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Advanced Materials for Gene Delivery

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Abstract. Gene therapy is a widespread and promising treatment of many diseases resulting from genetic disorders, infections and cancer. The feasibility of the gene therapy is mainly depends on the development of appropriate method and suitable vectors. For an efficient gene delivery, it is very important to use a carrier that is easy to produce, stable, non- oncogenic and non-immunogenic. Currently most of the vectors actually suffer from many problems. Therefore, the ideal gene therapy delivery system should be developed that can be easily used for highly efficient delivery and able to maintain long-term gene expression, and can be applicable to basic research as well as clinical settings. This article provides a brief over view on the concept and aim of gene delivery, the different gene delivery systems and use of different materials as a carrier in the area of gene therapy.

Introduction

Genes are specific sequences of bases, is the protein that carries out everyday life functions and even make up the majority of cellular structures. When genes are unable to carry out their normal functions, genetic disorders can results. Gene therapy is a technique where the disease has been treated by correcting defective genes. The process involves the introduction of a nucleic acid sequences into a cell to modify the expression of a gene in that cell. There are several approaches available that can modify the mutant or infected genes such as a) inserting normal gene into a non-specific location within the genome, and replace a non-functional gene, b) an abnormal gene could be removed from desired anatomical sources, and then swapped for a normal gene through homologous recombination, c) the defective gene could be repaired to its normal function through selective reverse mutation.

Gene therapy has attracted considerable interest to the researcher for the treatment of several diseases arising from genetic deficiencies or disorder such as cancer [1], cardiovascular disease [2], haemophilia [3], cystic fibrosis [4], β-thalassemia [5], muscular dystrophy [6], malignant tumors [7], including ovarian carcinoma [8] and HIV [9] for many years. Gene therapy has also been considered as a suitable substitute for conventional protein therapy [10]. Gene delivery for therapeutic application currently involves two strategies namely corrective or cytotoxic gene therapy. The corrective gene therapy involves the correction of genetic defects in target cells to treat a disease with single gene disorders. On the other hand, the cytotoxic gene therapy follows the destruction of target cells using a cytotoxic pathway, which can potentially be used for the treatment of uterine leimyomata and malignant tumors [4]. Despite the many research groups exploring this field, overall research progress has not lived up to the expectation in clinical trial since its first trial begins in 1970 [11, 12]. Current gene therapy is experimental, and so far the Food and Drug Administration (FDA)

in the United States has not yet approved any human gene therapy product for sale. Some of the reasons include the lack of efficient gene carriers, too rapid clearance from blood circulation, and safety concern in clinical trial. In general, all the research groups are working for the development of suitable gene therapy techniques with an objective to find an effective therapeutic gene [13] and an efficient and safe *in vivo* transfer of the genetic materials without premature degradation in systemic blood stream to the targeted tissues or organs [14].

DNA Delivery Process

The discovery of Deoxyribonucleic acid (DNA) and its function has offered us with an un-imaginable and endless possibility in various fields of science with an implication of progress of life that lead to diverse functional applications. Biotechnology is one of the many fields that has strongly benefitted from the possibilities of genetic engineering. Gene technology has facilitated the efficient production of pharmaceutical products. As a medicinal product, DNA can be delivered to patients target cells using either viral or non-viral mediated vectors. Viral vector is a virus that has been genetically altered to carry normal DNA by encapsulating and delivering their genes to target cells in a pathogenic manner. Different types of viruses are used as gene therapy vectors. On the other hand, non-viral vector method involves, the DNA molecules are usually condensed and/ or complexes with cationic transfection reagents, and then DNA constructs are engulf by the cell membrane through endocytosis and finally release the genetic materials into the target cells. Other non-viral mediated system involves creation of artificial lipids sphere with an aqueous core, which carries the therapeutic DNA, and reached to the patients target cell membrane. However, this delivery system tends to be less effective than other options.

DNA Transfection

Transfection is the delivery of DNA, Ribonucleic acid (RNA) and macromolecules into the patient target cells. A technique originally developed to allow the infected cells uptake of purified DNA rather than by intact viral or non-viral particles. In gene therapy, the DNA can be administered either by *in vivo* or *ex vivo*. *In vivo* process, the gene or vector can directly be administered into the patient or target organ, and potentially can be applied to any cell. The *ex vivo* administration includes harvesting and cultivation of cells from patients, with *in vitro* gene transfer and reintroduction of transfected cells. In pharmaceuticals or in basic research, the successes of gene therapy, either *ex vivo* or *in vivo*, mainly depends on DNA delivery to the target cells accompanied with a high level of transfection efficiency, which depends on the composition of the gene expression system. Usually the number of cells receives and express the DNA in the respective nucleus is small, and it is very difficult to improve the efficiency of DNA delivery. Moreover, correlation between the *in vitro* and *in vivo* transfection efficiency is not easy to establish [15, 16] and making conversion of positive results in cell culture into animals even more difficult. The low efficiency of DNA delivery from outside the cell to inside the nucleus is a natural consequence of this multi-step process, which involves low uptake across the plasma membrane, inadequate release of DNA molecules with limited stability, and lack of nuclear targeting. There are number of groups working using different methods for improving DNA uptake, enhancing penetration across the plasma membrane, approaches for optimizing protection and intracellular release of DNA, and ways of enhancing targeting of DNA to the nucleus.

Gene Delivery Systems

Gene therapy has the potential to improve therapeutic outcomes for currently untreatable diseases. The feasibility of widespread and well deserve conceptual gene therapy applications depends upon the development of appropriate methods for gene delivery. The main obstacle in the field of gene therapy is to find suitable vectors [17]. Currently most of the vectors actually suffer from many in directive problems such as lacking of specified cell targeting *in vivo*, inefficient long-term expression [18], and low transfection rates [19]. In addition, the vectors also grieve from achieving the optimum

packaging capacity and sufficient size of the vectors and immunogenicity. Therefore, the ideal gene therapy delivery system should have the following criterion; it should be injectable, targetable to specific sites *in vivo*, easy to be regulated, and able to maintain long-term gene expression, and should be non-immunogenic [13]. The conventional and currently engross gene delivery systems can be divided into viral and non-viral vectors with specific advantages and disadvantages [20].

Viral Vectors. Viral vectors were considered to be a potential vehicle to be explored due to its natural ability to transport their genomic DNA by encapsulating and delivering their genes to target host cells in a pathogenic manner. The process involves, the viral vector infected the patients target cells, and then viral vector off load its genetic materials (therapeutic gene) into the target cell, followed by the generation of functional cell from the therapeutic gene, and restores the target cells to a normal state. A variety of virus vectors [21, 22]such as retroviruses, lentiviruses, adenovirus, and adeno-associated virus have been extensively studied in laboratory-based research as well as in clinical trials, to deliver therapeutic genes into target living cells for displaying higher efficiencies (usually > 90%) in expression as well as delivery. Among the most studied viruses, retroviruses have been studied extensively [23]. The retrovirus entered into the cell through the interaction between receptor cell surface and the viral envelope glyco proteins [24]. Adenovirus offers an alternative way to introduce the genetic material into the target host cells. The adenovirus entered into the target host cells and produces a wide range of human infection that includes acute febrile upper respiratory infections, keratoconjunctivitis, and hemorrhagic cystitis, and then viral vector off load its genetic materials through cell lysis [25]. Considering its most attractive features, researchers made tremendous efforts to use the adenovirus in both differentiated and non-differentiated types of cells, as well as non-dividing cells and hematopoietic cells.The adenovirus is most effectively applied in cytotoxic gene therapy. The herpes simplex virus (HSV) along with thymidine kinase has also been studied in clinical trials using in situ retroviral delivery in malignant brain tumours [26] and in prostate cancer [27]. These trials have provided useful data but definitive clinical efficacy has not been marked [28]. Despite the promise of the technique, several issues like immunogenity and their oncogenic potential [29, 30], reproducibility [31], high risk in toxicity [32], cost effectiveness and non-specific uptake [33] must be resolved before consider them for long term applications.

Non-viral Vectors. As the concept of gene therapy expanded, gene therapy trigger its potential to treat currently untreatable disease that caused by genetic disorders. Viral vectors are naturally considered as an efficient vector for gene therapy, but have encountered several obstacles in clinical applications due to the issues such as high risk in toxicity, high production cost, non-specific uptake, immunogenicity, and potential gene integration into oncogenic regions. Considering these above mentioned concerns, non-viral gene delivery materials such as cationic lipids and polymers, have been extensively studied as gene delivery vectors and were found to be a promising attractive alternative to viral vectors [34]. In non-viral vector system, the genomic DNA molecules normally complexes with cationic transfection reagents, and then DNA are engulf by the cell membrane through endocytosis and release the genetic materials into the target cells. The non-viral vectors show advantageous properties in their low immunogenicity, the absence of endogenous virus recombination, low production cost and reproducibility [35]. In addition, Non-viral vectors do not depend on the size of DNA for packaging and the possibility of modification with ligands for tissue- or cell-specific targeting. Non- viral gene carriers are generally based on three categories: (i) direct DNA delivery, (ii) lipid-based and (iii) polymer-based delivery, which exist a steady driving force as gene delivery vehicles. In this review article, we will discuss the recent advances in gene delivery enlightening the focus of non-delivery vectors consisting of DNA and lipids or polymers for gene delivery.

Present Status of Non-viral Gene Delivery System

Naked DNA Delivery. Although all of the gene therapy techniques are currently under development, the simplest system involves the direct injection of naked DNA into target cells/organ, which has been under investigation for more than 40 years. The direct delivery method was useful in delivery of DNA into skeletal muscle [36], liver [37], heart muscle [38] and tumors [39]. Direct DNA delivery method has also been used in the development of the DNA tumor vaccines [40]. However, naked genes are rapidly degraded by nucleases and show poor cellular uptake [41]. Moreover, DNA and RNA are a negatively charged species and therefore don't freely passed through the negatively charged lipid cell membrane, due to electrostatic repulsion. To overcome these problems, an appropriate DNA carrier is one of the prerequisites for the success of gene therapy [42, 43]. In search for DNA carrier system that can provide safe and efficient gene delivery continues to be one of the most challenging tasks of gene therapy. The model DNA carrier should possess the following properties; a) should not be toxic or induce an immune response, and able to stabilize the DNA before and after intake, b) ability to assemble or form a stable complex or packed *in vitro*, c) must be stable enough to resist extra and intracellular enzyme (protects DNA from enzymatic degradation), d) capability of binding and passing or allowing penetration through target cell membrane by efficient nuclear targeting, e) capable to release the therapeutic DNA or RNA into the cell nuclei resulting in gene expression, f) the carrier should be mono-disperse, and less than 200 nm in diameter.

Wolff et al. [44] injected a 5% sucrose solution containing DNA into the quadriceps muscle of a number of mice and found dose-related expression that lasted for several days. However, this approach limited in its application because it can be used only with certain tissues and requires large amounts of DNA. Chang et al. used particle- mediated gene transfer (or particle bombardment) technique, which is based on coating microscopic gold particles with DNA and firing them into tissue [45]. However, it limits their long-term applications due to low level of expression and immunogeniety [46]. (Diethyl amino) ether (DEAE)-dextran [47] and calcium phosphate [48] chemicals were tried for interaction with DNA to form DEAE-dextran-DNA and Ca phosphate-DNA complex. However, both methods were hampered by cytotoxicity.

Lipid Based Gene Delivery. Lipid based gene delivery is one of the most intensively studied non-viral vectors, which appeared to be a promising technique to modulate cellular gene expression for therapeutic and research applications. They are positively charged at physiological pH, which are composed of a cationic lipid and a neutral lipid or cholesterol [49] and self-assemble with negatively charged DNA and form a positively charged complex, resulting in the formation of lipoplex [50]. Lipoplex interact with the cell membrane and internalize into the cell through endocytosis, which lead to the destabilization of the lipid complex, and results cytoplasmic delivery of the DNA. On the other hand, the use of anionic liposomes has been mainly restricted to the delivery of other therapeutic macromolecules [51].

Since 1983, researcher has been trying to develop and evaluate cationic lipids formulation for efficient gene transfer. N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) [52], [1,2-bis(oleoyloxy)-3-(trimethylammonio)propane] (DOTAP) [53], 3β[N-(N′, N′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol) [54], and dioctadecylamido glycylspermine (DOGS) [55] were widely used as a commercial reagent for cationic lipids. A neutral lipid, Dioleoylphosphatidylethanolamine (DOPE)is also often used in conjunction with cationic lipids to aid in endolysosomal escape [56].

N-[1-(2, 3-dioleyloxy) propyl]-N,N,N-trimethylammonium chloride, or DOTMA, was used as a lipofectin. To improve the transfection efficiencies of lipofectin, DOTMA was coupled with a neutral lipid dioleoylphosphatidylethanolamine (DOPE) in 1: 1 ratio. DOTMA was one of the first synthesized, well characterized and commercially available cationic lipids used for gene delivery. Different research groups were made their efforts to prepare modified DOTMA by varying the major functional moieties such as head group, linker, linkage bonds and hydrocarbon chains of DOTMA to reduce the toxicity and increase the transfection efficiencies [47, 57]. It was found that the

cytotoxicity associated with the formulated monovalent lipids were dependent on plated cell density and the structural aspects of the lipids [47]. {2, 3-dioleyloxy-N-[2(sperminecarboxamido) ethyl]-N, N-dimethyl-l propanaminiumtrifluoroacetate}, or DOSPA, is another cationic lipid synthesized as a derivative of DOTMA. DOSPA is structurally almost similar to DOTMA. The only difference between these two is that DOSPA has spermine group which is bound via a peptide bond to the hydrophobic chains that allows for a more efficient packing of DNA [58].

One of the best-studied cationic lipids in this setting is N- [1-(2, 3- dioleyloxy)-propyl]-N, N, N-trimethylammonium chloride (DOTAP) [52], which has been applied to *in vivo* genetic modification of a wide variety of organs in animals [59]. The main difference between DOTAP and DOTMA is that DOTAP linked the backbone by ester bonds rather than ether bonds, which is hydrolysable and help degrading the lipids and reducing toxicity. Wang et al were able to achieve good tumor specificity using a cationic lipid system consisting of DOTAP and cholesterol. This system results in at least 10-fold greater expression per mg of tumor tissue compared to liver tissue. When the suicide gene thymidine kinase from Herpes simplex virus is delivered systemically using this system, the tumors show a marked decrease in growth [60]. 3β [N-(N',N'-dimethylaminoethane)-carbamoyl] cholesterol, or DC-Chol, was first prepared by Gao and Huang in 1991 [61]. In contrast to DOTMA and DOTAP, DC-Chol contains a tertiary amine, which could help reducing the aggregation of lipolexes leading to higher transgene expression [62].

Di-octadecyl-amido-glycyl-spermine, DOGS, is commercially available under the name Transfectam, has a structure similar to DOSPA. DOSPA and DOGS both have a spermine group and two 18- carbon alkyl chains. The difference between these two is that DOSPA has a quaternary amine. In contrast, DOGS does not have quaternary amines, chains are saturated and linked to the head group through a peptide bond. DOGS has been used to transfect many cell lines. Behr et al. showed that DOGS is very effective in delivering the chloramphenicol acetyl transferase (CAT) reporter plasmid with no noticeable cytotoxicity [55].

Polyethylene glycol (PEG), one of the readily and widely used biomaterials with very low level of toxicity, presents many attractive qualities as a liposomal coating [63]. PEG has been used to modify the surface to improve the performance of lipofection, which can facilitate the protection from degradation *in vivo*. Kim et al. showed that serum containing PEGylated lipoplexes gives increased transfection efficiencies as compared to liposomal transfection without surface attachments [64]. In addition, the PEGylated lipoplexes display improved stabilities, decreased immune responses and longer circulation times in the blood, and allow the liposome to overcome aggregation problems through mutually repulsive interactions between the PEG molecules [65]. However, the main drawback with this liposomal system is the lack of specificity with regard to cellular targeting. Shi et al. reported that PEGylation inhibited endocytosis of the lipoplexes that depends upon the functional groups that were conjugated to the lipoplexes and the amount of PEG on the liposome [66]. These awkward findings trigger the research community to design novel PEG-containing liposomes where PEG will be removed following endocytosis via degradation. Alternative liposomal formulations utilizing polymers other than PEG are being produced with the goal of creating sterically protected lipoplexes. Metselaar et al. used L-amino-acid-based polymers for lipoplex modification. It was found that L-amino-acid-polymer based lipoplex extended circulation time and reduced clearance by macrophages at similar level to those modified with PEG [63]. In addition, theses oligopeptides showed increased biodegradability and favorable pharmacokinetics when lower concentrations are used.

Anionic lipoplexes are composed of physiologically safe components that include anionic lipids, cations, and plasmid DNA [67]. In general, gene delivery by anionic lipids is not very attractive and efficient. Commonly used lipids in this category are phospholipids that can be found naturally in cellular membranes such as phosphatidic acid, phosphatidylglycerol, and phosphatidylserine. Nicolau et al [68] generated liposomes consisting of phosphatidylcholine, phosphatidylserine and cholesterol, and loaded those with a DNA designed to express insulin, followed by intravenous injection into rats, a small increase in serum insulin was observed for less than one day. These

preliminary results demonstrated the feasibility of the approach, but restricted in next step due to difficulties involve the reproducible preparation of conventional liposomes.

Encapsulation of DNA into neutral and anionic liposomes has also been explored as non-viral delivery system. An anionic liposome cannot efficiently bind negatively charged DNA due to repulsive electrostatic forces that occur between the anionic head group of the lipids and the phosphate backbone of DNA. Therefore, DNA must be encapsulated to use the anionic lipids as cell specific targeting. However, the size and shape of the DNA matrix limits it encapsulated form of applications [69].

Various anionic liposomes have been well characterized for gene delivery in a small number of cell types including CHO cells and primary hippocampal neurons [69-71]. However, despite very promising and encouraging results, overall knowledge regarding anionic lipofection is as yet limited due to many difficulties, some of which will certainly challenge our scientific ingenuity. One of the main reasons is the lack of reproducibility. Moreover, due to their systematic administration, are associated with unwanted side effects due to non- specific cytotoxicity of the immune system that leads to a variety of undesired complications. Successful gene therapy of any disease will likely be an extraordinary task with the first successes involving gene therapy effectively combined with other conventional treatments. Therefore, the relative merits of any gene delivery system have to be evaluated carefully to achieve the final target.

Polymer Based Gene Delivery. Polymer based gene delivery carriers have been developed as a replacement of viral vectors for their avoidance of immunogenic and oncogenic problems. It gained significant attention in last two decades due to their great potential for the development of safe and efficient vectors [72]. It is well understood that polymers can be specifically designed for the proposed application as synthetic polymers offer the advantage of controlled synthesis, coupling of cell or tissue specific targeting moieties, and one can easily modify the properties that confer upon specific physiological or physiochemical properties by changing the condition of synthesis like hydrophobicity, charge density and cross linking density, and molecular weight.

A large variety of synthetic and natural polymers and copolymers have been explored to find their suitability for *in vitro* and *in vivo* gene delivery applications. Unfortunately, till to date no polymeric structure has been found to be a suitable one [73]. The insight into the relationship between the polymer structure and their biological performance, such as the DNA compaction, toxicity and transfection efficiency is still rather limited. Hence, the discovery of new potent materials still relies on empirical approaches rather than on a rational design.

The natural cationic polymer that includes cyclodextrins (CDs) [74], gelatin [75], collagen [76], dextran [77, 78], cellulose [79], chitosan [80, 81], and their modified derivatives has also been researched for gene delivery applications ranging from tissue engineering to vaccination therapy [82]. Cyclodextrins (CDs) are water soluble, relatively non-toxic, and non-immunogenic cyclic oligomers of glucose units. These unique properties of cationic CDs have been utilized for several therapeutic applications [83]. Gonzalez et al. synthesized and evaluated β-cyclodextrin- based cationic polymers as potential gene carriers [84]. It was found that the transfection efficiency of β-CD polymers was comparable to that of PEI and lipofectmine. Pun et al. studied linear and branched PEIs grafted β-CD as potential gene carriers [85]. It was found that the cytotoxicity of the modified polymer was greatly reduced along with increasing the grafting percentage of CD. However, the transfection efficiency was reduced with increasing the grafting percentage of CD. Gelatin, a natural polymer derived from collagen by acid or base treatment. It contains both positive and negative charges. However, the cationic property of gelatin appears due to the lysine and arginine residues. Gelatin is biodegradable and biocompatible in physiological environment, which makes it one of the versatile materials to be used in various pharmaceutical and medical applications [86, 87]. Morimoto et al. synthesized cationic gelatins by coupling ethylenediamine (EDA) or spermine through an EDC-mediated reaction that enable interactions with biomolecules of anionic nature [88]. Xu et al. applied cationic gelatin nanoparticles for the non-viral delivery to adult canine articular chondrocytes

in vitro [89]. It was found that chondrocytes transfected with IGF-1 maintain steady IGF-1 overexpression in cationic gelatin scaffolds for up to 2 weeks in three-dimensional culture. Inada et al. investigated the transfection of cationized gelatin and plasmid DNA complex into the monocyte-derived immature dendritic cells [90]. Fujii et al. synthesized a cationic gelatin conjugate using Hemagglutinating Virus of Japan Envelope and sodium borocaptate, and studied their safety, bio-distribution and effectiveness in a multiple liver tumor model for boron neutron capture therapy [91]. Dextran, an FDA- approved highly water-soluble branched polysaccharide composed of glucose units linked by α- 1, 6- linkages. The potential application of cationic dextran as a polymeric carrier has been exploited due to its biodegradability, wide availability, ease of modification and solubility in water.A series of cationic derivative of dextran namely glycidyltrimethylammonium chloride-dextran, diethylaminoethyl-dextran and dextran-spermine have been synthesized and evaluated their transfection efficiency for the delivery of nucleic acids [92]. Dextran- spermine and their derivatives have been found to be high transfection of plasmid DNA both *in vitro* and *in vivo* [93]. Cellulose, a linear β- 1, 4- D- glucan, possesses several unique characteristics that include hydrophilicity, biodegradability and antibacterial properties have been utilized for various therapeutic applications [94]. Song et al. reported the homogeneous quaternization of cellulose in aqueous solution by preparing a novel amphiphilic quaternized cellulose derivative, hydrophilically and hydrophobically modified quaternized cellulose, and evaluated as a potential gene delivery carrier [95]. The quaternized cellulose derivatives have been found to be promising materials for gene delivery applications.

Chitosan, a biodegradable linear polysaccharide composed of D-glucosamine and N-acetyl-d-glucosamine residues with β- 1, 4- linkage. Chitosan is one of the most reported non-viral naturally-derived cationic gene polymers, due to its biodegradability, biocompatibility, non-allergenicity, mucoadhesive property and strong affinity with DNA. Chitosan demonstrated low cytotoxicity and higher transfection efficiency than poly-L-lysine (PLL) in a series of tests performed on both experimental animals [96] and humans [97]. However, the low solubility of chitosan at physiological pH limits its therapeutic applications [98]. In order to increase the solubility of the chitosan, chemically modified chitosan derivatives have been synthesized and evaluated as a carrier for asialoglycoprotein receptor-targeted gene delivery [99]. The modified chitosan interact with DNA and formed spherical self-aggregates of an average diameter of 160 nm, showed efficient transfection for COS-7 cells. Belalia et al. synthesized and investigated quaternized chitosan. The quaternized chitosan was found to be improved the mucoadhesive properties depending on the degree of quaternization, which makes this modified chitosan derivative a good candidate for gene delivery [100]. Some neutral polymers such as pluronic block copolymers [101] have also been studied. They showed good transfection efficiency. However, the mechanism between these polymers and DNA has not been identified. The problem is complicated, since the neutral polymeric vectors are only efficient *in vivo* but not *in vitro*.

Among non-viral vectors, synthetic cationic polymers have been intensively studied for gene therapy applications for self-assembling ability with DNA, controlling the size to form desired nanosized particles, protects the DNA from degradation, easy to manufacture [102], conjugate to targeting ligands and to protect the genetic material during its transport through the blood stream to the target cells [103-105]. Various synthetic cationic polymers have been used to form polyplexes with DNA, which includes polyethyleneimine (PEI) [106], poly-L-lysine (PLL) [107], poly(amino-*co*-ester) (PAE) [108, 109], and polyamidoamine dendrimer (PAMAM) [110]. Polyethyleneimines (PEIs) , first introduced by Boussif et al. in 1995 [111], in linear or branched form, is one of the most commonly studied and considered to be the gold standard of non-viral vectors for delivering genes to the target cells due to high complex stability. PEIs offer a significantly more efficient transfection and protection against nuclease degradation than other polycations such as PLL. It could be due to the fact that PEIs have higher charge density and form more compact and efficient complexation. However, PEI showed significant cellular toxicities most likely due to its high amount of positive charges [112]. In addition, due to the non-degradable nature of PEI, it is not suitable for *in-vivo* use [113]. PLL with variable molecular weights is one of the first polymers that have been studied as a

potential non-viral gene delivery carrier [114]. Poly-L-lysine, lysine as the repeating unit, is linear polypeptides; thus they possess a biodegradable nature, which is very useful for *in vivo* applications. PLL has the ability to form a polyelectrolyte complex with DNA. PLL (pKa ~10.5) is protonated in physiological conditions, may ionically interact with negatively charged phosphate groups of DNA, and form a nano-particulate polyelectrolyte complex [115]. However, *in vivo* biocompatibility of PLL/DNA complexes may be limited since the use of PLL elicited an immune response [116], and rapidly bound to plasma proteins and cleared from the circulation [117]. Poly(amino-*co*-ester) is a class of synthetic cationic polymer and hydrolytically degradable polyamines with no negative effects on cell viability were developed and explored by Langer et al. for its potential application in DNA delivery [118, 119]. The functional groups at the chain end containing amine-terminated poly(amino-*co*-ester) enhanced cellular uptake and DNA delivery [112].

Polyamidoamine dendrimers are a class of highly branched cationic polymers, which are capable of condensing DNA, and delivering it to a variety of cell lines with minimum cytotoxicity [120]. Dendrimers are spherical, highly branched polymers, most commonly used in non-viral gene delivery. The 6- generation Starburst^{TM} PAMAM dendrimers, either in intact (Polyfect®) or fractured (Superfect®) form are the most commonly used dendrimers for non-viral gene delivery. The main advantage of structural feature of these dendrimers is that they have highly dense amine in the periphery of the molecule, which enable efficient condensation of nucleic acids, leaving the inner amine functions available for a proton sponge during endolysosomal acidification, thus enabling more efficient endosomal escape. These dendrimers show significantly enhanced (>50-fold) levels of reporter gene expression compared to the intact polymer. The reason for this finding is still unclear, however it could be one of the main reasons is that an increased flexibility of the polymer with a better ability to complex DNA helps increasing the gene expression [121].

Colloidal systems with a cationic poly(lactide-*co*-glycolide) (PLGA) surface containing microparticles coated with a cationic cetyltrimethylammonium bromide surfactant by a solvent evaporation process were also reported to deliver DNA vaccine [122]. However, the microparticles attached to DNA complexes were weak as the cationic surfactant was physically adsorbed onto the microparticle surface [123].

Amphiphilic copolymers can be considered as suitable gene/drug delivery carriers [124]. Numbers of systems containing block copolymers composed of dimethylaminoethyl methacrylate (DMAEMA) and PEG [125], a random copolymer of DMAEMA and poly(ethylene glycol) monomethyl ether methacrylate (PEGMA) [126], and DMAEMA-Oligo EGMA [126] have been studied for gene delivery as PEG based polymers show a high degree of biocompatibility, and their flexible chain can easily create a favourable atmosphere, which can minimize interaction with the blood. These intriguing properties allow the PEG coated particles to circulate in the blood for an extended period of time for preferential accumulation in affected areas in the body [127]. These copolymers showed a good ability to encapsulate genes. However, they were not stable, and disintegrating into unimers especially at high salt concentrations [126], or at a lower or physiological pH [128]. The stabilization of self-aggregates could be achieved in several ways by core cross-linking such as (a) entrapment of low molecular weight monomers with the hydrophobic block followed by polymerization, which is based on the synthesis of the polymer or polymeric gel in the core to form a kind of semi-IPN core. Entanglement of the core segments to the formed gel stabilizes the particle. However, it still takes physical force to stabilize the assembly [129], (b) introduction of the polymerizable group as the side chain of the hydrophobic segment followed by cross-linking reaction [130] or polymerization in the core [131]. However, this approach limits the DNA loading capacity and also affects in drug release [132]. The stability of the micelle could also enhance through covalent cross-linking in the shell without affecting the loading capacity in the core [133]. However, the degree of incorporating functional groups into the hydrophilic shell of cross-linked micelle is low and there is no test result available with the efficiency of presenting these molecular targeting elements on the micelle. Yang et al [134, 135] developed cationic core shell micelles from an amphiphilic copolymers consisting of cholesteryl side chains in the core and a cationic amine (quaternary and tertiary amine) in the main chain as formed the shell of the micelles for co-delivery

of drug and gene. However, grafting density of the neutral lipid cholesteryl was low as they are grafted on to the backbone of the polymer chain. MW cannot be controlled as they degrade during grafting at higher temperature. Moreover, DNA binding capacity appeared to low at physiological conditions as they bind to the rigid backbone of the polymer.

Conclusion

Gene delivery is a multi-step process, in which an appropriate property of carriers would be needed to go through each step. Therefore, a major motivation for gene therapy research has been in need to rationally designed multifunctional polymeric vectors, which can overcome a series of extra- and intra-cellular barriers to develop novel treatments for diseases with no effective conventional treatment.

Viral vector is one of the important transfection vector systems, which used as an effective way of DNA delivery and efficient target cell- specific transfection. In fact, viral vector based gene therapy involves ~75% of recent clinical protocols. However, except few, no definitive success has been credited for the clinical effectiveness of viral system [136, 137]. Moreover, toxicity, restricted targeting of specific cell types, limited DNA carrying capacity, production and packaging problems, and high cost limits the application of viral medicated delivery system [138-141].

The non-viral systems, especially polymeric DNA delivery systems, have become increasingly desirable and popular research tool for elucidating gene structure, regulation, and function in both basic research and clinical applications. There are several advantages in the non-viral medicated system that includes easy and scale up production, cost effectiveness, transport, store, and easy to modulate DNA loading capacity. Moreover, the non-viral vectors can easily protect DNA from degradation in the lysosome and blood stream. The majority of non-viral vectors involve synthetic materials that offer the advantage of easy and controlled synthesis, and can easily tuned the properties of the material such as block structure, hydrophobicity, charge density, cross-linking content, and molecular weight. Additionally, synthetic polymers are available with functional groups, which can be easily modified with surface ligands to achieve targeted gene delivery via receptor- mediated endocytosis. Although, these non-viral delivery systems achieve gene transfer *in vitro* and *in vivo*, they usually show some awkward behaviour due to the size and /or the positive charge of these complexes [142]. The positively charged complexes aggregate with negatively charged blood compounds such as albumin and erythrocytes, and these large aggregates get captured in the capillary blood vessels of different organs such as lungs and liver.

The main common disadvantage of both types of vectors, which limits their *in vivo* applications, is the rapid clearance from the blood circulation to the target organs. Usually cationic polymer-DNA complexes has the tendency to form aggregated particles, which results of large size particles (~μm) with broad distribution and get captured in the capillary blood vessel, and are not stable at physiological conditions. However, for an efficient gene delivery, it is obligatory to use a carrier that is stable, mono-disperse in size, not captured in the blood vessel and can prolong its circulation time in the blood. Therefore, the relative merits of any gene delivery system have to be evaluated carefully to achieve the final target by developing an ideal system with an efficient bio-distribution to first-pass organs, rapid clearance of complexes, lack of tissue-targeting, toxicity, nonspecific interactions etc. for gene delivery that can be easily used for highly efficient delivery/ expression and can applicable to basic research as well as clinical settings.

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