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Engineered Smart Biomaterials for Gene Delivery

Review Article

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Summary

The delivery technology of gene into cells has been increasingly paid attention for gene therapy and the generation of genetically engineered cells. If it is possible to artificially introduce exogenous genetic materials into cells at a high transfection efficiency by a delivery technology, the technology will give academically, clinically, and practically great impacts to gene therapy, cell and molecular biology or pharmaceutical and food industries for bio-productions. The major aim of gene therapy is to effectively deliver the genetic materials into cells, genetically modifying and repairing cell functions, which may induce therapeutic healing of disease conditions. The genetic material involves DNA, RNA, antisense, DNA decoy, and ribozyme, and it is expected that their appropriate transfection allows disease cells to turn to a good direction of recovery. The genetic manipulation often manifests the mechanisms of intracellular machineries of gene and protein, while it may play an important role in making clear the appropriate genes associated with various diseases. Based on the basic and scientific knowledge, the delivery technology of gene is applicable to produce various proteins pharmaceutically valuable, e.g. cytokines, growth factors, and antibodies as well as seeds strong against harmful insects and cold weather damage. In other words, the cells genetically innovated work as the microfactory to produce valuable pharmaceutical and food products. This review provides a critical view of different approaches of gene therapy with a major focus on smart biomaterials transfection agent technologies to control the *in vitro* and *in vivo* localization and function of administered genes.

I. Introduction

Gene therapy is the correction of genetic defects for treatment or prevention of diseases associated with defective gene expression, by administration of a functional gene in to cells followed by expression and production of the required proteins. Nucleic acids (DNA or RNA), as a therapeutic agents, have been used to deliver genes as DNA plasmids, to modify pre-mRNA splicing to improve disease-causing mutations, or to mediate gene knockdown via RNA interference (RNAi) technology (Anderson, 1998 ;Yiqi Seow). The application of gene delivery for therapeutic purpose is currently based on two strategies including corrective or cytotoxic gene therapy. The corrective therapy which results in correction of genetic defects in target cells and is applied for the treatment of diseases with single gene disorders such as severe combined immunodeficiency syndromes (Cavazzana-Calvo, et al., 2000), cystic fibrosis (Alton, et al., 1999), hemophilia (Dwarki, et al., 1995), sickle cell anemia (Larochelle, et al., 1995), β-thalassemia (Bank, Dorazio, & Leboulch, 2005), muscular dystrophy (Chamberlain, 2002), and malignant tumors, including ovarian cancer (Coukos & Rubin, 1998), prostate cancer (Harrington, Spitzweg, Bateman, Morris, & Vile, 2001), and breast cancer (Obermiller, Tait, & Holt, 1999). The second strategy, cytotoxic gene therapy, which has been developed by investigation of novel targeted cytotoxic genes in cancer gene therapy (Fielding, et al., 2000) results in destruction of target cells using a cytotoxic pathway which is used for treatment of malignant tumors, including ovarian (Deshane, et al., 1995), breast (Lazennec, Alcorn, & Katzenellenbogen, 1999), and prostate cancer (Rodriguez, et al., 1997).

Gene therapy strategies for cancer treatment may possibly improve the body's ability to fight cancer or make the cancer more sensitive to chemotherapy. It can apply to induce or augment an antitumor immunological reaction, to correct a genetic deficiency in the tumor cells, to make the malignant disease more susceptible to conventional therapies, and to make the normal host cells more resistant to conventional therapies (Harris, Gutierrez, Hurst, Sikora, & Lemoine, 1994; Rosenberg, 2001; Schmidt-Wolf, 2003). One of the main objectives of gene therapy is to treat disease which caused by loss or gain- of- function mutations by delivering a therapeutic gene into the cell nucleus, to improve and express the deficient gene product at physiological levels. However, is important that a therapeutic gene can be expressed over long periods of time, therefore, it must be maintained within the nucleus, replicated and passed on through subsequent cell divisions. To date, the difficulties in achieving sustained gene expression and in developing safe and efficient gene-delivery methods are the greatest obstacle gene therapy approaches (Glover, Lipps, & Jans, 2005).

Eukaryotic cells are protected against the uptake of exogenous nucleic acids by a series of cellular barriers that must be overcome before a delivered gene can be expressed in the target cell nucleus. Plasma membrane constitutes a major barrier for the entry of hydrophilic molecules into the cell interior. Selective and reversible permeabilization of this barrier is a prerequisite for many biotechnological applications (Hapala, 1997). The barriers for gene delivery include (a) degradation of exogenous nucleic acids in the extracellular space, (b) internalization of the gene- transfer vehicle, (c) intracellular trafficking from the endosome to the lysosome and the escape from the endosome, (d) dissociation of exogenous nucleic acids from the vector and (e) entry of the exogenous nucleic acids into the nucleus.

Further factors need to be considered, for systemic delivery purposes (in vivo), including physicochemical properties of vector that affect stability of therapeutic gene in the blood (i. e. particle size and zeta potential of the vector) and the role of immune system (i.e. trapping of the vector by reticuloendothelial system) (S-D Li and L Huang, 2006). Considering barriers to transfer gene into cells is needed to improve the design of gene therapy vectors and achieve an efficient gene therapy. These barriers can be generally classified as extracelluar, cell entry-related, or intracellular.

II. Classification of cellular barriers systems

2.1 Extracellular Barriers

The extracellular barriers come across with gene therapy vectors are dependent on the method of administration. Successful engagement of gene delivery involves avoiding inactivation of transgene in the extracellular compartment and initial favorable interactions with the cell surface. The administration of genes for therapeutic purposes can be made in vivo or ex vivo. In vivo administration includes systemic administration of the gene or vector into the patient or into the target organ, and potentially can be applied to any cell. While the ex vivo administration includes harvesting and cultivation of cells from patients, with in vitro gene transfer and reintroduction of transfected cells (Corbel & Rossi, 2002; Moisset & Tremblay, 2001).

Ex vivo treatments, in which a patient's cells are removed from his body for transduction, limit the potential obstacles by allowing the vector to interact with the target cell immediately and in the presence of fewer complicating agents. It is not surprising that most of the early clinical successes in gene therapy have involved ex vivo application (Gersting, et al., 2004). In vivo administration requires vectors to evade a greater variety and quantity of enzymes, immune cells, and clearing agents that can compromise their effectiveness.

On the other hand, the route of intravascular administration, injection through the artery or vein, may affect vector distribution as it provides the first capillary bed that the vector will meet. As the particle extravasation and interaction with endothelial cells will be drastically enhanced in the microcirculation because of the permeability characteristics of these vessels and the reduced velocity of blood flow (Lu, et al., 1999). Due to the extracellular barriers affecting in vivo gene delivery the delivery vehicles should be designed to overcome these obstacles. In systemic gene delivery the vectors should be designed to effectively recognize their target tissues and be ignored by other tissues. The size of vectors is important to consider which must be small enough to pass through small blood vessels without causing a blockage (Kwak, Kriven, Wallig, & Choy, 2004).

Moreover, the interactions between the vector and the blood components are limiting parameters affecting the stability of DNA complexes in blood, limiting the half-life and targetability of DNA complexes. The components of the complement system mediate the interactions through opsonization of intravenously administered DNA complexes. ; Plank, Mechtler, Szoka Jr, & Wagner, 1996). Further, the cellular component of blood may mediate rapid clearance of positively charged particles from the blood. Electrostatic binding of positive surface-charge particles to negatively charged erythrocyte membranes will usually end up in the lung (Kircheis, R. et al. (1999), while their opsonization with proteins of complement systems and coagulation cascade, Immunoglobulins, albumin, will be recognized by macrophages and will eventually be uptaken by the liver [Dash, P.R. et al. (1999); Mahato, R.I. et al. (1995)].

2.2. Cellular uptake

Cellular uptake by target cells following gene delivery is another concern to achieve a successful delivery. The cells surface molecules and also the size of vector can affect internalization of synthetic gene carriers. For synthetic vectors, which lack an inherent targeting mechanism, cell uptake is based on nonspecific interactions. Although the endocytosis has been established as the main mechanism for the internalization of non- viral vectors into the cells (Goodman, et al., 1996; Labat-Moleur, et al., 1996; Zuhorn, Kalicharan, & Hoekstra, 2002), this process is less efficient than receptor-mediated uptake (Zabner, Fasbender, Moninger, Poellinger, & Welsh, 1995). The application of receptor-mediated endocytosis is a promising approach to transfer the therapeutic gene into target cells.

For example, binding and internalization of asialoglycoproteins through high-affinity cell surface receptors on hepatocytes (Perales, et al., 1997). Also, targeting of transferrin receptors (Tf), an iron-binding glycoprotein, on the rapidly dividing cells has been used as a tumor-targeting ligand for gene delivery systems (Kakudo, et al., 2004; Wagner, Plank, Zatloukal, Cotten, & Birnstiel, 1992). The folate receptor is another example of receptors overexpressed in tumor cells, and it can be used for tumor targeting (Cho, Kim, Jeong, & Park, 2005; R. J. Lee & L. Huang, 1996).

2.3. Intracellular Barriers

After internalization of DNA complexes by receptor-mediated or adsorptive endocytosis, vector will face some intracellular barriers which should be overcome in order to achieve a successful gene delivery. In the cytoplasm a gene transfer vehicle needs to be enclosed within the endosomal or lysosomal membrane which can be separated from the cytoplasm this bottleneck in gene delivery can be responsible for the degradation of the internalized DNA.

Later, it should escape from the endocytic environment and be released to the cytosol and be transported from the cell cytoplasm to the region near the nucleus. Therefore, DNA complexes should be unpacked that will provide the nucleic acid access to the nucleus for transgene expression. Therefore, protecting plasmid DNA from cytosolic nucleases is one of the important characteristics for an ideal gene transfer vector (Lechardeur, et al., 1999). Viruses have mechanisms to overcome the endosomal barrier, viral proteins often contain membrane-active domains which mediate the delivery of the viral genome to the cytoplasm after their activation in the endosome (Plank, Zauner, & Wagner, 1998).

It has been indicated that the movement of DNA from the cytoplasm to the nucleus may be one of the most important limitations to successful gene transfer in vitro and in vivo (Brinster, Chen, Trumbauer, Yagle, & Palmiter, 1985; Capecchi, 1980; Escriou, et al., 1998; Mirzayans, Aubin, & Paterson, 1992; Zabner, et al., 1995). Based on the type of cells, between 30 to 60% of the intracellular DNA can transfer to the nuclear compartment. Therefore, different cells may provide different capacities for nuclear localization of DNA (James & Giorgio, 2000). To explain, degradation of free DNA in presence of cytoplasmic nucleases suggested to be the main reason that majority of cytoplasmic plasmids fail to reach the nucleus. Accordingly, the long persistence of DNA in the cytoplasm, results in less nuclear entry of DNA.

On the other hand, dissociation of DNA from the vector before nuclear uptake will be different based on the type of vector which DNA is complex with. It is reported that in lipoplex-mediated transfections, in the nucleus the DNA, that results in gene expression, is probably free of lipid while for certain polyplexes such as PEI, it can remain in complex with the DNA after transport into the

nucleus and leads to gene expression (Pollard, et al., 1998; Zabner, et al., 1995).

The final hindrance to the nuclear entry of plasmid DNA is nuclear envelope. The incompetent nuclear internalization of plasmid DNA from the cytoplasm was found more than 20 years ago. It is reported that comparison of gene expression by plasmid DNA injected into the nucleus and cytosol, resulted in transcription of 0.1- 0.001% of the cytosolically injected plasmid DNA.With regard to, the size of plasmid DNA (2–10 MDa), nuclear entry occurs less likely by passive diffusion in post mitotic cells. The plasmid DNA can pass the NPC through the same mechanism used for the active transport of polypeptides larger than 60 kDa (Delphine Lechardeura, 2005).

In dividing cells, the nuclear envelope rupture during mitosis allows for the nuclear importation of DNA from cytoplasm. The plasmids can be transported into the nuclei of nondividing cells via the nuclear pore complex (NPC) (Dowty, Williams, Zhang, Hagstrom, & Wolff, 1995). The macromolecular exchange between the nucleus and the cytoplasm occurs through the nuclear pore complex (NPC) which is signal-dependent, and involved a series of receptor proteins (Mattaj & Englmeier, 1998). The proteins necessary for plasmid nuclear uptake include importin α and β, and RAN (Wilson, Dean, Wang, & Dean, 1999). The Short nucleic acids, such as oligonucleotides, diffuse freely through the NPC, While, DNA entry in the nucleus is mediated by an active transport process through the NPC and is energy dependent (Hagstrom, et al., 1997; Ludtke, Zhang, Sebestyén, & Wolff, 1999; Wilson, et al., 1999).

Considering the barriers limiting gene delivery, the main objective in gene therapy via a systemic pathway is the development of a stable and non-toxic gene vector that can encapsulate and deliver foreign genetic materials into specific cell types such as cancerous cells with an efficient transfection efficiency. Synthetic gene therapy vectors are characteristically mixed of DNA with cationic lipid (lipoplexes) (X. Gao & Huang, 1996), cationic polymer (polycation) (K. W. Leong, et al., 1998; Sosnowski, et al., 1996), or polymer-lipid agents (lipopolyplex) (R. Lee & L. Huang, 1996; S Li, Rizzo, Bhattacharya, & Huang, 1998) so that condense the nucleic acid and protect it from damage in transport, and facilitate its uptake and processing by target tissues. The naked DNA is designed for direct intra-tissue injection while other types of non-viral vectors are designed for systemic or airway administration (Hart, 2000; Niidome & Huang, 2002; C. Wiethoff & C. Middaugh, 2003)..

III. Classification of gene delivery systems

Due to limitation affecting effective naked DNA delivery the development of gene transferring systems should be critical to successful gene transfer. In general, gene delivery systems are divided into two classes: non-viral transfection systems and viral transduction systems (Xiujuan Zhang, W.T. Godbey).

3.1. Viral Systems

Viral vector has been shown high transfection efficiency in a variety of human tissues such as kidney, heart, muscle, eye, and ovary. Viruses used their innate mechanism of infection for cell entry and release the expression cassette (Kamiya H. 2001). The viral vectors investigated for delivery of therapeutic genes include adenoviruses, retroviruses, lentiviruses, and adeno-associated viruses. Viruses were used in more than 70% of human clinical gene therapy trials world-wide. Gene therapy using viral systems has made considerable progress for the treatment of a wide range of diseases, such as muscular dystrophy, AIDS, and cancer (Siddhesh D.2005).

3.1.1. Adenovirus vectors

The adenoviruses are non-envelpoed, double-stranded DNA viruses, known as the most commonly used vector (24.7% of all trials) (Michael L. Edelstein, 2007). Among 42 serotypes of adenovirus infecting humans; the serotype 5 is typically used in gene therapy, which the majority of the E1a and E1b regions removed to prevent virus replication. Adenoviruses are capable of carrying a larger DNA (up to 36 kilobase) load than retroviruses however their capacity is still too small to carry the genes required for certain clinical applications. Although transgene expression by adenoviral vector is transient, the main advantages of adenoviral vectors are their high efficiency of transduction and high level of gene expression.

These vectors also can infect non-dividing cells. Among the safety issues concerning the application of adenoviral vectors including; the presence of neutralizing antibodies in paients, the inflammatory and immune response caused by the vector, and receptor-independent uptake of vector by the reticuloendothe-lial system (RES) and the liver, the main one is the risk of stimulating a severe immune and inflammatory response, as was tragically occurred in an unsuccessful trial for OTC deficiency (Raper SE.2003; Yanzheng Liu, 2006). In order to reduce the function of RES to uptake adenoviral vectors several strategies have been studied such as inhibiting function of reticulendothelial cells by bisphosphonates and Gadolinium, application of reactive polymers to cover the charges of the adenoviral vector, and mutational modification of the vector fiber protein and the penton base proteins, to reduce uptake of vector into the hepatocytes (Fisher KD, 2000; Green NK, 2004; van Beusechem VW, 2005).

In addition the macrophages/dendritic cells and the complement system, several non-specific interactions occur between the Ad capsid and cellular

and non- cellular blood components, such as interaction Ad vectors with platelets, leading to thrombocytopenia after intravenous delivery and interactions with human erythrocytes. It has also been revealed that interaction of Ad with plasma proteins, in particular vitamin K–dependent coagulation zymogens, significantly affect vector biodistribution and transduction after systemic delivery (Morral, N. 2002; Wolins, N, 2003; Lyons, M, 2006; Shayakhmetov, DM, 2005; Parker, AL, 2006; Baker, AH, 2007; Stone, D, 2007). The chemical modification of adenovirus (Ad) gene transfer vectors with synthetic polymers promises to overcome most of the obstacles mentioned above (Florian Kreppel, 2008).

3.1.2. Retroviral vectors

Retroviral vectors are derived from wild type retroviruses and are engineered to carry a foreign gene of interest into a target cell. Retroviruses belong to a class of enveloped viruses containing a single-stranded RNA molecule as the genome. The principle specification of retroviruses is their replicative strategy, through reverse transcription of the viral RNA into linear double-stranded DNA following integration of the dsDNA into the genome of the host cell (John M. Coffin, 1997).

Two categories of retroviruses are based on the complexity of their genome; the simple retroviruses have only three genes, gag, pol, and env, whereas the complex retroviruses contain genes which code additional proteins responsible for regulating viral replication and interacting with the host cell immune response. For example, in addition to gag, pol, and env, the human immunodeficiency virus type1 (HIV-1) genome contains six additional genes: tat, rev, nef, vpr, vpu, and vif. Pathological patterns of retroviruses include three basic subfamilies: oncogenic retroviruses, lentiviruses, and spumaviruses. Murine leukemia virus, most oncogenic retroviruses, belongs to the simple retrovirus classification, while lentiviruses and spumaviruses (for instance, human immunodeficiency virus and human foamy virus) belong to complex group (John M. Coffin, 1997).

To prevent production and release of virions and subsequent pathogenic effects of retroviruses the vectors should be replication defective. Commonly, the retroviral vectors are composed of the therapeutic gene and the cis-acting elements of the viral genome, while the trans - acting viral genes (gag, pol, and env) are removed (K.A. Delviks, 1999). With regard to, oncovirus-based vectors, such as the murine leukemia virus, the cis-acting elements refer to att, LTR (U3, R, U5), the primer binding site (PBS), the packaging signal c, and the polypurine tract (PPT) which are necessary for viral gene expression and replication are included. For lentivirus-based vectors such as HIV-1, in addition to the above cis- acting elements, sequences that extend into the gag open reading frame are essential for packaging. Hence, HIV-1 vectors also have the relevant portion of gag in which the translational initiation codon for gag itself has been mutated. HIV-1 based vectors also contain a portion of the env gene that includes the Rev Response Element (R RE) (Wei-Shau Hu, 2000). Most retroviral RNA is maintained in bacterial plasmids therefore the manipulation and propagation of the vector DNA can be facilitated, it also contain a drug resistance gene that often used as marker genes to assist the selection of viral vectors much easier. The bacterial neomycin phosphotransferase gene (neo) is a frequently used marker gene in retroviral vectors (K.A. Delviks, 1999).

Retroviral vectors are known as the first vectors used in gene therapy however were used in only 28% and 22.8% of the trials in 2004 and 2007, respectively. Retroviruses integrate into the genome of the target cell and provide long term transgene expression. This type of vectors is capable of targeting dividing cells due to their intrinsic selectivity for proliferating cells (access to the cell nucleus during breakdown of the nuclear membrane). It is reported that retroviral vectors are able to transfect high populations (45%-95%) of primary human endothelial and smooth muscle cells which are generally extremely difficult to transfect. The Q vectors or self-inactivating (SIN) retroviral vectors are engineered to minimize the risk for occurrence of replication-competent retrovirus (RCR) (Michael L. Edelstein, 2007; Garton KJ, 2002). The studies has been shown that the modification of retroviral vectors by tumor vasculature targeting motifs containing Asn-Gly-Arg, could improve the binding efficiency and transduction of the vector to both human umbilical vein epithelial cells (HUVECs) and KSY1 endothelial cells (Liu L 2000).

Lentiviruses are a subclass of retroviruses. Lentiviral vectors are developed from the human immunodeficiency virus (HIV) by deletion of the nonessential regulatory genes and sequences through which homologous recombination can lead to the recombination of therapeutic vector with HIV. Different from retroviral vectors, lentiviral vectors can target both dividing and nondividing cells. Consequently these vectors are able to transduce terminally differentiated cells such as neurons, macrophages, and hematopoietic stem cells (Barker E. 2003).

3.1.3. Adeno-associated vectors

The adeno-associated viruses (AAV) are single-strand DNA virus and replication defective. They belong to the family of parvoviruses and require a "helper virus" for efficient replication (usually adenovirus or HSV-1). The short arm of chromosome 19 is the preferred integration site for this virus. The viral replication proteins (Rep) are essential for integration of AAV. In the presence of helper virus, the AAV viral genome replicates episomally, followed by viral protein synthesis while the intact AAV vectors integrate into the host-cell genomic DNA. The replication of viral genome in case of recombinant AAV vectors, used in gene therapy studies, is predominantly episomal (Yanzheng Liu, 2006).

AAV2 is known as the most widely used serotype in gene therapy, though other efficient

types such as AAV8 are under development. The serotype-specific differences in transduction are probably related to the differences in viral uptake and/or intracellular trafficking (GHOSH, 2007). The cell entry take place through the internalization of clathrin-coated pits and escapes endosomal degradation via acidification of the late endosome (F. P. Manfredsson, 2010; J. E. Ziello, 2010). AAV has been of interest, because of their broad transduction range in tissues such as liver, muscle, retina and the central nervous system and for their long-term expression mode (Rabinowtz, J. E., 1998). AAV causes either a low or undetectable level of immune response. The small capacity (5 kb) and the difficulties encountered in production of the vector for clinical trials are features that limit the use of AAV in gene therapy. The great feature of the AAV is the absence of a vigorous host immune response against the vector-infected cells result in prolonged periods of transgene expression in the target cells following infection (Yanzheng Liu, 2006).

3.2. Non-viral systems

Early efforts in vector design have focused primarily on genetically engineered viruses, such as retrovirus, adenovirus, and adeno-associated virus. Nevertheless, intrinsic hindrances related to viral vectors, such as immunogenicity and safety concerns have limited their clinical approval (Cusack & Tanabe, 2002). Non-viral synthetic vectors are, consequently, being constructed as substitutes to viral vectors (F. Liu & Huang, 2002). Compared to viral vectors, non-viral vectors are potentially less immunogenic, are comparatively easy to produce in clinical quantities, and are associated with fewer safety concerns (Mohanraj & Chen, 2007).

However, the strong promoters in viral vectors mediate high level of transgene expression and they are more efficient at introducing and maintaining foreign gene expression compared with non-viral vectors. Non-viral systems are cationic in nature which can interact with negatively charged DNA through electrostatic interactions. Their positive total charge enable them of efficiently interacting with the negatively charged cell membranes and internalizes into the cell, which occurs mainly through the endocytosis pathway (J. P. Behr, 1994). Various types of synthetic vectors have been developed for gene transfer. Several cationic lipid and polymer based vectors have been established to make complex with DNA for cellular internalization and protect DNA against degradation (Laura De Laporte, Jennifer Cruz Rea, 2004).

We have reported on the use of non-viral gene vectors in combination with three dimensional (3D) culture systems to enhance in vitro gene transfection [9-50].

3.2.1. Cationic polymers

Cationic polymers were introduced by Wu and Wu in 1987 (G. Y. Wu & C. H. Wu, 1987) and were further expanded by a second generation, PEI, by Behr, et al in 1995 (O. Boussif, et al., 1995) Polycationic vector neutralize the negative charge of DNA and decreases the electrostatic repulsion between DNA and cells, also they protect DNA from enzymatic digestion by nucleases in serum and extracellular fluids (Putnam, Gentry, Pack, & Langer, 2001). Compared with the cationic lipids, cationic polymers do not have hydrophobic moieties, but they can condense the DNA more efficiently resulting in smaller DNA condensed particles (Khalil, Kogure, Akita, & Harashima, 2006). The direct mixing of cationic polymers with DNA, results in electrostatic interactions between the cationic charge of the polymer and the negative charge of the DNA and the formation of particles, as small as 20 to 40 nm in some cases. The size and the charge of the polyplexes depend on more the ratio between the polymer and DNA than on the properties of the polymer (Choosakoonkriang, Lobo, Koe, Koe, & Middaugh, 2003).

Two ways are hypothesized for endosomal release of DNA by cationic polymer-based vectors. The first one is based on the physical disruption of the negatively charged endosomal membrane after direct interaction with the cationic polymer. This mechanism depends on cell type in terms of the target membrane composition. Such a mechanism has been suggested for both PAMAM dendrimers and poly-L-lysine (PLL) (Zhang & Smith, 2000). Another hypothesis is based on endosomal disruption by cationic polymers with ionizable amine groups which is called the ‘‘pro-ton-sponge’’ hypothesis (O. Boussif, et al., 1995) **(Figure. 1).** The ATPase enzyme in endosomal membranes actively transports protons from the cytosol into the vesicle which results in acidification of the compartment (Grabe & Oster, 2001).

The accumulation of protons in the vesicle leads to an influx of counter ions which causes osmotic swelling and the endosomal membrane rupture, following by releasing the polyplexes into the cytoplasm (O. Boussif, et al., 1995; Maxfield & Yamashiro, 1987; Yamashiro, Fluss, & Maxfield, 1983). The polymers such as PEI, containing a large number of secondary and tertiary amines, may act through this mechanism as they can buffer the pH, causing the ATPase to transport more protons to reach the desired pH.

Polycations commonly used in gene delivery and transfection include polyethylenimine (Remy, et al., 1998), poly(L-lysine) (G. Wu & C. Wu, 1987), poly- brene (Mumper, et al., 1996), gelatin (K. Leong, et al., 1998), and cationic polysaccharides (Azzam, et al., 2002). Among the many cationic polymers available, the most frequently used in gene delivery are poly-L-lysine (PLL)- and PEI-based polymers (O. Boussif, et al., 1995; G. Wu & C. Wu, 1987).

Most polycations are toxic to cells and nonbiodegradable, in the advanced polymeric gene delivery systems, high cationic charge density macromoleculs act as endosomal buffering systems, as a result they suppress the endosomal enzyme activities and protect the DNA from degradation.

The high cationic charge mediates DNA condensing and buffering capacities therefore the requirement for the addition of endosomolytic agents will decrease (J. Behr, 1997; O Boussif, et al., 1995). The activity of polycation is related to their molecular weight, polymer type, polymer-DNA ratio and molecular structural. Cationic polysaccharides are known to be the most attractive candidates among the various polycations which tested in gene delivery and transfection. They are natural, non-toxic, biodegradable, and biocompatible materials and can be modified easily for improved physicochemical properties (Berscht, Nies, Liebendörfer, & Kreuter, 1995; Carreño-Gómez & Duncan, 1997).

A new type of biodegradable polycation were constructed based on grafted oligoamine residues on natural polysaccharides, they are effective in delivering plasmids for a high biological effect. The biodegradable polysaccharide carriers are particularly appropriate for transfection and biological applications for the reason that they are water soluble, can be readily transported to cells in vivo by known biological processes, and perform as effectual vehicles for transporting agents complexed with them (Azzam, Eliyahu, Makovitzki, & Domb, 2003).

The Dextran-spermine polycations are prepared by reductive amination synthesis between oxidized dextran (dialdehyde derivatives) and the naturally occurring tetramine spermine (Hosseinkhani, Azzam, Tabata, & Domb, 2004). The spermine residues in Dextran-spermine (D-SPM) polycations play a crucial role in cell transfection. Therefore, D-SPM conjugate are active in transfecting a wide range of cell lines in vitro. Modification of D-SPM conjugates with polyethylene glycol (PEG) induced high gene expression in liver after intravenous injection compared to D-SPM which showed no expression in all organs. Generally, PEGylation of dextran–spermine polycations showed remarkable increase in the complex stability and transfection efficiency of the polycations in serum-rich media (Hosseinkhani, et al., 2004).

Polyethylenimines (PEI) is a polycation based transfection vector. PEI is stable, easy to handle, and inexpensive cationic polymer. The high positive charge density of PEI facilitated the binding of anionic DNA within the physiological pH range. Therefore, PEI is able to form smaller complexes with DNA. Taken together, PEI is considered as a notable vector for non-viral gene delivery (O Boussif, et al., 1995; Dunlap, Maggi, Soria, & Monaco, 1997; Kichler, Leborgne, Coeytaux, & Danos, 2001). PEI can mediate efficient gene transfer without the use of an endosome-disruption component possibly due to its intrinsic endosome-buffering property. PEI has been used for in vivo gene transfer via different routes of administration such as lung instillation, kidney perfusion, intracerebral injection and i.v. administration. Targeted gene delivery has also been reported by conjugating a ligand to PEI. Interestingly, PEI of different molecular weights and isoforms (branched or linear) differs with respect to their in vivo transfection efficiency and toxicity. Further study on the structure–function relationship may lead to the discovery of a new class of polymers that are more efficient and less toxic in vivo. Many factors affect the efficiency/cytotoxicity profile of PEI polyplexes (and almost any non-viral vector) such as molecular weight, degree of branching, ionic strength of the solution, zeta potential and particle size (Kircheis, et al., 1999; Kunath, et al., 2003).

PLL polymers are one of the first cationic polymers employed for gene transfer (G. Y. Wu & C. H. Wu, 1987). They are linear polypeptides with the amino acid lysine as the repeat unit; thus, they possess a biodegradable nature. This property is very useful for in vivo applications. PLL polyplexes are, however, rapidly bound to plasma proteins and cleared from the circulation (Dash, Read, Barrett, Wolfert, & Seymour, 1999; Ward, Read, & Seymour, 2001). PLL polyplexes have poor transfection ability when applied alone or without modifications (Brown, et al., 2000; Shewring, et al., 1997). One popular modification that can increase both the transfection ability and the circulation half-life of these vectors is coating with PEG (Lee, Jeong, & Park, 2002; Ward, Pechar, Oupicky, Ulbrich, & Seymour, 2002). Also, receptor-mediated strategies can improve the transfection efficiency of these vectors (Brown, et al., 2000).

Recent studies have shown that natural biopolymers such as Dendrimers, gelatin, and chitosan polymers can also form stable nanoparticles upon mixing with DNA (Roy, Mao, Huang, & Leong, 1999; Tang, Redemann, & Szoka Jr, 1996). Improved gene expression has been observed as compared with naked DNA when these DNA nanoparticles are administered intratracheally, intramuscularly, or intragastrically.

3.2.2. Cationic lipids

Gene transfer using cationic-lipid–pDNA complexes (also known as cationic lipoplexes) has emerged as one of the most versatile tools for delivering therapeutic genes and many other drugs, and is being tested in preclinical and clinical trials (S. Li & Ma, 2001). Cationic lipoplexes are easy and inexpensive to produce; they are made up of non-toxic and non-immunogenic precursors and they can deliver large polynucleotides into somatic cells. The

cationic lipids were used for first time in 1987 as a synthetic carrier to deliver gene into cells (Felgner, et al., 1987). Cationic lipids are composed of three basic domains, the polar head group which is positively charged, a linker which connects the cationic head group with the hydrophobic anchor (the nature and length of linker may impact on the stability and the biodegradability of the vector), and a hydrophobic part which composed of a steroid or of alkyl chains.

The role of cationic head group is to promote interaction with DNA, while the self-association of hydrophobic part involves in constitution of either micelles or liposomes in the presence of a helper lipid, such as dioleylphosphatidylethanolamine (DOPE) or cholesterol. Modifications of the hydrophobic domain can be involved in optimal vector structure moiety. DOPE is frequently useful because it can fuse with other lipids when exposed to a low pH, such as in endosomes, which aid in the release of the associated DNA into the cytosol (Cullis, Hope, & Tilcock, 1986; Farhood, Serbina, & Huang, 1995). Cholesterol provides structural stability, and there is evidence that it can influence targeting in vivo via scavenger receptors (Allen & Chonn, 1987; Hug & Sleight, 1991). Linkers are sensitive to various biological stimuli, inducing DNA release at defined time-points during the intracellular trafficking of the lipoplex. Lipoplexes form a multilayered structure containing plasmid sandwiched between the cationic lipids (Ewert K, Ahmad A, 2005; Martin, M. Sainlos, A. 2005). Different design of the cationic head group have been described (e.g. type of amine, linear or T-shape), hydrophobic domain (e.g. two linear C8–C18, cholesterol), and linker (e.g. degradable, tunable) for optimized transfection (Tranchant I, Thompson B. 2004).

Cationic lipids are typically used in the form of cationic liposomes. however, cationic lipid emulsions have been described and evaluated as potential non-viral gene carriers (Yi, et al., 2000). In early studies, DNA was encapsulated in neutral or anionic liposomes without changing the structures of the liposomes (Guo & Lee, 2000). Polyplexes are made from direct mixing between cationic liposomes and DNA solutions lead to binding of positively charged liposomes to negatively charged phosphate molecules on the DNA backbone through electrostatic interactions (Felgner, et al., 1987). The ratio between the cationic charge of the liposome and the negative charge of the DNA usually controls the size of lipoplexes (Radwan Almofti, et al., 2003).

The size of cationic liposomes are typically around (100 nm) and after forming complex with DNA, it change to the range from as small as 200 nm to structures as large as 2 μm (Wasan, Reimer, & Bally, 1996). Commonly used cationic lipids are 1, 2-dioleoyl-3-trimethylammonium propane (DOTAP), 3β (N-(N', N'-dimethylaminoethane)-carbamoyl) cholesterol (DC-Chol), N-[1-(2, 3-dioleyloxy) propyl]-N, N, N-trimethylammonium chloride (DOTMA). Cationic lipids in liposomal formulation serve the same function as cationic polymers to form a complex with anionic DNA and also enhance complex association due to the positive charges. The positively charged lipoplexes can cause more efficient gene expression through ionic interactions with the negative cell surface. In addition to the compaction and neutralization of DNA, cationic liposomes provide a protective role against extra- and intracellular nucleases. This can be attributed to the compaction and covering of DNA by the lipid bilayers (Eastman, et al., 1997).

Cationic-lipid–plasmid complexes are effective in vitro transfection agents, but they have limited capacity for systemic application. In addition, the positive charge of cationic lipids, which improves transfection efficiency in vitro, they are susceptible to interaction with negative constituents in the blood circulation including various proteins, which is significant limitation for their systemic administration. Cellular uptake represents the first barrier to cell transfection, it is generally accepted that the lipoplex is taken up by cells mainly through endocytosis although some studies suggest that the complex can directly penetrate the cell membrane (Whitmore, 2000). The cell surface molecules involved in the interaction with lipoplexes have not been thoroughly identified. It is believed that Proteoglycans (PGs) on the cell membrane may play an important role as cells deficient in PG synthesis are more difficult to transfect. Although it is expected that smaller-sized lipoplexes are more efficiently internalized via endocytosis, it is reported that larger lipoplexes have more ability to improve transfection activities which can be due to their ability to sediment onto the cell surface. However, the larger complexes are more susceptible to interaction with extracellular components. Therefore, it affect their ability to reach the target cells and make their in vivo transfection activity weak (Khalil, et al., 2006; Y. Liu, et al., 1997).

To overcome the barrier involved in the release of DNA from endosome cationic lipids may facilitate DNA release through their detergent and/or buffering properties. It is hypothesized that the endosomal escape of cationic lipids may be through the electrostatic interactions between the cationic lipids and the endosomal membrane leading to the displacement of anionic lipids from the cytoplasm-facing monolayer of the endosomal membrane, by way of the so-called flip-flop mechanism **(Figure. 1).**

A neutral ion pair formation between anionic lipids of the endosomal membrane and the cationic lipids results in decomplexation of the DNA and finally its release into the cytoplasm (Xus & Szoka Jr, 1996; Zelphati & Szoka, 1996). Addition of a fusogenic helper lipid such as DOPE facilitate the formation of a destabilizing hexagonal phase with the endosome membrane, and enhance gene expression by promoting the release of DNA from the endosomal compartment (Zabner, et al., 1995). The in vivo gene transfer efficiency of lipoplxes varies depending on

the route of administration affecting the physical properties of lipoplexes due to their interaction with biological fluids. For intratumor injection due to limited contact with biological fluids, the physical properties of lipoplexes may not change before encountering tumor cells. In contrast, by i.v. administration, the size of lipoplex, structure, and net charge is expected to change considerably before reaching to the target cells.

The biological fluids interactions with lipoplexes such as the effect of serum on the rate of aggregation and subsequent disintegration may depend on the structure of cationic and helper lipids. This can be explained, why cationic lipidic vectors of different lipid compositions have a dramatic difference in transfection efficiency following i.v. administration. Another limitation with systemic gene delivery of lipoplex is the rapid clearance of lipoplexes by the reticuloendothelial system (RES) or their accumulation within the lung tissue.(Ishiwata, Suzuki, Ando, Kikuchi, & Kitagawa, 2000) which this limitation can be improved by incorporating polyethylene glycol (PEG) lipids into the lipoplex, leading to increased circulation time of the lipoplexes and allowing protein expression in distal tumors (Anwer, et al., 2000). Understanding of the obstacles in systemic delivery of lipoplexes has led to the development of several novel formulations that are more efficient.

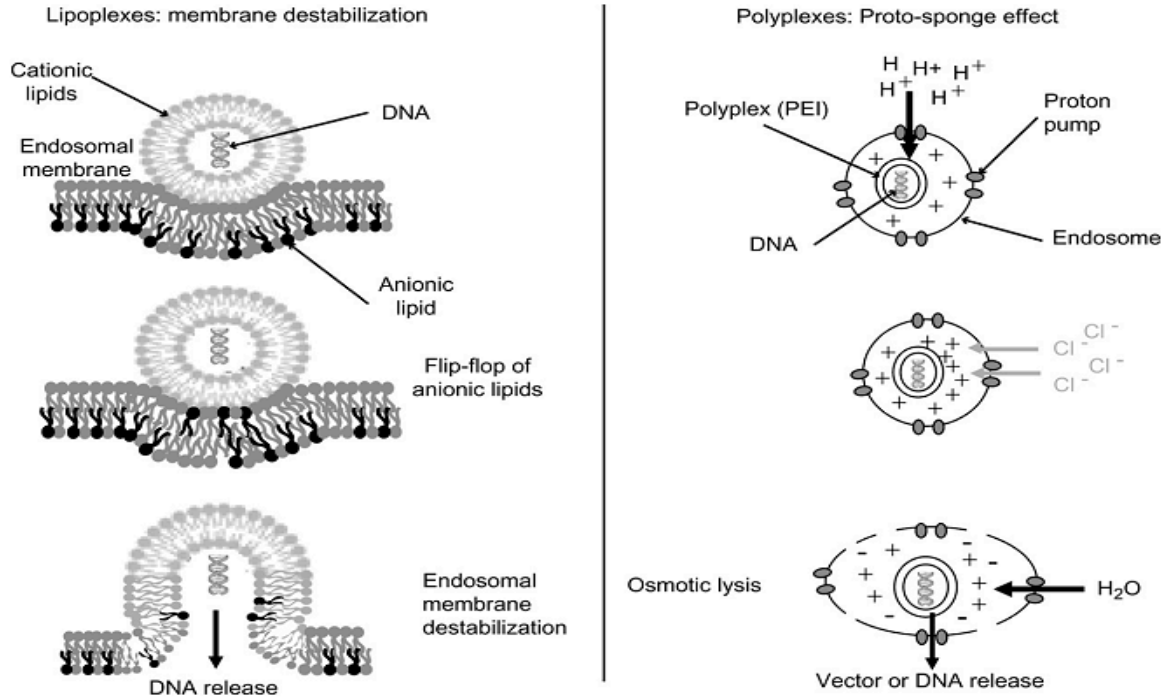

Figure 1: Hypothesis of endosomal escape of lipoplexes' and polyplexes' gene delivery systems Biomaterials,2008, 29(24-25).

3.2.3. Lipopolyplexes

Lipid-polymer-DNA complexes known as LPDs or lipopolyplexes are developing nonviral vectors for gene delivery. Lipopolyplexes are triplex complexes made with liposomes, a cationic polymer and nucleic acids which reported to be efficient construction to deliver DNA ([Gao and Huang, 1996], [Li and Huang, 1997], [Dileo et al., 2003] and [Vangasseri et al., 2005]). It is reported that packaging of therapeutic gene with combination of polycation and liposomes causes less toxic and more efficient in vitro gene transfer which protects DNA to a greater extent from nuclease degradation compared with cationic liposomes alone (Ibanez et al., 1996; Shangguan et al., 2000). LPDs should usually prepare just before their use (Mathieu Berchelc, 2012). Recently, a number of studies have attempted to analyze physicochemical properties of lipopolyplexes with different design of polycations and liposomes to enhance transfection efficiency of these vectors (Mathieu Berchelc, 2012, Federico Perche, 2011; Nie Y, 2010).

Conclusion:

Considering the role of gene therapy as an effective strategy to treat disease, understanding intracellular trafficking of exogenous DNA is essential to overcome many of barriers hindering efficient transgene expression. Non-viral gene delivery using liposomal and polymer based systems has been studied extensively, the main issues associated with these vectors is their efficiency. A number of studies evaluating various designs of viral and non-viral vectors have been performed to develop appropriate gene delivery systems that give high

levels of gene expression with monitoring harmful side effects when administered to treat a specific disease. Therefore, it is likely to achieve further gene therapy successes in the near future by developing perfect vectors with properties described for a successful and safe gene therapy.

Discussed in this article are a few applications and the advantages of biomaterials in gene therapy. Even though there are certain technological hurdles, these can be overcome by understanding the drawbacks of theindividual systems and finding an alternative to overcome these drawbacks. However, there is necessary to create new and other alternative methods if we face to any problems using the current technology in delivering biomolecules; such as proteins, growth factors, and DNA. Such systems have previously been created as one alternative method to enhance the *in vitro* and *in vivo* localization of biomolecules. [1-27].

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