

Figure 6: Schematic representation of lamellar or inverted hexagonal phase structure in the formation of lipid/DNA complex (lipoplex).

lipoplex protects the DNA from degradation (Uyechi-O' *et al.,* 2003), facilitate penetration via the negatively-charged cell membrane as they can fuse with the plasma membranes of cells, facilitate the transfer of complexes into the cytoplasm via endocytosis (Zelphati and Szoka, 1996; Xu and Szoka, 1996), destabilize the endosomal membrane and they facilitate the release of plasmid DNA into the cytoplasm, thus bypass the lysosomal degradation pathway (Patil *et al.,* 2005, Gardlik *et al.,* 2005). They are biodegradable and do not elicit cellular immune response but are less efficient than viral vectors, exhibit significant variability in transfection efficiency, usually quickly silenced and have limited use in vivo due to their inherent toxicity (Conwell and Huang, 2005).

The toxicity of lipoplexes has been a major limitation for their use *in vivo* gene delivery systems. The excessive positive charges, facilitating the electrostatic interactions with negatively charged DNA, also promote cytotoxicity. The toxicity of cationic lipids is mainly determined by theircationic nature, which is determined by the structure of its hydrophilic group. It is closely associated with the charge ratio between the cationic lipid species and the nucleic acids, as well as the dose of lipoplexes administered (Conwell and Huang, 2005; Dass, 2002). Higher charge ratios are generally more toxic to a variety of cell types,The quaternary amine headgroup is more toxicthan tertiary one. The import of a heterocyclic ring as thesubstitution of the liner amine headgroup, such as pyridiniumand guanidine, can spread the positive charge of the cationic head, and significantly decreased the toxicity.

Neutral lipids are often a component for cationic liposome formulations in which they play an assistant role. Three neutral lipids often incorporated into formulations are dioleoylphosphatidyl-ethanolamine (DOPE), dioleoylphosphatidylcholine (DOPC) and cholesterol. The usage of neutral lipids allowsto decrease toxicity and attain higher transfection levels *in vivo* (Hong *et al.,* 1997; Lasic, 1997). For instance, DOPE can facilitate membrane fusion and aid the destabilization of the plasma lemma or endosome.

Most of the linker bonds in the synthesizedlipids are ether, ester or amide bond. Although compounds with ether linker render better transfection efficiency, they are toostable to be biodegraded thus cause toxicity. Cationic lipids withester bonds such as DOTAP in the linker zone are morebiodegradable and associated with less cytotoxicity in culturedcells (Freedland *et al.,* 1996; Leventis and Silvius, 1990), but those with ester or amide linkers are liable todecompose in the circulation.

Although lipid - based vectors do not trigger a cellular immune response, however these vectors may

be recognized as foreign and initiate the production of cytokines such as tumor necrosis factor‑α (TNF‑α), interferon‑γ (INF‑γ), interleukin‑6 (IL‑6), and IL‑12. It havebeen shown that the sequential injection of liposome and plasmid DNA can significantly reduce the inflammatory response induced by systemic gene delivery (Tan *et al.,* 2001). Liu *et al.* (2002b) described the preparation of a non-immunostimulatory lipid‑based vector which contains lipid, DNA and an inflammatory suppressor molecule that specifically inhibits the production of the cytokine, NF‑κB. Thus, the addition of an immune suppressor molecule within the lipoplex can significantly reduce the toxicity associated with lipid‑based non‑viral vectors.

For development of more effective and safer lipoplex systems useful in vivo transfection significant advances have been made. Lipoplexes have been modified to contain ligands that are recognized by the target cells (e.g., folate and transferrin) (Dauty 2002 and Zuber 2003). In addition short chain fragments of antibodies as transferrin antibodies have also been used to target tumor cells (Xu *et al.,* 2002). Both systems showed an increased affinity for binding tumor tissues in vitro and in vivo (Dauty *et al.,* 2002, Xu *et al.,* 2002 and Zuber *et al.,* 2003). The attachment of polyethylene glycol (PEG) to the vectors prior to delivery (i.e., PEGylation) promote the stabilization in the bloodstream and enhance the binding of lipoplexes to the target cells as compared to unmodified lipoplexes (Zuber *et al.,* 2003; Yu *et al.,* 2004).

To achieve successful transgene expression, a series of extracellular and intracellular transport barriers as DNA protection, internalization, intracellular trafficking and nuclear transport need to be overcome by delivery vectors (Fig 7). Viral vectors have already showed their great success in addressing each challenge. Non-viral vectors, however lack one or several of the necessary functions. Understanding the barriers encountered by delivery vectors is a prerequisite to design more efficient carriers for gene therapy.

Complexation of nucleic acids with non-viral vectors aims to neutralize the negatively charged phosphate backbone of DNA to prevent charge repulsion against the anionic cell surface, reduce their sizes to appropriate scales for cellular internalization (i.e., nanometersfor receptor-mediated endocytosis; micrometers for macropinocytosis or phagocytosis); and toprotect the DNA from pH and both extracellar and intracellular nuclease degradation (Abdelhady *et al.,* 2003; Lechardeur *et al.,* 1999; Schaffer and Lauffenburger, 1998) Three main packaging methods are reported: electrostatic interaction, encapsulation, and adsorption (Wong *et al.,* 2007).

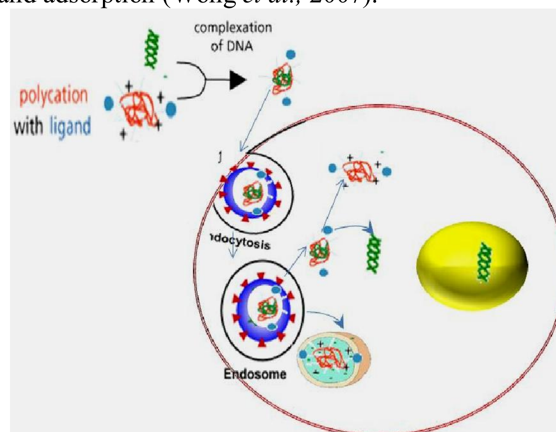

Fig. 7. Barriers to gene delivery: (I) package therapeutic genes; (II) Bindingto plasma membrane and entry into the cell (III) escape the endo-lysosomal pathway; (IV) effective DNA/vector release; (V) traffic through the cytoplasm and intothe nucleus; (VI) enable gene expression; and (VII) remain biocompatible

Complexation of DNA mediated by electrostatic interactions between the negatively charged phosphate backbone of DNA and cationic molecules leads to charge neutralization and a compaction of the nucleotide fragment. Cationic polymers typically interact with DNA in anstronger manner which leads to formation of complexes containing multiple DNA molecules. It has been shown that the size of the complex formed varies significantly depending on the type of cationic structure used and preparation conditions including concentration of DNA, pH, type of buffer, and N/P ratio (Meredith *et al.,* 2009). Additionally, the size of polymer-DNA complexes has been correlated with the molecular weight of the polymer e.g. high molecular weight polylysine (224 kDa) form DNA complexes with diameters ranging from 100 to 300 nm, while low molecular weight polylysine (□4 kDa) form complexes with diameters between 20-30 nm (Wolfert and Seymour, 1996). The morphology of DNA complexes formed with cationic polymers is independent of the polymer used. For example, complexes derived from DNA and polylysine, polyethylenimine, or various dendrimers form toroidal structures of similar diameters (Tang and Szoka, 1997).

In systemic non-viral gene delivery, the complex usually has to be injected into the blood stream and enter into the circulation before reaching the cells of interest. Complexation of nucleic acids with non-viral vectorsimparts a positive charge which is important for both cellular-binding and internalizationhowever it cause instability of the cationic complex as positively

charged complexesinteract withnegatively charged blood components (e.g., serum proteinsas serum albumin, lipoproteins ;HDL and LDL)promoting their aggregation and blood clearance (Tros de Ilarduya *et al.,* 2010). Additionally, the physiological salt concentration of serum (150 mM) often promotes aggregation ofthe cationic complexes, which potentially could lead to vascularblockage (Ogris *et al.,* 1999). Cationicpolyplexes canalsoactivate the complement system,becomerecognizedand cleared by the reticuloendothelial system (Zhang *et al.,* 2005, (Muzykantov and Torchilin, 2003). The induction of any one of these events can lead to premature elimination of the polyplex preventing delivery of the genetic cargo to its final destination.

The *in-vivo* gene delivery can be improved by reducing salt/serum effects. The incorporation of a hydrophilic moieties particularly poly(ethylene glycol) (PEG) into the vector creating a hydrophilic shell that effectively increase the solubility, masks the cationic charges, reduces aggregation, and minimizes interactions with serum proteins and other serum components resulting in prolonged circulation time (Medina-Kauwe *et al.,* 2005).

Internalization of the cationic carriers, from the exterior of the cell through plasma membrane is considered the most critical limiting step for an efficient DNA transfection. They pass through various active cell uptake mechanisms such as endocytosis, pinocytosis, or phagocytosis (El Ouahabi *et al.,* 1999). Transfection of nonviral DNA complexes based on whether or not the complex is conjugated to targeting ligands.

Studies of electron and fluorescence microscopy have shown that lipoplexes and polyplexes can be detected in intracellular vesicles beneath the cell membrane, suggesting that they enter cells by endocytosis and will thus be directed toward the endolysosomal compartment (Merdan *et al.,* 2002; Zhou *et al.,* 1994; Mislick *et al.,* 1996).

The predominant way of entry of cationic gene delivery systems seems to be by non-specific adsorptive endocytosis followed by the clathrin-coated pit mechanism, because negatively charged glycoproteins, proteoglycans and glycerophosphates, present on the cell membrane, are able to interact with the positively charged systems (Mounkes *et al.,* 1998; Rejman *et al.,* 2004). Using specific inhibitors of different endocytosis pathways, Rejman et al., 2004 conclude that lipoplex (DOTAP/DNA) uptake can be proceeded only by clathrin-mediated. endocytosis,whilepolyplexes (PEI/DNA) can be taken up by two mechanisms, one involving caveolae and the other clathrin-coated pits. However, the internalization pathway seems to be dependent on the system used and the cells to transfect (Zuhorn *et al.,* 2002; Simoes *et al.,* 2000; Prabha *et al.,* 2002). It has been shown that the size of the complex affects cellular uptake in various cell lines. These suggest that optimal size for gene transfer of non-targeting cationic vector-DNA complexes is between 70 and 90 nm (Xu *et al.,* 2007; Xu *et al.,* 2007; Schatzlein *et al.,* 2003).

Numerous polyplexes have been designed to gain cellular entry via receptor-mediated endocytosis. The ability to bind a particular cell type with highspecificity is especially significant in a systemic deliveryin which appropriate biodistribution and tissue targeting are essential (Wong *et al.,* 2007). Vector systems attached with highly specific cell ligands that bind the cell-surface receptors present in the target cells but absent in any other cell, promote delivery to specificcells and tissues, as in most diseased conditions the therapeutic genes must be delivered to a certain cell type.Thesetargating ligands include endogenous ligands as transferrin and RGD peptide, carbohydrates as galactose, mannose, lactose, antibodies as anti CD3 and antiEGF, cell penetrating peptide as HIV Tat and polyarginine sequences, biomolecules, and antibodies (Gao *et al.,* 2005; Mellman, 2007).

Carrier systems containing a specific targeting moiety could enter cells via both adsorptive endocytosis and receptor-mediated endocytosis (Akinc *et al.,* 2005). The optimal size for targeting cationic vector-DNA complexes found to be between54-60 nm (Akinc *et al.,* 2005).

Upon being taken up via endocytosis, macromolecules captured within the endosomes usually transform into digestive lysosomes unless some escape mechanisms are used to intercept this maturation process.

A \`proton-spong\` mechanism has been attributed to the endosomal escape of polyplexes based on cationic polymers with amino groups for gene delivary. Weak amine compounds such as chloroquine and cationic polymers polyethyleneimines and partially degraded polyamidoaminedendrimers) absorb protons and slow down the acidification process that is essential for endosome–lysosome transition (Xu *et al.,* 1996). Consequently, the influx of chloride counter ions builds up osmotic pressure inside the endosomes causing osmotic swelling and eventual endosomolysis and release of its contents into the cytosol.

In cationic lipid-based vectors, for local endosomal membrane destabilization, the electrostatic interactions between the cationic lipids and the endosomal membrane induce the displacement of anionic lipids from the cytoplasm-facing monolayer of the endosomal membrane, by way called flip-flop mechanism. The formation of a neutral ion pair between anionic lipids present in the endosomal

membrane and the cationic lipids of the vector will thencause subsequent decomplexation of the DNA and finally its release into the cytoplasm (Hafez *et al.,* 2001). Additionally, non-cationic helper lipids such as neutral DOPE facilitate membrane fusion and help destabilize the endosomal membrane (Ellens *et al.,* 1986; Gao and Huang, 1995; Li *et al.,* 2004).

For polyester-based carriers such as poly(lactic-co-glycolic acid), the breakdown products by hydrolysis can also build up the osmotic pressure inside the endosome which leads to the release of the contents trapped therein. Several attempts have been used to increase the rate of endosome release. The incorporation of membrane-destabilizing peptides, such as synthetic N-terminal peptides of Rhinovirus VP-1 or influenza virus HA-2, into the cationic complex can mediate endosomal release. Under acidic conditions, these peptides arrange to form an amphipathic α-helical structure that induce membrane interaction and disruption leading to subsequent release of the endosomal contents into the cytosol (Curiel *et al.,* 1991). The co-delivery of inactivated viral particles or recombinant viralcapsule proteins that possess endosomolyticactivities (Kloeckner *et al.,* 2004), and the use of photochemically generated free radicals to cause membrane damage (Dauty *et al.,* 2005) were also reported.

Once DNA molecules in their free form or as DNA/ carrier vector complexesreleased into the cytoplasm, they must traffic through the cytoplasm and enter the nucleuswhere transcription takes place. Diffusion of large molecules is limited in the cytoplasm because of the presence of the highly dense cytoskeleton. The mobility of large molecules, such as pDNA, is extremely low in the cytoplasm, making them an easy target for cytoplasmic nucleases (Lukacs *et al.,* 2000).The mobility of DNA/ carrier vector complexes depends on the size and spherical structure of the molecule (circular plasmid DNA >linear DNA D) (Mao *et al.,* 2010). Small DNA fragments <250 bp with an extended linear length of approximately 85nm able to diffuse widely in the cytoplasm, whilst plasmid DNA >2 kb is unable to diffuse freely from the site of injection (Lukacs *et al.,* 2000; Shimizu *et al.,* 2005).

Large molecules are transported along microtubule (MT) components of the cytoskeleton towards the nucleus. MTs are long, hollow cylinders made of tubulin that extend from the vicinity of the plasma membrane to the MT organizing centre (MTOC), a structure that is typically in close proximity to the nucleus (Cavistonand and Holzbaur, 2006). Most MT transport towards the MTOC is catalyzed by the molecular motor dynein, whereas molecules may be moved away from the MTOC along MTs by the motor kinesin.

Non-viral vectors can be trafficked in MT-dependent fashion, although the mechanism(s) are poorly understood.The primary mode of transport appears to be mediated by the natural endocytic mechanism of the cell (Soldati and Schliwa, 2006). Whilst still within endosomes, both liposome and PEI vector/DNA complexes appear to be rapidly transported through the cell along MTs dependent on the action of dynein (Hasegawa *et al.,* 2001; Suh *et al.,* 2003; Kulkarni *et al.,* 2005). Although it has been reported that the endosomal trafficking of PEI/DNA complexes is directed towards the perinuclear region (Suh *et al.,* 2003), over a longer time course, the movement appears to be a back-and-forth motion about their starting positions (Kulkarni *et al.,* 2005, 2006), most likely as a result of switching between dyneinorkinesin directed transport (Kural *et al.,* 2005); this ultimately ends when the complex exits the endosome (Lakadamyali *et al.,* 2003).

Naked plasmid DNA in the cytoplasm may be trafficked along MTs through the action of adaptor proteins that bind both the DNA and dynein (Vaughan and Dean 2006). The identity of the adaptor proteins and mechanism whereby DNA is linked to dynein is unknown, as is whether this MT trafficking is specific to certain DNA sequences. It is known that viruses use specific protein sequences for interaction with dynein to promotetransport to the nucleus (Dohner *et al.,* 2005; Radtke *et al.,* 2006). The attachment of dynein-association sequences directly to non-viral vectors or DNA can enhance cytoplasmic transport to the perinuclear region.

In order to minimize residence time within the cytosol and promote transport toward and into the nucleus, researchers have recruited natural endogenous cytosolic factors to facilitate the shuttling of either polyplexes or DNA itself.

The nuclear envelope (NE) represents the final barrier for the entry of DNA in order to permit transcription of the therapeutic gene. Nuclear import of pDNA may be more challenging for transfection of non-dividing cells. Non-dividing cells showed a 90% lower expression level compared to actively dividing cells (Fasbender *et al.,* 1997). During cell division, the NE is temporarily disassembled and DNA-vector complexes can be sequestered within the daughter cell nuclei (Vaughan and Dean, 2006).This is the basis of the ease with which conventional non-viral vectors transfect rapidly dividing immortalized cell lines, but show only poor transfection rates in non-dividing cells (Fasbender *et al.,* 1997).

This double-membrane nuclear envelope is interrupted by large protein structures called nuclear pore complexes (NPC) which regulate transport through nuclear envelope. Proteins less than 40 kDa in MW or 9 nm in diameter or nucleic acids of up

to□300 bp,can passively diffuse through NPC channels, but larger macromolecules cannot pass through freely (Bastos *et al.,* 1995). Proteins greater in size are trafficked into the nucleus in an ATP-dependent process triggered by reorganization of short peptide sequences known as the nuclear localization sequence(NLS). The NLS is a major player that shuttles protein–plasmid complexes through the nuclear pore (Cartier and Reszka, 2002). Cytosolic proteins destined for the nucleus contain NLS, that are recognized by import proteins which direct their subsequent transport into the nucleus.

Identification of the NLSs, such as SV40 from the larger tumor antigen Simian virus 40 and M9 from nuclear ribonucleoprotein, enabled design of first generation of nuclear targeting non-viral vectors (Cartier and Reszka, 2002). Over the past few years, a wide range of potential NLS sequences,which may be applied to delivery systems were identified (Jans *et al.,* 1998).

NLSs as a short, cationic peptide sequences, can directly interact and condense DNA without the need for a polycation condensing agent (Kichler *et al.,* 2000) or can be attached to a polymer vector that is subsequently complexed with its DNA cargo (Moffatt *et al.,* 2006; Talsma *et al.,* 2006).

The ability of NLSs to enhance cytosolic trafficking and nuclear uptake may be limited by the size and type of DNA used (i.e., linear, plasmid), the method of NLS incorporation (i.e., covalent conjugation to DNA, electrostatic complexation with DNA, or conjugation to a polymer vector), type of NLS peptide employed, the number of NLSs incorporated, and the type of polymer vector used (e.g., liposomes, PEI) (Bergen *et al.,* 2005). Covalent conjugations may reduce transcription activity of pDNA because of the chemical modifications. To overcome such problem, a peptide nucleic acid (PNA) has been proposed as a bi-functional linker to tether NLS to pDNA. The SV40 NLS-PNA-pDNA tertiary complex can mediated nuclear import of pDNA both *in vitro* and *in vivo* without the influence on transcription activity of the nucleic acid (Branden *et al.,* 2001., 1999).

Because of the potential immune response associated with the use of an exogenous NLS, numerous efforts were made to modify plasmid DNA so that it can be recognized by cellular factors as a nuclear import substrate. Modifications include addition of specific DNA sequences recognized by transcription factors (Bremner *et al.,* 2004). A DNA targeting sequence (DTS), which is able to associate with cytoplasmic transcription factors that contain the endogenous NLS, is attached to a DNA vector for active nuclear import of DNA (Lam and Dean, 2010; Miller and Dean, 2009). The DTS leads to the formation of a tertiary complex DNA–DTS-transcription factor and nuclear import of pDNA.

The DNA dissociation from its vector is important for efficient gene expression (Honore *et al.,* 2005; Schaffer *et al.,* 2000).

It was found that the lipoplexes released The DNA during endosomal release, thus deliveringonly naked DNA to the cytosol which results in poor gene expression compared with injection of naked DNA (Sun *et al.,* 2005). In contrast the DNA isreleased from the polyplexes in the nucleus (Cheng *et al.,* 2006).

The DNA-loaded nanoparticles coupled with microtubule-directed transport mechanism was reported to be disintegrated in nucleus (Li and Huang, 2006; Aris and Villaverde, 2004). So,the gene expression can be enhanced if dissociation occurs within the nucleus to minimize the residence time of unprotected DNA within the cytosol (Pollard *et al.,* 2004). Several strategies have been developed to effect the intracellular release of DNA, by incorporating thermo responsive properties or hydrolytically degradable or reducible linkages within the polymeric vector.

The long term expression of exogenous genes is achieved through transgenic insertion into the host genome while transient short term expression results from episomal transgene, as in most cases, where DNA molecules stays in the nucleus without integration into the host genome by incorporating a transposon sequencewithin the delivery system either on the same plasmid as the transgene or on a separate plasmid with the transgenic plasmid, chromosomal insertion may be induced resulting in longer transgene expression (Belur *et al.,* 2003; Hamlet *et al.,* 2006; Huang *et al.,* 2006; Ohlfest *et al.,* 2004; Keravala *et al.,* 2006). Several transposon systems for gene therapy applications have been studied; the Sleeping Beauty (SB) transposon (Belur *et al.,* 2003; Huang *et al.,* 2006; Ohlfest *et al.,* 2004; Keravala *et al.,* 2006). Tol2 (Hamlet *et al.,* 2006) Piggybac (Ohlfest *et al.,* 2004) and Himar 1 (Keravala *et al.,* 2006; Wu *et al.,* 2006)).

The use of transposon-mediated genome integration may lead to random insertion into the host genome which can result inunwanted side effects. Sitespecific integration to the host genome have been developed with the use ofintegrase enzymes derived from bacteriophages (e.g., fC31 integrase) and engineered hybrid systems composed of a transposable element and a DNA sequence recognition element (e.g. DNA binding domain). These systems provide a certain degree of site specificity through their ability to recognize and dock the transgenic plasmid to a known genome locus after which transgene insertion occurs. To regulate

transcription of the transgene,efforts have been focused on introducing transcription-regulating promoters upstream of the therapeutic gene sequence eg Mammalian and viral promoters and stimuli-responsive promoter (Izsvak and Ivics, 2004; Al-Dosari *et al.,* 2006) .

Cationic polymers are efficient gene delivery vectors in vitro conditions, but these carriers may fail in vivo because they can not overcome the multiple barriers to successful gene transfer and frequently,only low transfection efficiencies and poor in vivo stabilities were achieved. As a consequence, very few nonviral vectors have so far reached clinical trials.

Cationic polymers can condense DNA efficiently and are more stable (Zhang *et al*., 2004), while cationic lipid-based liposomes have better biocompatibility and are quite effective for the delivery of DNA into the cytosol through endosomal pathway but the entry of the DNA into the nucleus is very inefficient (Hideyoshi *et al*., 2001). Polycation liposomes i.e. a ternary complex of cationic liposomes, cationic polymer and DNA; PCLs are the second generation of non-viral gene delivery vectors that can improve gene transfer compared to the first generation of non-viral gene delivery vectors represented by lipoplexes and polyplexes. They combined the advantages of both cationic polymers and cationic lipids and can be prepared by condensing DNA with a polycation such as poly-L-lysine or polyethylenimine and entrapping this polyplex within anionic or neutral liposomes. This method of packaging DNA would increase the DNA loading, showed a lower cytotoxicity and a higher transfection efficiency which are favorable for the in vivo application of gene delivery systems (Chen *et al*., 2007).

Garcia et al (2007) developed a lipopolyplexby combining PEI and DOTAP/Chol liposomes (lipopolyplex at three different lipid/DNA molar ratios). They found that these vectors were highly effective in protecting DNA from attack by DNAse I. Transfection activity was maximal by using a lipid/DNA molar ratio of 17/1. These complexes showed high efficiency in gene delivery of DNA to liver cancer cells.On the other hand, complexes formed with linear PEI (22 kDa) were more effective than lipopolyplexes containing branched PEI (800 or 25 kDa). Thesignificant advantages conferred by these complexesinclude small particle size to improve transfection efficiency in vivo, decreased cytotoxicity, efficient transfection of livercancer cells in the presence of 60% of serum, and stabilityof the complexes which indicated that they are much more efficient than conventional lipoplexes and polyplexes.

Chen et al (2009) synthesized a polycation liposomes (PCLs) from PEI (Mw = 800)-cholesterol (PEI 800-Cho) and dioleoylphosphatidylethanolamine (DOPE) was incorporated to increase the transfection activity of PCLs.They reported that PEI 800-Chol/DOPE liposomes significantly enhanced the growth inhibition effects of antisense oligodeoxynucleotides (ASODN) against tumor cells, suggesting that it would be a promising vector for ASODN delivery and improved antisense therapy.

In another Study, cationic liposomes. (pDNA/PEI/DOTMA lipopolyplexes) containing polyethylenimine (PEI) lipopolyplexes with N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethlylammonium chloride (DOTMA) and pDNA with different charge ratio of the complex to pDNA, was investigated for in vitro and in vivo gene delivery. After intravenous injection of the lipopolyplexes into mice, high-gene expression in the liver, spleen, and lung was observed Theselipopolyplexes showed little cytotoxicity. Thus, theseresults confirm the usefulness of PEI lipopolyplex with DOTMA and pDNAinvitroand in vivo gene delivery (Matsumoto *et al*., 2008).

Alipopolyplexes containing N-lauroylsarcosine (LS) as a hybrid vector for pulmonary gene delivery via the systemic route.Lipopolyplexes were composed of polyethylenimine (PEI), N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethlylammonium chloride (DOTMA), LS, and plasmid DNA (pDNA). The addition of LS decreased the high zeta potential and showed little aggregation with erythrocytes and low cytotoxicity. After intravenous injection of the complexes into mice, the lipopolyplexes showed extremely high transgene efficiency in the lung. These results suggest that lipopolyplexes containing LS are safe and useful gene delivery vectors with lung directivity (Kurosaki *et al*., 2009}.

Multicomponent nonviralvectors, incorporating more than one type of carrier molecule, andmore closely imitating viruses in their assembly and cell targetingproperties, have been investigated and have been shownto have enhanced transfection properties (Demeneix *et al*., 2004; Miller, 2003; Kostarelos and Miller, 2005). Several groupshave reported the use of ternary lipid-peptide-DNA lipopolyplexvectors incorporating receptor-targeting moieties (Hart *et al*., 1998; Tagawa et al., 2002) by coatingthe surface of these complexes with polymeric poly(ethyleneglycol) (PEG) units prevents aggregation, lowers residualtoxicity, and prolongs the circulation time (Lasic, and Needham, 1995; Allen, 1994; Wheeler et al.,1999).

A pegylatedimmuno-lipopolyplexes (PILP) was developed by combining PEI/DNA complexes, anionic liposomes and strands of polyethylene glycol (PEG) was incorporated on the surface of the

lipopolyplexestopromote stabilization in the bloodstream. The tips of the PEGstrands are conjugated with a targeting monoclonal antibody (MAb). The pattern of gene expression in vivo is determined by the receptor specificity of the targeting MAb (Palmer *et al*., 2003).These complexes showed high efficiency in gene delivery to liver cancer cells with no significant cytotoxicity. The significant advantages conferred by these complexes include (i) small and stable particle sizes to improve transfection efficiency, reproducibility of transfection efficacy, decreased cytotoxicity, and efficient transfection of liver cancer cells in vitro and in vivo. They concluded that the PILP are promising gene delivery systems which may be used to target the liver cancer.the strategy for developing pegylatedimmuno-lipopolyplexes is a combination of the strengths of conventional polyplexes and liposomes, as well as pegylation technology and monoclonal antibody targeting technology, which results in a more stable and efficient gene delivery system with minimal cytoxicity (Zhang *et al*., 2003).

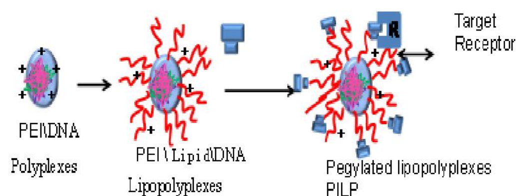

Figure 8: Polycation liposomes.

Regardless of the great improvement of the transfection efficiency for non-viral vectors, in most cases, non-viral systems still can not reach the high transfection efficiency as viral vectors, nor long-term transfection. To optimize the transfection efficiency, several other transfection methods, as 3D systems; scaffold mediated gene delivery, were developed. Scaffold mediated gene delivery enables localized and sustained DNA deliveryandprovide a long-term expressionin target sites, especially in those cases in which the transgenic expression must be prolonged and localized. DNA delivered from the scaffold is principally taken up by the surrounding cells at the implant site, therefore limiting unwanted exposure to other cells (Yurong *et al*., 2010). The degradation rate of the scaffold material can be designed so that the required release rate is achieved (Jang *et al*., 2005; Scherer *et al*., 2002). The entrapment of DNA into 3D scaffolds protects the DNA against extracellular denaturation or degradation by nucleases or proteases (Holladay et al., 2009).

Different sizes (from the macro to the nano scale) (Roy et al., 2003; Saul et al., 2007; Capan et al., 1999) and structures (films, pellets, sponges, hydrogels and spheres) (Kang *et al*., 2008; Berry *et al*., 2002)) for scaffolds used in gene delivery have been developed and investigated depending on the targeted clinical application. These variations allows the scaffold to be uniquely designed for each required purpose regarding degradation rate, pore size and mechanical stiffness (Lei and Segura, 2009).

Two different polymeric materials are utilized; natural polymeric scaffoldsas; collagen and hyaluronan and synthetic polymeric scaffoldsas; poly(lactideco- glycolide) (PLG/PLGA) and poly(l-lactic acid) (PLA).DNA can be incorporated in the polymer either in the naked or condensed state (O'Brien *et al*., 2005).The highest transgene expression and the longest sustained release in vitro and in vivo were exhibited by the scaffold with encapsulated polyplexes (Chen and Mooney, 2003; Hosseinkhani *et al*., 2008; Hosseinkhani *et al*., 2006).

The main principle in scaffold design is to mimic the natural environment; therefore natural materials are an obvious choice for scaffold fabricationto mimic the natural environment. The advantages of these materials over synthetic polymeric systems usually include, good cellular adhesion,lower toxicity during degradation and lower immune response on implantation (Hosseinkhani *et al*., 2006). However natural polymeric scaffolds have poor mechanical properties and a fast degradation rate, so the natural polymer can be crosslinked to improve their properties (Pietrzak *et al*., 2008).

Collagen and atelocollagen; a denatured form of collagen in which antigenic epitopes have been removed, are the most widely used natural material. Naked plasmid can be encapsulated by absorption from an aqueous solution onto preformed collagen sponges to form a `gene activated matrix` (GAM) capable of DNA delivery in vivo (Yu *et al*., 2006; Fang et al., 1996). Attempts to improve GAMs include the use of condensing agents to protect the DNA encapsulated into collagen scaffolds. DNA has been condensed with PLL or PEI, followed by absorption onto collagen sponges, or condensed by cross-linking PLL to collagen sponges and then adsorbing DNA (Yurong *et al*., 2010). PLL condensation increased DNA incorporation and reduced the release rate of DNA from the scaffold (Bonadio *et al*., 1999). Hybrid collagen–hyaluronic acid hydrogels, and collagen–glycosylaminoglycan scaffolds have demonstrated enhanced activity over GAMs (Ochiya *et al*., 1999; Ochiya *et al*., 2001).

Hyaluronic acid (HA) or hyaluronan which is a major component of the extracellular matrix,is used as a scaffolding biomaterial due to its low immunogenicity, biodegradability and good viscoelasticity (Malafaya *et al*., 2007; Yong-Hong *et*

al., 2005). Collagen has been mixed with HA to alter the scaffold characteristics (O'Brien *et al*., 2005). Segura et al. have examined the HA-collagen hydrogels for gene delivery applications and reported that approximately 50% of polyplexes were released from the scaffold after 48 h in conditioned media and transfection only occurred in cells in direct contact with the hydrogel. Spatial control of gene expression could play an important role where directional specificity is required (O'Brien *et al*., 2005).

In another gene delivery study, crosslinked-collagen sponges were loaded with polyplexes and tested in transfection studies, using ten different cell types (Hosseinkhani *et al*., 2008). These scaffolds were found to release 25% of the polyplexesby day 7. High levels of gene expression were recorded throughout a 3-week period which may be an indication of the cells infiltrating the scaffold and reaching the encapsulated polyplexes. This study showed that the scaffold delivery method sustain expression significantly longer than a non-scaffold method and each gene delivery system must be optimized for the specific target as some targets may be easier to transfect than others (Kim B-S *et al*., 1998).

Synthetic materials have many advantages which make them suitable candidates for gene delivery scaffolds. They can be produced in bulk with a high quality of batch reproducibility, can be fabricated with specific controlled mechanical and chemical characteristics as degradation rates, mechanical stiffness, strength and surface functional groups, which can all be precisely designed for the scaffold application. Synthetic polymeric scaffolds suffer from some drawbacks as their lack of innate cell binding sites, toxicity and immunogenicity in vivo (Silva *et al*., 2004).

Poly(lactic-co-glycolic acid) (PLGA); the biodegradable synthetic copolymer is the most investigated synthetic material used in gene delivery scaffolds. PLGA films have been tested as polyplex reservoirs (Chumakova *et al*., 2008). Approximately 10–30% of polyplexes were retained on the film after incubation in the polyplex soak. These polyplexes successfully transfected cells on the film and also detached from the scaffold and transfected cells at the bottom of the well, with no cytotoxicity effects in vitro (De Rosa *et al*., 2003). Different types of PLGA were tested, the use of a high molecular weight hydrophobic PLGA increased the encapsulation efficiency to approximately 46% and the release profiles changed too. The macro, micro and nano scale PLGA-based systems have been investigated in vivo (Silva *et al*., 2004). Capan *et al*. 1999 have reported on microspheres that had an approximate 30% encapsulation efficiency for the polyplexes. The microspheres were shown to protect the DNA in the polyplex from enzymatic degradation in vitro (Capan *et al*., 1999).

A final strategy for substrate mediated gene delivery is attempting to incorporate a blend of natural and synthetic polymers. There has been limited research performed on this design principle in relation to polyplex gene delivery. One example is collagen/polyglycolide (collagen/PGA) scaffold (Lei *et al*., 2009).

Many variables can affect the efficiency of DNA incorporation in the scaffold. Increasing the polymer MW or the use of polymers at high concentration allows for longer retention of DNA in the scaffold, and can increase the duration of release by affecting the microsphere morphology through the creation of more dense polymer networks or increasing particle wall thickness (Luo *et al*., 1999; Tinsley-Brown *et al*., 2000). Encapsulation of plasmid DNA in macroporous polymer scaffolds may provide efficient delivery and expression of the plasmid without compromising DNA bioactivity (Wang *et al*., 1999). This approach is based on the providing both a sustained release of DNA and allowing cells to migrate into the delivery vehicle which lead to multiple opportunities for transfection and a high level of expression for a controlled period of time. The mechanism by which porous polymeric scaffolds increase transfection efficiency may also relate to the presentation of a large surface area from which to deliver DNA to cells in vivo. By maintaining an available pool of DNA on a surface, without allowing for polyplex aggregation, transfection efficiency may not only be increased, but may also be sustained for longer periods of time (Jang and Shea, 2003).

The physical and mechanical properties of scaffold materials can affect the ability of cells to endocytose plasmid DNA and express the encoded genes (Genes *et al*., 2004; Kong *et al*., 2005). The effect of material stiffness on gene delivery has been investigated by monitoring the transfection efficiency of PEI–DNA polyplexes on cells adhered to hydrogels with varying elastic modulus. Increasing stiffness led to an increase in polyplex uptake, decondensation, and delivery to the cell nucleus, which was accompanied by an increase in cell proliferation (Tseng *et al*., 1999). Proliferating cells are believed to be easier to transfect due to the disruption of the nuclear membrane that occurs during cell division and enhancing cell proliferation may be broadly useful for enhancing gene transfection (Escriou *et al*., 2001)

Localizing polyplexes at the cell surface has also been shown to influence transfection efficiency. PEI–DNA polyplexes can be complexed with silica nanoparticles that concentrate polyplexes to the cell surface, without increasing the concentration of DNA

in the media, and the transfection efficiency was significantly increased (Luo *et al*., 2000).

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References

1. Abbasi M, Uludaˇg, Incani V, Yu Ming Hsu, C, Jeffery A. Further investigation of lipid-Substituted poly(l-lysine) polymers for transfection of human skin fibroblasts. Biomacromolecules 2008; 9: 1618–1630.
2. Abdelhady HG, Allen S, Davies MC, Roberts CJ, Tendler SJ, Williams PM. Direct real-time molecular scale visualization of the degradation of condensed DNA complexes exposed to DNase I. Nucleic Acids Res 2003; 31: 4001–5.
3. Al-Dosari M, Zhang G, Knapp JE, Liu D. Evaluation of viral and mammalian promoters for driving transgene expression in mouse liver. Biochem Biophys Res Commun 2006; 3392: 673–678.
4. Al-Dosari MS, Gao X. Nonviral gene delivery: principle, limitations, and recent progress. AAPS J 2009; 11: 671–681.
5. Ahn C, Chae S, Bae Y, Kim S. Synthesis of biodegradable multi-block copolymers of poly(L-lysine) and poly(ethylene glycol) as a non-viral gene carrier. J Controlled Release 2004; 97: 567–574.
6. Akinc A, Thomas M, Klibanov AM, Langer R. Exploring polyethylenimine-mediated DNA transfection and the proton sponge hypothesis. J Gene Med 2005; 7: 657–63.
7. Allen TM. Long-circulating (sterically stabilized) liposomes for targeted drug-delivery. Trends Pharmacol Sci. 1994; 15: 215–220.
8. Aillon KL, Xie Y, El-Gendy N, Berkland CJ, Forrest ML. Effects of nanomaterial physicochemical properties on in vivo toxicity. Adv Drug Deliv.Rev 2009; 61: 457–466.
9. Alshamsan A, Haddadi A, Incani V, Samuel J, Lavasanifar A, Uludag H. Formulation and delivery of siRNA by oleic acid and stearic acid modified polyethylenimine. Mol Pharmaceut 2009;6:121–33.
10. Aris A, Villaverde A. Modular protein engineering for nonviral gene therapy. Trends Biotechnol 2004; 22: 371–377.
11. Aspden TJ, Mason JD, Jones NS, Lowe J, Skaugrud O, Illum L. Chitosan as a nasal delivery system: the effect of chitosan solution on in vitro and in vivo mucociliary transport rates in human turbinates and volunteers. Journal of Pharmaceutical Sciences 1997; 86: 509–513.
12. Bastos R, Pante N, Burke B. Nuclear pore complex proteins. Int Rev Cytol 1995; 162B: 257–302.
13. Belur LR, Frandsen JL, Dupuy AJ, Ingbar DH, Largaespada DA, Hackett PB. Gene insertion and long-term expression in lung mediated by the Sleeping Beauty transposon system. Mol Ther 2003; 8: 501–507.
14. Bergen JM, Pun SH. Peptide-enhanced nucleic acid delivery. MRS Bull 2005; 30: 663–667.
15. Bhavsar MD, Amiji MM. Polymeric nano- and microparticle technologies for oral gene delivery. Expert Opin Drug Deliv. 2007; 4: 197–213.
16. Bonadio J, Smiley E, Patil P, Goldstein S. Localized, direct plasmid gene delivery in vivo: prolonged therapy results in reproducible tissue regeneration. Nat Med 1999; 5: 753– 759.
17. Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix P, Behr J. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine, Proc Natl Acad Sci 1995; 92: 7297–7301.
18. Bremner KH, Seymour LW, Logan A, Read ML. Factors influencing the ability of nuclear localization sequence peptides to enhance nonviral gene delivery. Bioconjug Chem 2004; 15: 152–161.
19. Brown MD, Scha¨tzlein A, Brownlie A, Jack V, Wang W, Tetley L, Gray AI, Uchegbu IF. Preliminary characterization of novel amino acid based polymeric vesicles as gene and drug delivery agents. Bioconj Chem 2000; 11: 880–891.
20. Burton EA, Glorioso J, Fink DJ. Gene therapy progress and prospects: Parkinson's disease. Gene Ther 2003; 10: 1721–1727.
21. Canine BF, Arash H. Development of recombinant cationic polymers for gene therapy research. Advanced Drug Delivery Reviews. 2010; 62: 1524–1529
22. Capan Y, Woo BH, Gebrekidan S, Ahmed S, DeLuca PP. Preparation and characterization of poly(d,l-lactide-co-glycolide) microspheres for controlled release of poly(l-lysine) complexed plasmid DNA. Pharm Res 1999; 16: 509–513.
23. Capan Y, Woo BH, Gebrekidan S, Ahmed S, DeLuca PP. Influence of formulation parameters on the characteristics of poly(-lactideco- glycolide) microspheres containing poly(-lysine) complexed plasmid DNA. J Control Release 1999; 60: 279–86.
24. Caracciolo G, Pozzi D, Caminiti R, Congiu Castellano A. Structural characterization of a new lipid/DNA complex showing a selective transfection efficiency in ovarian cancer cells. Eur Phys J 2003; 10: 331–336.
25. Cartier R, Reszka R. Utilization of synthetic peptides containing nuclear localization signals for nonviral gene transfer systems. Gene Ther 2002; 9: 157–167.
26. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, Down J, Denaro M, Brady T, Westerman K, Cavallesco R, Gillet-Legrand B, Caccavelli L, Sgarra R, Maouche-Chretien L, Bernaudin F, Girot R, Dorazio R, Mulder GJ, Polack A, Bank A, Soulier J, Larghero J, Kabbara N, Dalle B, Gourmel B, Socie G, Chretien S, Cartier N, Aubourg P, Fischer A, Cornetta, K, Galacteros F, Beuzard Y, Gluckman E, Bushman F, Hacein-Bey-Abina S, Leboulch P. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature 2010; 467: 318–322.
27. Cheng N, Liu W, Cao Z, Ji W, Liang D, Guo G. A study of thermoresponsive poly (N-isopropylacrylamide) polyarginine bioconjugate non-viral transgene vectors. Biomaterials 2006; 27: 4984–92.
28. Colosimo A, Serafino A, Sangiuolo F, Di Sario, S, Bruscia F, Amicucci P, NovelliG, Dallapiccola B, Mossa G. Gene transfection efficiency of tracheal epithelial cells

by DC-chol-DOPE/DNA complexes. Biochim Biophys Acta 1999;1419: 186–194.
29. Conwell CC, Huang L. Recent advances in non-viral gene delivery. Adv Genet 2005; 53: 3–18.
30. Cui Z, Mumper RJ. Chitosan-based nanoparticles for topical genetic immunization. Journal of Controlled Release 2001; 75: 409–419.
31. Caviston JP, Holzbaur EL. Microtubule motors at the intersection of trafficking and transport. Trends in Cell Biology 2006; 16: 530-537.
32. Chollet, P, Favrot M, Hurbin A, Coll J. Side-effects of a systemic injection of linear polyethylenimine-DNA complexes. J Gene Med 2004; 4: 84–91.
33. Chen J, Wang H, Jian- Gao Q, Chen H, Liang W. Liposomes modified with polycation used for gene delivery: Preparation, characterization and transfection in vitro. International Journal of Pharmaceutics 2007; 343: 255–261.
34. Chen JL, Hu Y, Shuai WP, Chen HL, Liang WQ, Gao JQ. Telomerase targeting antisense oligonucleotides carried by polycation liposomes enhance the growth inhibition effect on tumor cells. J Biomed Mater Res B Appl Biomater 2009; 89B: 362–368.
35. Chen RR, Mooney DJ. Polymeric growth factor delivery strategies for tissue engineering. Pharm. Res. 2003; 20: 1103– 1112.
36. Chumakova OV, Liopo AV, Andreev VG, Cicenaite I, Evers BM, Chakrabarty S, et al. Composition of PLGA and PEI/DNA nanoparticles improves ultrasound-mediated gene delivery in solid tumors in vivo. Cancer Lett 2008; 261: 215–25.
37. Curiel DT, Agarwal S, Wagner E, Cotten M. Adenovirus enhancement of transferrin–polylysine-mediated gene delivery. Proc Natl Acad Sci USA 1991;88: 8850–8854.
38. Dass CR. Vehicles for oligonucleotide delivery: therapeutic applicability against tumors. J Pharm Pharmacol 2002; 54: 3–27.
39. Dauty E, Remy J, Zuber G, Behr J. Intracellular delivery of nanometric DNA particles via the folate receptor. Bioconjugate Chem 2002; 13: 831–839.
40. Dauty E, Verkman AS. Actin cytoskeleton as the principal determinant of size-dependent DNA mobility in cytoplasm: a new barrier for non-viral gene delivery. J Biol Chem 2005; 280: 7823–7828.
41. De Rosa G, Quaglia F, La Rotonda MI, Besnard M, Fattal E. Biodegradable microparticles for the controlled delivery of oligonucleotides. Int J Pharm 2002;242:225–8.
42. De Rosa G, Quaglia F, Bochot A, Ungaro F, Fattal E. Long-term release and improved intracellular penetration of oligonucleotide; polyethylenimine complexes entrapped in biodegradable microspheres. Biomacromolecules 2003; 4: 529–36.
43. del Pozo-Rodríguez A, Pujals S, Delgado D, Solinís, MA, Gascón AR, Giralt E. A proline -rich peptide improves cell transfection of solid lipid nanoparticle-based non-viral vectors. J Control Release 2009; 133: 52–59.
44. Demeneix B, Hassani Z, Behr JP. Towards multifunctional synthetic vectors. Curr Gene Ther 2004; 4: 445–455.
45. Dennig J, Duncan E. Gene transfer into eukaryotic cells using activated polyamidoamine dendrimers. J Biotechnol 2002; 90: 339–47.
46. Dergunova MA, Alexeenko TV, Zhanaeva SY, Filyushina EE, Buzueva I, Kolesnikova OP. Characterization of the novel chemically modified fungal polysaccharides as the macrophage stimulators. International Immunopharmacology 2009; 9: 729–733.
47. Dias RS, Lindman B, Miguel MG. DNA interaction with catanionic vesicles. J Phys Chem B 2002; 48: 12600-12607.
48. Ding JJ, Guo CY, Cai QL, Lin YH, Wang H. In vivo expression of green fluorescent protein gene and immunogenicity of ES312 vaccine both mediated by starburst polyamidoamine dendrimers. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2005; 27:499–503.
49. Dohner K, Nagel CH, Sodeik B. Viral stop-and-go along microtubules: taking a ride with dynein and kinesins. Trends in Microbiology 2005; 13: 320-327.
50. El Ouahabi A, Thiry M, Schiffmann S, Fuks R, Nguyen-Tran H, Ruysschaert JM. Intracellular visualization of BrdU-labeled plasmid DNA/cationic liposome complexes. J Histochem Cytochem 1999; 47: 1159–66.
51. Ellens H, Bentz J, Szoka FC. Destabilization of phosphatidylethanolamine liposomes at the hexagonal phase transition temperature. Biochemistry 1986; 25: 285–94.
52. Elouahabi A, Ruysschaert JM. Formation and intracellular trafficking of lipoplexes and polyplexes, Mol Ther 2005; 11: 336–347
53. Elouahabi A, Thiry M, Pector V, Fuks R, Ruysschaert JM, Vandenbranden M. The role of endosome destabilizing activity in the gene transfer process mediated by cationic lipids. FEBS Lett 1997; 414: 187–192.
54. Escriou V, Carriere M, Bussone F, Wils P, Scherman D. Critical assessment of the nuclear import of plasmid during cationic lipid-mediated gene transfer. J Gene Med 2001; 3: 179– 187.
55. Fang J, Zhu YY, Smiley E, Bonadio J, Rouleau J, Goldstein S, McCauley L, Davidson B, Roessler B.Stimulation of new bone formation by direct transfer of osteogenic plasmid genes. Proc Natl Acad Sci USA 1996; 93: 5753– 5758.
56. Fang N, Chan V, Mao HQ, Leong KW. Interactions of phospholipid bilayer with chitosan: effect of molecular weight and pH. Biomacromolecules 2001; 2: 1161–1168.
57. Fasbender A, Zabner J, Zeiher BG, Welsh MJ. A low rate of cell proliferation and reduced DNA uptake limit cationic lipid-mediated gene transfer to primary cultures of ciliated human airway epithelia. Gene Therapy 1997; 4: 1173-1180.
58. Felgner PL, Gadek TR, Holm M Roman R, Chan HW, Wenz M, Northrop JP, Ringold GM, Danielsen M. Lipofection: a highly efficient,lipid-mediated DNA-transfection procedure, Proc Natl Acad Sci 1987; 84: 7413–7417.
59. Fischer D, Bieber T, Li Y, Elasasser HP, Kisssel T. A novel non-viral vector for DNA delivery based on low molecular weight, branched polyethyleneimine: effect of molecular weight on transfection efficiency and cytotoxicity, Pharm Res 1999; 16(8):1273-1279.
60. Floch V, Loisel S, Guenin E, Hervé AC, Clement JC, Yaouanc JJ, des Abbayes H, Férec C. Cation substitution in cationic phosphonolipids: a new concept to improve transfection activity and decrease cellular toxicity. J Med Chem 2000; 43: 4617–4628.
61. Freedland SJ, Malone RW, Borchers HM, Zadourain Z, Malone JG, Bennett MJ, Nantz MH, Li JH, Gumerlock PH, Erickson KL. Toxicity of cationic lipid- ribozyme complexes in human prostate tumor cells can mimic ribozyme activity. Biochem Mol Med 1996; 59: 144–153.

62. Fu HL, Cheng SX, Zhang XZ, Zhuo RX. Dendrimer/DNA complexes encapsulated in a water soluble polymer and supported on fast degrading star poly (dl-lactide) for lo calized gene delivery. J Control Release 2007; 124: 181–8.
63. Gao H, Hui KM. 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 2001; 8: 855–863.
64. Gao H, Shi W, Freund LB. Mechanics of receptor-mediated endocytosis, Proc Natl Acad Sci 2005; 102: 9469-9474.
65. Gao X, Huang L. Cationic liposome-mediated gene transfer. Gene Ther 1995; 2:710–722.
66. Gao X, Huang L. Potentiation of cationic liposome-mediated gene delivery by polycations. Biochemistry 1996; 35: 1027–1036.
67. Gao X, Kuruba R, Damodaran K, Day B, Liu D, Li S. Polyhydroxylalkyleneamines: A class of hydrophilic cationic polymer-based gene transfer agents. Journal of Controlled Release 2009; 137: 38–45.
68. Garcia L, Bunuales M, Duzgunes N, DeIlarduya CT. Serum-resistant lipopolyplexes for gene delivery to liver tumour cells. Eur J Pharm Biopharm 2007; 67: 58–66.
69. Gardlik R, Palffy J, Hodosy J, Lukacs J, Turna J, Celec P. Vectors and delivery systems in gene therapy. Med Sci Monit 2005; 11 (4): RA110–RA121.
70. Gascón AR, Pedraz, JL. Cationic lipids as gene transfer agents: a patent review. Expert Opin Ther Pat 2008; 18: 515–524.
71. Genes N, Rowley J, Mooney DJ, Bonassar L. Effect of substrate mechanics on chondrocyte adhesion to modified alginate surfaces. Arch Biochem Biophys 2004; 422: 161– 167.
72. Gershon H, Ghirlando R, Guttman SB, Minsky A. Mode of formation and structural features of DNA-cationic liposome complexes used for transfection. Biochemistry 1993; 32: 7143–7151.
73. Godbey W, Wu K, Mikos A. Size matters: molecular weight affects the efficiency of poly(ethyleneimine) as a gene delivery vehicle. J. Biomed Mater Res 1999; 45: 268–275.
74. Godbey W, Wu K, Mikos A. Poly(ethylenimine) and its role in gene delivery. J Control Release 1999; 60: 149–160.
75. Haensler J, Szoka FC. Polyamidoamine cascade polymers mediate efficient transfection of cells in culture. Bioconjug Chem 1993; 4: 372–379.
76. Hafez IM, Maurer N, Cullis PR. On the mechanism whereby cationic lipids promote intracellular delivery of polynucleic acids. Gene Ther 2001; 8: 1188–96.
77. Hamlet MR, Yergeau DA, Kuliyev E, Takeda M, Taira M, Kawakami K. Tol2 transposon-mediated transgenesis in Xenopus tropicalis. Genesis 2006; 44: 438–445.
78. Hart SL, Arancibia-Ca´rcamo CV, Wolfert MA, Mailhos C, O'Reilly NJ, Ali RR, Coutelle C, George AJT, Harbottle RP, Knight AM, Larkin DFP, Levinsky RJ, Seymour LW, Thrasher AJ, Kinnon C. Lipid-mediated enhancement of transfection by a nonviral integrin-targeting vector. Hum Gene Ther 1998; 9: 575 585.
79. Hasegawa S, Hirashima N, Nakanishi M. Microtubule involvement in the intracellular dynamics for gene transfection mediated by cationic liposomes. Gene Therapy 2001; 8: 1669-1673
80. Hejazi R, Amiji M. Chitosan-based gastrointestinal delivery systems. J Controlled Release 2003; 89: 151–65
81. Hideyoshi H, Yasuo S, Hiroshi K. Intracellular control of gene trafficking using liposomes as drug carriers. Eur J Pharm Sci 2001; 13: 85–89.
82. Holladay C, Keeney M, Greiser U, Murphy M, O'Brien T, Pandit A. A matrix reservoir for improved control of non-viral gene delivery. J Control Release 2009; 136: 220–5.
83. Hong K, Zheng W, Baker A. Papahadjopoulos. Stabilisation of cationic liposome/DNA complexes by polyamines and polyethyleneglycol– phospholipid conjugates for efficient in vivo gene delivery. FEBS Lett 1997; 414: 187–192.
84. Honore I, Grosse S, Frison N, Favatier F, Monsigny M, Fajac I. Transcription of plasmid DNA: influence of plasmid DNA/polyethylenimine complex formation. J Control Release 2005; 107: 537–46.
85. Horobin WR, Weissig VA. QSAR-modeling perspective on cationic transfection lipids. Predicting efficiency and understanding mechanisms. J Gene Med 2005; 7: 1023–1034.
86. Hosseinkhani H, Azzam T, Kobayashi H, Hiraoka Y, Shimokawa H, Domb A. Combination of 3D tissue engineered scaffold and non-viral gene carrier enhance in vitro DNA expression of mesenchymal stem cells. Biomaterials 2006; 27: 4269–78.
87. Hosseinkhani H, Yamamoto M, Inatsugu Y, Hiraoka Y, Inoue S, Shimokawa H. Enhanced ectopic bone formation using a combination of plasmid DNA impregnation into 3-D scaffold and bioreactor perfusion culture. Biomaterials 2006; 27: 1387.
88. Hosseinkhani H, Hosseinkhani M, Gabrielson NP, Pack DW, Khademhosseini A, Kobayashi H. DNA nanoparticles encapsulated in 3D tissue-engineered scaffolds enhance osteogenic differentiation of mesenchymal stem cells. J Biomed Mater Res 2008; 85A: 47–60.
89. Huang M, Fong CW, Khor E, Lim LY. Transfection efficiency of chitosan vectors: Effect of polymer molecular weight and degree of deacetylation. Journal of Controlled Release 2005; 106: 391-406.
90. Huang X, Wilber AC, Bao L, Tuong D, Tolar J, Orchard PJ. Stable gene transfer and expression in human primary T cells by the Sleeping Beauty transposon system. Blood. 2006; 107: 483–91.
91. Huang YC, Riddle K, Rice KG, Mooney DJ. Long-term in vivo gene expression via delivery of PEI DNA condensates from porous polymer scaffolds. Hum Gene Ther 2005; 16: 609-617.
92. Illum L, Jabbal-Gill I, Hinchcliffe M, Fisher AN, Davis SS. Chitosan as a novel nasal delivery system for vaccines. Adv Drug Deliv Rev 2001; 51: 81–96.
93. Ishii T, Okahata Y, Sato T. Mechanism of cell transfection with plasmid/chitosan complexes. Biochimica et Biophysica Acta 2001; 1514: 51–64.
94. Itaka J, Harada A, Yamasaki Y, Nakamura K, Kawaguchi H, Kataoka K. In situ single cell observation by fluorescence resonance energy transfer reveals fast intracytoplsmic delivery and easy release of plasmid DNA complexed with linear polyethylenimine. J Gene Med. 2004; 6: 76– 84.
95. Izsvak Z, Ivics Z. Sleeping beauty transposition: biology and applications for molecular therapy. Mol Ther 2004; 9: 147–56.
96. Jain NK, Gupta U. Application of dendrimer-drug complexation in the enhancement of drug solubility and

bioavailability. Expert Opin.Drug Metabol Toxicol 2008; 4: 1035–1052.
97. Jang JH, Shea L. Controllable delivery of non-viral DNA from porous scaffolds, J Control Release 2003; 86: 157–168.
98. Jang J-H, Rives CB, Shea LD. Plasmid delivery in vivo from porous tissue-engineering scaffolds: transgene expression and cellular transfection. Mol Ther 2005; 12: 475-83.
99. Jans DA, Chan CK, Huebner S. Signals mediating nuclear targeting and their regulation: Application in drug delivery. Med Res Rev 1998; 18: 189–223.
100. Jeong JH, Kim SW, Park TG. Molecular design of functional polymers for gene therapy. Prog Polym Sci 2007;32:1239–74.
101. Kabanov AV. Taking polycation gene delivery systems from in vitro to in vivo. Pharm Sci Tech Today 1999; 2: 365–72.
102. Kang SW, Lim HW, Seo SW, Jeon O, Lee M, Kim BS. Nanosphere mediated delivery of vascular endothelial growth factor gene for therapeutic angiogenesis in mouse ischemic limbs. Biomaterials 2008; 29: 1109–1117.
103. Kay MA, Glorioso JC, Naldini L. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. Nat Med 2001; 7: 33–40.
104. Keravala A, Liu D, Lechman ER, Wolfe D, Nash JA, Lampe DJ, et al. Hyperactive Himar1 transposase mediates transposition in cell culture and enhances gene expression in vivo. Hum Gene Ther 2006; 17: 1006–1018.
105. Kerr D. Clinical development of gene therapy for colorectal cancer. Nat Rev Cancer 2003; 3: 615–622.
106. Kichler A, Pages JC, Leborgne C, Druillennec S, Lenoir C, Coulaud D. Efficient DNA transfection mediated by the C-terminal domain of human immunodeficiency virus type 1 viral protein R. J Virol 2000; 74: 5424–31.
107. Kim, A, Lee E.H, Choi SH, Kim CK. In vitro and in vivo transfection efficiency of a novel ultradeformable cationic liposome. Biomaterials 2004; 25: 305–313.
108. Kim B-S, Mooney DJ. Development of biocompatible synthetic extracellular matrices for tissue engineering. Trends Biotechnol 1998; 16: 224–230.
109. Kim TH, Ihm JE, Choi YJ, Nah JW, Cho CS. Efficient gene delivery by urocanic acid-modified chitosan. J Control Release 2003; 93: 389–402.
110. Kim YH, Gihm SH, Park CR, Lee KY, Kim TW, Kwon IC. Structural characteristics of size-controlled self-aggregates of deoxycholic acid-modified chitosan and their application as a DNA delivery carrier. Bioconjugate Chem 2001; 12: 932–938.
111. Kloeckner J, Prasmickaite L, Hogset A, Berg K, Wagner E. Photochemically enhanced gene delivery of EGF receptortargeted DNA polyplexes. J Drug Target 2004; 12: 205–213.
112. Kohn DB. Update on gene therapy for immunodeficiencies. Clin Immunol. 2010; 135: 247–254.
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. Kong H, Liu J, Riddle K, Matsumoto T, Leach K, Mooney DJ. Non-viral gene delivery regulated by stiffness of cell adhesion substrates. Nat Mater 2005; 4: 460–464.
115. Kong H, Polte T, Alsberg E, Mooney DJ. FRET measurements of cell-traction forces and nano-scale clustering of adhesion ligands varied by substrate stiffness, Proc Natl Acad Sci USA 2005; 102: 4300–4305.
116. Koping-Hoggard M, Mel'nikova YS, Varum KM, Lindman B, Artursson P. Relationship between the physical shape and the efficiency of oligomeric chitosan as a gene delivery system in vitro and in vivo. Journal of Gene Medicine 2003; 10: 130-141.
117. Kostarelos K, Miller, AD. Synthetic, selfassembly ABCD nanoparticles; a structural paradigm for viable synthetic non-viral vectors. Chem Soc Rev 2005; 34: 970–994.
118. Kramer M, Stumbe J, Grimm G, Kaufmann B, Kruger U, Weber M, Haag. Dendritic polyamines: Simple access to new materials with defined treelike structures for application in non-viral gene delivery. Chem biochem 2004; 5: 1081–1087.
119. 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. Proc Natl Acad Sci USA 1996; 93: 4897–902.
120. Kulkarni RP, Castelino K, Majumdar A, Fraser SE. Intracellular transport dynamics of endosomes containing DNA polyplexes along the microtubule network. Biophysical Journal 2006; 90: L42-L4.
121. Kulkarni RP, Wu DD, Davis ME, Fraser SE. Quantitating intracellular transport of polyplexes by spatio-temporal image correlation spectroscopy. Proceedings of the National Academy of Sciences of the USA 2005; 102: 7523-7528.
122. Kural C, Kim H, Syed S, Goshima G, Gelfand VI, Selvin PR. Kinesin and dynein move a peroxisome in vivo: a tug-of-war or coordinated movement?. Science 2005; 308: 1469-1472.
123. Kurosaki T, Kishikawa R, Matsumoto M, Kodama Y, Hamamoto T, To H, Niidome T, Takayama K, Kitahara T, Sasaki H. Pulmonary gene delivery of hybrid vector, lipopolyplex containing N-lauroylsarcosine, via the systemic route. J Control Release. 2009; 136: 213-219.
124. Jevprasesphant R, Penny J, Jalal R, Attwood D, McKeown NB, D'Emanuele A. The influence of surface modification on the cytotoxicity of PAMAM dendrimers. Int J Pharm 2003; 252, 263–266.
125. Lakadamyali M, Rust MJ, Babcock HP, Zhuang X. Visualizing infection of individual influenza viruses. Proceedings of the National Academy of Sciences of the USA 2003; 100: 9280-9285.
126. Lam AP, Dean DA. Progress and prospects: nuclear import of nonviral vectors. Gene Ther 2010; 17: 439–447.
127. Lasic DD, Strey H, Stuart MCA, Podgornik R, Frederik PM. The structure of DNA-liposome complexes. JACS 1997; 119: 832-833.
128. Lasic DD. Recent developments in medical applications of liposomes: sterically stabilized liposomes in cancer therapy and gene delivery in vivo. Journal of Controlled Release 1997; 48:203-222
129. Lasic DD, Needham D. The "stealth" liposome: A prototypical biomaterial. Chem 1995; 9: 2601–2628.
130. Lavertu M, Methot S, Tran-Khanh N, Buschmann MD. High efficiency gene transfer using chitosan/DNA nanoparticles with specific combinations of molecular weight and degree of deacetylation. Biomaterials 2006; 27: 4815-4824.
131. Lechardeur D, Sohn KJ, Haardt M, Joshi PB, Monck M, Graham RW. Metabolic instability of plasmid DNA in

the cytosol: a potential barrier to gene transfer. Gene Ther 1999; 6: 482–97.

132. Lei Y, Segura T. DNA delivery from matrix metalloproteinase degradable poly(ethylene glycol) hydrogels to mouse cloned mesenchymal stem cells. Biomaterials 2009; 30: 254–65.
133. Leventis R, Silvius JR. Interactions of mammalian cells with lipid dispersions containing novel metabolizable cationic amphiphiles, Biochim. Biophys. Acta. 1990; 1023; 124–132.
134. Lihua L, Wanga S, Shana B, Sanga M, Liub S, Guiying W. Advances in viral-vector systemic cytokine gene therapy against cancer vaccine. 2010; 28(23): 3883–3887 Li S, Huang L. In vivo gene transfer via intravenous administration of cationic lipid–protamine–DNA (LPD) complexes. Gene Ther 1997; 4: 891– 900.
135. Li W, Nicol F, Szoka Jr. FC. GALA: a designed synthetic pH-responsive amphipathic peptide with applications in drug and gene delivery. Adv Drug Deliv Rev 2004; 56: 967–85.
136. Li XM, Ma YL, Liu XJ. Effect of the Lycium barbarum polysaccharides on age-related oxidative stress in aged mice. Journal of Ethnopharmacology 2007; 111: 504–511.
137. Liua Z, Zhanga Z, Zhoua C, Jiao Y. Hydrophobic modifications of cationic polymers for gene delivery. Progress in Polymer Science 2010; 35: 1144–1162
138. Liu WG, Yao KD. Chitosan and its derivatives—A promising nonviral vector for gene transfection. Journal of Controlled Release 2002; 83: 1–11.
139. Liu F, Huang L. A syringe electrode device for simultaneous injection of DNA and electrotransfer. Mol Therapy 2002; 5: 323–328.
140. Liu Z, Zhang Z, Zhou C, Jiao Y: Hydrophobic modifications of cationic polymers for gene delivery. Progress in Polymer Science. 2010; 35: 1144–1162
141. Lukacs GL, Haggie P, Seksek O, Lechardeur D, Freedman N, Verkman AS. Size-dependent DNA mobility in cytoplasm and nucleus. J Biol Chem 2000; 275: 1625–1629.
142. Luo D, Woodrow-Mumford K, Belcheva N. Saltzman WM. Controlled DNA delivery systems. Pharm. 1999; 16(8): 1300– 1308.
143. Luo D, Saltzman W. Enhancement of transfection by physical concentration of DNA at the cell surface. Nat Biotechnol 2000; 18: 893–895.
144. Malafaya PB, Silva GA, Reis RL. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. Adv Drug Deliv Rev 2007; 59: 207–33.
145. Mansouri, S, Lavigne, P, Corsi K, Benderdour M, Beaumont E, Fernandes JC. Chitosan–DNA nanoparticles as non-viral vectors in gene therapy: Strategies to improve transfection efficacy. European Journal of Pharmaceutics and Biopharmaceutics 2004; 57: 1–8.
146. Mao S, Sun W, Kissel T. Chitosan-based formulations for delivery of DNA and siRNA.Adv Drug Deliv Rev 2010; 62: 12–27.
147. Matsumoto M, Kishikawa R, Kurosaki T, Nakagawa H, Ichikawa N, Hamamoto T, To H, Kitahara T, Sasaki H. Hybrid vector including polyethylenimine and cationic lipid, DOTMA, for gene delivery. Int J Pharm 2008; 363: 58-65.
148. McNeish IA, Bell SJ, Lemoine NR. Gene therapy progress and prospects: cancer gene therapy using tumour suppressor genes. Gene Ther 2004; 11: 497–503.
149. Medina-Kauwe LK, Xie J, Hamm-Alvarez S. Intracellular trafficking of nonviral vectors. Gene Ther. 2005; 12: 1734–1751.
150. Mellman I. Endocytosis and molecular sorting. Annu Rev Cell Dev Biol 1996; 12:575–625.
151. Meredith A, Mintzer Eric E, Simanek K. Nonviral Vectors for Gene Deliver. Chem Rev 2009; 109: 259–302
152. Merdan T, Kunath K, Fischer D, Kopecek J, Kissel T. Intracellular processing of poly(ethylene imine)/ribozyme complexes can be observed in living cells by using confocal laser scanning microscopy and inhibitor experiments. Pharm Res 2002; 19: 140–146.
153. Midoux P, Monsigny M. Efficient gene transfer by histidylated polylysine/pDNA complexes. Bioconjug Chem 1999; 10: 406–411.
154. Miguel MG, Pais AA, Dias RS, Leal C, Rosa M, Lindman, B. DNA cationic amphiphile interactions. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2003; 228: 43-55.
155. Miller, AD. The problem with cationic liposome/micelle-based non-viral vector systems for gene therapy. Curr.Med. Chem. 2003; 10: 1195–1211.
156. Miller AM, Dean DA. Tissue-specific and transcription factor-mediated nuclear entry of DNA. Adv. Drug Deliv 2009; 61: 603–613.
157. Mislick KA, Baldeschwieler JD. Evidence for the role of proteoglycans in cation-mediated gene transfer. Proc Nat Acad Sci USA 1996; 93: 12349–12354.
158. Mitomo K, Griesenbach U, Inoue M, Somerton L, Meng C, Akiba E, Tabata T, UedaY, Frankel GM, Farley R, Singh C, Chan M, Munkonge F, Brum A, Xenariou S, Escudero-Garcia S, Hasegawa M, Alton EW. Toward gene therapy for cystic fibrosis using a lentivirus pseudotyped with Sendai virus envelopes. Mol Ther 2010; 18: 1173–1182.
159. Moffatt S, Wiehle S, Cristiano RJ. A multifunctional PEI based cationic polyplex for enhanced systemic p53-mediated gene therapy. Gene Ther 2006; 13: 1512–23.
160. Mounkes LC, Zhong W, Cipres-Palacin G, Heath TD, Debs RJ. Proteoglycans mediate cationic liposome–DNA complex-based gene delivery in vitro and in vivo. J BiolChem 1998; 273: 26164–26170.
161. Mumper RJ, Wang JJ, Claspell JM, Rolland AP. Novel polymeric condensing carriers for gene delivery. Proceedings of the International Symposium on Controlled Release. Bioactive Materials 1995; 22: 178-179.
162. Muzykantov VR, Torchilin V. Biomedical Aspects of Drug Targeting. Kluwer Academic Publishers 2003; pp. 211–78
163. Nabel EG. Gene therapy for cardiovascular diseases. J Nucl Cardiol 1999; 6: 69–75.
164. Nishikawa M, Huang L. Nonviral vectors in the new millennium: delivery barriers in gene transfer. Hum Gene Ther 2001;12:861–70.
165. Newkome GR, Yao ZQ, Baker GR, Gupta VK. 1985. Micelles.Part 1. Cascade molecules. A new approach to micelles. J Org Chem 1985; 50, 2003–2004.
166. O'Brien FJ, Harley BA, Yannas IV, Gibson LJ. The effect of pore size on cell adhesion in collagen-GAG scaffolds. Biomaterials 2005; 26: 433–441.
167. Ochiya T, Takahama Y, Nagahara S, Sumita Y, Hisada A, Itoh H, Nagai YM. Terada. New delivery system for plasmid DNA in vivo using atelocollagen as a carrier material: the Minipellet, Nat Med 1999; 5; 707– 710.

168. Ochiya T, Nagahara S, Sano A, Itoh H, Terada M. Biomaterials for gene delivery: atelocollagen-mediated controlled release of molecular medicines, Curr Gene Ther 2001; 1:31–52.
169. Ogris M, Brunner S, Schuller S, Kircheis R, Wagner E. PEGylated DNA/transferrin-PEI complexes: reduced interaction with blood components, extended circulation in blood and potential for systemic gene delivery. Gene Ther 1999; 595; 605.
170. Ohlfest JR, Lobitz PD, Perkinson SG, Largaespada DA. Integration and long-term expression in xenografted human glioblastoma cells using a plasmid-based transposon system. Mol Ther 2004; 10: 260–8.
171. Palmer LR, Chen T, Lam AMI, Fenske DB, Wong KF, MacLachlan I, Cullis PR. Transfection properties of stabilized plasmid-lipid particles containing cationic PEG lipids. Biochim Biophys Acta 2003; 1611: 204–216.
172. Persiani S, Shen, WC. Increase of poly(l-lysine) uptake but not fluid phase endocytosis in neuraminidase pretreated Madin–Darby canine kidney (MDCK)cells. Life Sciences 1989; 45: 2605–2610.
173. Prabha S, Zhou WZ, Panyam J, Labhasetwar,V. Size-dependency of nanoparticle-mediated gene transfection: studies with fractionated nanoparticles. Int J Pharm 2002; 244: 105–115.
174. Park IK, Kim TH, Park YH, Shin BA, Choi ES, Chowdhury EH, Akaike T, Cho CS. Galactosylated chitosan-graft-poly(ethylene glycol) as hepatocytetargeting DNA carrier. J Control Release 2001; 76, 349–362.
175. Park TG, Jeong JH, Kim SW. Current status of polymeric gene delivery systems. Adv Drug Deliver Rev 2006;58:467–86.
176. Patil SD, Rhodes DG, Burgess DJ. DNA-based therapeutics and DNA delivery systems: a comprehensive review. AAPS J 2005; 7: E61–E77.
177. Pietrzak WS,editor. Musculoskeletal tissue regeneration: biological materials and methods. Totowa, NJ: Humana Press 2008; 81–92
178. Pollard H, Remy JS, Loussouarn G, Demolombe S, Behr JP, Escande D. Polyethylenimine but not cationic lipids promotes transgene delivery to the nucleus in mammalian cells. J Biol Chem 1998; 273: 7507–11.
179. Pouton, CW, Seymour LW. Key issues in non-viral gene delivery. Advanced Drug Delivery Reviews 1998; 34: 3–19.
180. Radtke K, Dohner K, Sodeik B. Viral interactions with the cytoskeleton: A hitchhiker's guide to the cell. Cellular Microbiology 2006; 8: 387-400.
181. Radler JO, Koltover I, Salditt T, Safinya CR. Structure of DNA-cationic liposome complexes: DNA intercalation in multilamellar membranes in distinct interhelical packing regimes. Science 1997; 275: 810-814.
182. Rejman J, Bragonzi A, Conese M. Role of clathrin- and caveolae-mediated endocytosis in gene transfer mediated by lipo- and polyplexes. Mol Ther 2005; 12: 468–74.
183. Rao SB, Sharma CP. Use of chitosan as a biomaterial: studies on its safety and hemstatic potential. Journal of Biomedical Materials Research 1997; 34: 21–28.
184. Romoren K, Thu BJ, Evensen O. Immersion delivery of plasmid DNA. II. A study of the potentials of a chitosan based delivery system in rainbow trout (Oncorhynchus mykiss) fry. J Controlled Release 2002; 85: 215–25.
185. Roy K, Wang D, Hedley ML, Barman SP. Gene delivery with in-situ crosslinking polymer networks generates long-term systemic protein expression. Mol Ther 2003; 7: 401–408.
186. Ruponen M, Yla-Herttuala S, Urtti A. Interactions of polymeric and liposomal gene delivery systems with extracellular glycosaminoglycans: physicochemical and transfection studies. Biochim Biophys Acta 1999; 1415: 331-41.
187. Ruozi B, Tosi G, Leo E, Vandelli MA. Application of atomic force microscopy to characterize liposomes as drug and gene carriers. Talanta 2007; 73: 12-22.
188. Russ V, Gunther M, Halama A, Ogris M., Wagner, E. Oligoethylenimine grafted polypropylenimine dendrimers as degradable and biocompatible synthetic vectors for gene delivery. J Control Rel 2008; 132: 131–140.
189. Salditt, T, Koltover I, Radler JO, Safinya CR. Two-dimensional smectic ordering of linear DNA chains in self-assembled DNA-cationic liposome mixtures. PRL 1997; 79: 2582-2585.
190. Saul JM, Linnes MP, Ratner BD, Giachelli CM, Pun SH. Delivery of non-viral gene carriers from sphere-templated fibrin scaffolds for sustained transgene expression. Biomaterials 2007; 28: 4705–4716.
191. Schaffer DV, Fidelman NA, Dan N, Lauffenburger DA.Vector unpacking as a potential barrier for receptormediated polyplex gene delivery. Biotechnol Bioeng 2000; 67: 598–606.
192. Schaffer DV, Lauffenburger DA. Optimization of cell surface binding enhances efficiency and specificity of molecular conjugate gene delivery. J Biol Chem 1998; 273: 28004–28009.
193. Schatzlein AG. Targeting of synthetic gene delivery systems. J Biomed Biotechnol 2003; 2003:149–58.
194. Schepetkin IA, Quinn, MT. Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. International Immunopharmacology 2006; 6: 317–333.
195. Scherer F, Schillinger U, Putz U, Stemberger A, Plank C. Nonviral vector loaded collagen sponges for sustained gene delivery in vitro and in vivo. J Gene Med 2002; 4: 634–43.
196. Shi F, Rakhmilevich AL, Heise CP, Oshikawa K, Sondel PM, Yang NS, et al. Intratumoral injection of interleukin-12 plasmid DNA, either naked or in complex with cationic lipid, results in similar tumor regression in a murine model. Mol Cancer Ther 2002; 1: 949–57.
197. Shimizu N, Kamezaki F, Shigematsu S. Tracking of microinjected DNA in live cells reveals the intracellular behavior and elimination of extrachromosomal genetic material. Nucleic Acids Research 2005; 33: 6296-6307
198. Silva EA, Mooney DJ, Gerald PS. Synthetic extracellular matrices for tissue engineering and regeneration. In: Schatten G, editor. Current topics in developmental biology. 2004; 64: 181–205.
199. Simoes S, Slepushkin V, Pires P, Gaspar R, Pedroso de Lima MC, Duzgunes N. Human serum albumin enhances DNA transfection by lipoplexes and confers resistance to inhibition by serum. Biochim Biophys Acta 2000; 15: 459–469.
200. Soldati T, Schliwa M. Powering membrane traffic in endocytosis and recycling. Nature Reviews Molecular Cell Biology 2006; 7: 897-908.
201. Somia N, Verma M. Gene therapy: Trials and tribulations. Nature Review Genetics. 2000; 1: 91–99.
202. Sternberg B, Sorgi FL, Huang L. New structures in complex-formation between DNA and cationic liposomes

visualized by freeze-fracture electron-microscopy. Febs Letters 1994; 356: 361-366.
203. Strand SP, Lelu S, Reitan NK, de Lange Davies C, Artursson P, Varum KM. Molecular design of chitosan gene delivery systems with an optimized balance between polyplex stability and polyplex unpacking. Biomaterials 2010; 31: 975-87.
204. Suh J, Wirtz D, Hanes J. Efficient active transport of gene nanocarriers to the cell nucleus. Proceedings of the National Academy of Sciences of the USA 2003; 100: 3878-3882
205. Sun S, Liu W, Cheng N, Zhang B, Cao Z, Yao K. A thermoresponsive chitosan-NIPAAm/vinyl laurate copolymer vector for gene transfection. Bioconjug Chem 2005; 16: 972–80.
206. Takeuchi H, Yamamoto H, Niwa T, Hino T, Kawashima Y. Enteral absorption of insulin in rats from mucoadhesive chitosan-coated liposomes. Pharmaceutical Research 1996; 13: 896–901.
207. Tagawa T, Manvell M, Brown N, Keller M, Perouzel E, Murray KD, Harbottle RP, Tecle M, Booy F, Brahimi-Horn MC, Coutelle C, Lemoine NR, Alton EWFW, Miller AD. Characterisation of LMD virus-like nanoparticles self-assembled from cationic liposomes, adenovirus core peptide μ (mu) and plasmid DNA. Gene Ther 2002; 9:564–576.
208. Talsma SS, Babensee JE, Murthy N, Williams IR. Development and in vitro validation of a targeted delivery vehicle for DNA vaccines. J Control Release 2006;112: 271–279.
209. Tan Y, Liu F, Li Z, Li S, Huang L. Sequential injection of cationic liposome and plasmid DNA effectively transfects the lung with minimal inflammatory toxicity. Mol Thera 2001; 3: 673–682.
210. Tang MX, Redemann CT, Szoka Jr FC. In vitro gene delivery by degraded polyamidoamine dendrimers. Bioconjugate Chem 1996; 7: 703–14.
211. Tang MX, Szoka FC. The influence of polymer structure on the interactions of cationic polymers with DNA and morphology of the resulting complexes. Gene Ther 1997; 4:823–32.
212. Tang MX, Szoka FC. Activated polyamidoamine dendrimers, a non-viral vector for gene transfer to the corneal endothelium. Gene Ther 1997; 4: 823–832.
213. Thanou M, Florea BI, Geldof M, Junginger HE, Borchard G. Quaternized chitosan oligomers as novel gene delivery vectors in epithelial cell lines. Biomaterials 2002; 23: 153–159.
214. Thomas CE, Ehrhardt A, Kay MA. Progress and problems with the use of viral vectors for gene therapy. Nat Rev Genet 2003; 4: 346-358.
215. Tinsley-Brown A, Fretwell R, Dowsett AB, Davis S, Farrar G. Formulation of poly(d,l-lactic-co-glycolic acid) mircoparticles for rapid DNA delivery, J Control Release 2000; 66: 229–241.
216. Tokunaga M, Hazemoto N, Yotsuyanagi T. Effect of oligopeptides on gene expression: comparison of DNA/peptide and DNA/peptide/liposome complexes. Int J Pharm 2004; 269: 71–80.
217. Tomanin R, Scarpa M. Why do we need new gene therapy viral vectors? Characteristics, limitations and future perspectives of viral vector transduction. Curr Gene Ther 2004; 4: 357–372.
218. Tomalia DA, Baker H, Dewald J, Hall M, Kallos G, Martin S, Roeck J, Ryder J, Smith P. A new class of polymers: starburst-dendritic macromolecules. Polym J 1985; 17: 117–132.
219. Tros de Ilarduya C, Sun Y, Düzgünes N. Gene delivery by lipoplexes and polyplexes.European Journal of Pharmaceutical Sciences 2010; 40 (3): 159-170,
220. Tseng W, Helston F, Giorgio T. Mitosis enhances transgene expression of plasmid delivered by cationic liposomes. Biochim Biophys Acta 1999; 1445: 53–64.
221. Tuszynski MH. Growth-factor gene therapy for neurodegenerative disorders. Lancet Neurol 2002; 1: 51–57.
222. Uyechi-O' LS, Brien FC, Szoka Jr. Mechanisms for cationic lipids in gene transfer, in: A. Rolland, S.M. Sullivan (Eds.), Pharmaceutical Gene Delivery Systems, Marcel Dekker, New York. 2003, pp79–108.
223. Vanderkerken S, Vanheede T, Toncheva V, Schacht E, Wolfert MA, Seymour L. Synthesis and evaluation of poly(ethylene glycol)-polylysine block copolymers as carriers for gene delivery. Journal of Bioactive and Compatible Polymers 2000; 15: 115–138.
224. Vaughan EE, Dean DA. Intracellular trafficking of plasmids during transfection is mediated by microtubules. Molecular Therapy 2005. 13; 422-428.
225. Vigneron JP, Oudrhiri N, Fauquet M, Vergely l, Bradley JC, Basseville M, Lehn P, Lehn JM. Guanidinium-cholesterol cationic lipids: efficient vectors for the transfection of eukaryotic cells. Proc Natl Acad Sci USA 1996; 93: 9682–9686.
226. Walsh CE. Gene therapy progress and prospects: gene therapy for the hemophilias. Gene Ther 2003; 10: 999–1003.
227. Walther W, Stein U, Voss C, Schmidt T, Schleef M, Schlag PM. Stability analysis for long-term storage of naked DNA: impact on nonviral in vivo gene transfer. Anal Biochem 2003; 15: 230–235.
228. Wang D, Robinson D, Kwon G, Samuel J. Encapsulation of plasmid DNA in biodegradable poly(d,l-lactic-co-glycolic acid) microspheres as a novel approach for immunogene delivery. J Control Release 1999; 57: 9 –18.
229. Ward BB, Baker JR, In Majoros IJ, Baker Jr. JR (ed). Targeted Drug Delivery in General, New Technology in Medicine.Dendrimer Based Nanomedicine. Pan Stanford Publishing. 2008; USA. pp: 1-16.
230. Ward CM, Read ML, Seymour LW. Systemic circulation of poly(Llysine)/ DNA vectors is influenced by polycation molecular weight and type of DNA: differential circulation in mice and rats and the implications for human gene therapy. Blood 2001; 97: 2221–2229.
231. Weiss SI, Sieverling, N, Niclasen M, Maucksch C, Thunemann AF, Mohwald H. Uronic acids functionalized polyethyleneimine (PEI)-polyethyleneglycol (PEG)-graft-copolymers as novel synthetic gene carriers. Biomaterials 2006; 27: 2302–2312.
232. Wheeler JJ, Palmer L, Ossanlou M, MacLachlan I, Graham RW, Zhang YP, Hope M, Scherrer P, Cullis PR. Stabilized plasmid-lipid particles: construction and characterization. Gene Ther 1999; 6: 271–281.
233. Wolfert MA, Seymour LW. Atomic force microscopic analysis of the influence of the molecular weight of poly(L)lysine on the size of polyelectrolyte complexes formed with DNA. Gene Ther 1996; 3: 269–73.
234. Woods NB, Bottero V, Schmidt M, von Kalle C, Verma IM. Gene therapy: therapeutic gene causing lymphoma. Naturel 2006; 440 (7088): 1123-1130.

235. Wong SY, Pelet JM, Putnam D. Polymer systems for gene delivery past, present, and future. Prog Polym Sci 2007;32:799–837.
236. Woude I, Wagenaar A, Meekel AA, ter Beest MB, Ruiters MH, Engberts JB, Hoekstra D. Novel pyridinium surfactants for efficient, nontoxic in vitro gene delivery. Proc Natl Acad Sci USA 1997; 94:1160–1165.
237. Wiseman J, Goddard C, McLelland D, Colledge W. A comparison of linear and branched polyethyleneimine (PEI) with DCChol/DOPE liposomes for gene delivery to epithelial cells in vitro and in viv. Gene Ther 2003; 10: 1654– 1662.
238. Wightman L, Kircheis R, Rossler V, Carotta S, Ruzicka R, Kursa M, E. Wagner E. Different behavior of branched and linear polyethylenimine for gene delivery in vitro and in vivo. J Gene Med 2001; 3: 362–372.
239. Wu GY, Wu CH. Receptor-mediated in vitro gene transformation by a soluble DNA carrier system. J Biol Chem. 1987; 262: 4429–4432.
240. Wu SC, Meir YJ, Coates CJ, Handler AM, Pelczar P, Moisyadi S. piggyBac is a flexible and highly active transposon as compared to sleeping beauty, Tol2, and Mos1 in mammalian cells. Proc Natl Acad Sci USA 2006; 103(41): 15008–15013.
241. Xu D, Hong J, Sheng K, Dong L, Yao S. Preparation of polyethyleneimine nanogels via photo-Fenton reaction. Radiat Phys Chem 2007; 76: 1606–1611.
242. Xu DM, Yao SD, Liu YB, Sheng KL, Hong J, Gong PJ, Dong L. Size-dependent properties of M-PEIs nanogels for gene delivery in cancer cells. Int J Pharm 2007, 338: 291–296.
243. Xu L, Huang C, Huang W, Tang W, Rait A, Yin Y, Cruz I, Xiang L, Pirollo K, Chang E. Systemic tumor-targeted gene delivery by anti-transferrin receptor scFvimmunoliposomes. Mol Cancer Thera 2002; 1: 337–346.
244. Xu Y, Szoka Jr. Mechanism of DNA release from cationic liposome/DNA complexes used in cell transfection. Biochemistry 1996; 35: 5616–5623.
245. Yong-Hong L, Jones S, Forbes B, Martin GP, Brown MB. Hyaluronan: pharmaceutical characterization and drug delivery. Drug Deliv 2005; 12: 327–342.
246. Young LS, Searle PF, Onion D, Mautner V. Viral gene therapy strategies: from basic science to clinical application. J Pathol 2006; 208: 299–318.
247. Yu H, Chen X, Lu T, Sun J, Tian H, Hu J, Wang Y, Zhang P, Jing X. Poly(l-lysine)-graft-chitosan copolymers: synthesis, characterization, and gene transfection effect. Biomacromolecules 2007; 8: 1425–1435.
248. Yu L, Dean K, Li L. Polymer blends and composites from renewable resources. Prog Polym Sci 2006; 31: 576–602.
249. Yurong H, Kun L, Lei W, Shasha Y, Zhenzhong Z, Yun Z. Pegylated immuno-lipopolyplexes: A novel non-viral gene delivery system for liver cancer therapy Journal of Controlled Release 2010; 144: 75–81.
250. Zelphati O, Szoka Jr, Mechanism of oligonucleotide release from cationic liposomes. Proc Natl Acad Sci USA 1996; 93: 11493–11498.
251. Zhang G, Gurtu V, Smith TH, Nelson P, Kain SR. A cationic lipid for rapid and efficient delivery of plasmid DNA into mammalian cells. Biochem Biophys Res Commun 1997; 236: 126–129.
252. Zhang JS, Liu F, Huang L. Implications of pharmacokinetic behavior of lipoplex for its inflammatory toxicity. Adv Drug Deliv Rev 2005; 57: 689–698.
253. Zhanga S, Xua Y, Wanga B, Qiaob W, Liub D, Zongshi L. Cationic compounds used in lipoplexes and polyplexes for gene delivery. Journal of Controlled Release 2004; 100: 165–180
254. Zhang Y F, Schlachetzki WM. Pardridge, Global non-viral gene transfer to the primate brain following intravenous administration. Mol Ther 2003; 7: 11–18.Zhu N, Liggitt D, Liu Y, Debs R. Systemic gene expression after intravenous DNA delivery into adult mice. Science 1993; 261: 209-211.
255. Zhou X, Huang L. DNA transfection mediated by cationic liposomes containing lipopolylysine: characterization and mechanism of action. Biochim Biophys Acta 1994; 19: 195–203.
256. Zuhorn IS, Kalicharan R, Hoekstra D. Lipoplex-mediated transfection of mammalian cells occurs through the cholesterol-dependent clathrin-mediated pathway of endocytosis. J Biol Chem 2002; 17: 18021–18028.
257. Zuber G, Zammut-Italiano L, Dauty E, Behr J. Targeted gene delivery to cancer cells: Directed assembly of nanometric DNA particles coated with folic acid. Angew.Chem 2003; 42: 2666–2699.
258. Zuhorn IS, Bakowsky U, Polushkin E, Visser WH, Stuart MC, Engberts JB, Hoekstra D. Nonbilayer phase of lipoplex-membrane mixture determines endosomal escape of genetic cargo and transfection efficiency. Mol Ther 2005; 11: 801–810.

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