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Medical Polymer-Based Gene Therapy

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1. Introduction

Gene therapy provides great opportunities for treatment of diseases resulting from genetic disorders, infections, and cancer (Park, et al., 2006). Gene therapy has also been regarded as a suitable substitute for conventional protein therapy, since it can overcome inherent problems associated with administration of protein drugs in terms of bioavailability, systemic toxicity, *in vivo* clearance rate, and manufacturing cost (Ledley, 1996). Gene therapy refers to local or systemic administration of a nucleic acid construct capable of prevention, treatment, and even cure of disease through change of expression of genes responsible for the pathological condition (Bhavsar & Amiji, 2007). In theory, gene therapy is a simple concept that holds great promise as a cure for disease. However, in practice, considerable obstacles need to be overcome, including problems associated with safe and efficient gene delivery and stable gene expression. Many problems need to be solved in development of any gene therapy approach, including definition of cells that constitute the target, entry of DNA into those cells, expression of useful levels of gene product over an appropriate time period, and avoidance of the almost inevitable response of the host to the introduced materials, and so on (Grosshans, 2000, Smith, 1995).

Current gene therapy consists of two key factors: a gene that encodes a specific therapeutic protein, and a gene delivery system that controls delivery of gene expression plasmids to specific locations within the body (Mahato, et al., 1999, Park, et al., 2006). Due to several problems, including their instability in body fluids, non-specificity to target cells, degradation by enzymes, and low transfection efficiency, the lack of effective vectors is a major barrier to progress in gene therapy. Therefore, the ideal gene delivery method will be capable of high efficiency transfection of genes to a specific cell type; delivery to the nucleus, where it will become integrated into the host genome in a non-mutagenic fashion and be expressed or regulated; efficient transduction of cells, independent of the mitotic potential of the recipient; be non-infectious, non-toxic, and non-immunogenic; and be easy to manufacture and apply clinically (Chaum & Hatton, 2002).

Vehicles for gene delivery can be divided into two major groups: viral and non-viral vectors. Although such viral vectors have been commonly employed in clinical trials due to their high transfection efficiency, compared with non-viral vectors (Quong & Neufeld, 1998), their application to the human body is often frustrated by immunogenicity, potential infectivity, complicated production, and inflammation (Smith, 1995). Non-viral vectors involving use of cationic polymer and cationic lipid based carriers continue to enjoy a high profile due to the advantages offered by these systems, including safety, lower immunogenicity, and the ability to transfer larger DNA molecules, when compared with viruses (Anderson, 1998, Brown, et al., 2001). Previous efforts have focused primarily on cationic liposomes, such as *N*-[1-(2,3-dioleyloxy)propyl]-*N,N,N*-trimethyl ammonium chloride (DOTMA) (Felgner, et al., 1987), *N*-[1-(2,3-dioleoyloxy) propyl]-*N,N,N*-trimethyl ammonium ethyl sulphate (DOTAP) (Alexander & Akhurst, 1995), dimethylaminoethane-carbamoyl cholesterol (DC-Chol) and/or dioleoyl phosphatidylethanolamine (DOPE) (Farhood, et al., 1995) which incorporate with DNA and are transferred effectively into cell membranes. However, the major limitation of liposomes is their fast elimination from the bloodstream and localization in the reticuloendothelial system, primarily Kupfer cells of liver (Klibanov, et al., 1990). In addition, DNA/liposome complexes have been restricted due to cellular toxicity. Cellular changes, including cell shrinkage, reduced number of mitoses, and vacuolization of the cytoplasm (Friend, et al., 1996, Lappalainen, et al., 1994) and consequently leading to cell death via the apoptosis pathway, caused by lipoplexes, already been reported (Nguyen, et al., 2007). An alternative approach to development of non-viral vectors has been proposed for cationic polymers. In general, cationic polymers are widely accepted because of their ability for efficient condensation of DNA and interaction with cells due to the charge interaction between positively charged polymer/DNA complexes and negatively charged cellular membranes. Polymer/DNA complexes are more stable than those involving cationic lipids. In addition, they protect DNA against nuclease degradation (Jiang, et al., 2007, Jiang, et al., 2009).

Therefore, the objective of this chapter was to summarize the use of medical polymers, such as cyclodextrin, chitosan, polyethylenimine, poly(β-amino ester)s (PAEs), and their derivatives as non-viral vectors in the area of gene therapy.

2. Medical polymer-based gene therapy

2.1 Cyclodextrin

Cyclodextrins (CDs) are naturally occurring cyclic oligosaccharides composed of (1-4)-linked glucose units arising from enzymatic degradation of starch, which have been approved by the FDA for use as food additives (Mellet, et al., 2011). CDs comprised of 6, 7, and 8 glucose units are called α-, β-, and γ-CDs, respectively. Table. 1 shows the chemical structure and properties of α-, β-, and γ-CDs.

They feature a basket-shaped topology in which glucose hydroxyls orient to the outer space flanking the upper and lower rims, while methinic protons (H-5 and H-3), which point to the inner cavity cup-shaped cyclic oligomers of glucose, can form inclusion complexes with small, hydrophobic molecules (Forrest, et al., 2005). Due to their unique capability for formation of inclusion complexes in inner cavities, as well as many other favourable physicochemical and biological properties, natural CDs, and their derivatives have been applied in both drug delivery systems (Loftsson, et al., 2005, Uekama, et al., 1998) and gene delivery systems (Challa, et al., 2005, Dass, 2002, Redenti, et al., 2001).

The capability of CDs and their derivatives to interact with nucleotides is of great importance for exploitation of their properties of increasing resistance to nucleases as well

as delivery of genes. CDs can improve cellular uptake of genes and can also delay their degradation by increasing their stability against endonucleases. Zhao et al. reported that CDs can increase the cellular uptake of phosphorothioate ODNs (Zhao, et al., 1995). Cellular uptake of ^{35}S- and fluorescence-labeled antisense agents has been studied in human T cell leukemia cell lines (H9, CEM, or Molt-3) in the presence of CDs, including α-, β-, γCD, methyl-βCD, trimethyl sulfated βCD, HPγCD, HPβCD, hydroxyethyl βCD (HECD), trimethyl, sulfated βCD, and a mixture of various HPβCDs. Cellular uptake was found to be concentration and time dependent in the presence of CDs, and up to a two- and three-fold increase in cellular uptake was observed within 48 h. Interaction between βCD and cellular cholesterol in living cells was well reviewed by Zidovetzki et al. (Zidovetzki & Levitan, 2007). CDs can solve many of the problems associated with *in vivo* delivery of genetic materials, such as their limited ability to extravasate from the blood stream and traverse cellular membranes, high degree of susceptibility to endonucleases with potential toxicity of their breakdown products, polyanionic nature leading to nonspecific interactions with extracellular and intracellular cationic molecules, and potential immunogenicity (Challa, et al., 2005). For further efficient gene delivery, CDs were conjugated with cationic polymers. The most important feature of the CD-containing cationic polymer gene delivery system is that formation of polyplexes between polymers and DNA can be further modified by formation of inclusion complexes, since there are a large number of CD moieties (Davis & Brewster, 2004, Pack, et al., 2005). The first example of cationic polymers containing β-CD in the polymer backbone for gene delivery was reported by Davis and co-workers (Gonzalez, et al., 1999). β-CD containing cationic polymers efficiently condensed DNA to small particles and showed nontoxic and high gene transfection efficiency. The same group has developed a set of such CD-containing polymers and studied the structural effects of the polymers on gene delivery (Popielarski, et al., 2003, Reineke & Davis, 2003, 2003). In general, the CD-containing cationic polymers showed lower cytotoxicity and efficient gene transfection *in vitro*.

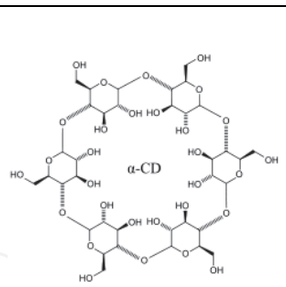
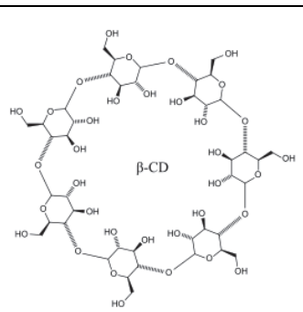
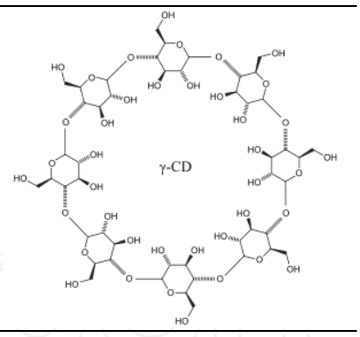
Chemical structure			
Cavity diameter (Å)	4.7 – 5.3	6.0 – 6.5	7.5 – 8.3
Molecular weight (Da)	972	1135	1297
Solubility (g/100 mL)	14.5	1.85	23.2

Table 1. Chemical structures and characterizations of CDs. CDs comprised of 6, 7, and 8 glucose units are called α-, β-, and γ-CDs, respectively.

The Uekama group synthesized dendrimer conjugates with α-, β-, and γ-CDs [Fig. 1], in anticipation of the following synergic effect; i.e., (1) dendrimer has the ability to complex

with plasmid DNA (pDNA) and to enhance cellular uptake of pDNA and (2) CDs have a disruptive effect on biological membranes by complexation with membrane constituents, such as phospholipids and cholesterols (Arima, et al., 2001). Dendrimer-conjugated CD (CDE) provided the greatest transfection activity (approximately 100 times higher than those of dendrimer alone and the physical mixture of dendrimer and α-CD) in NIH3T3 fibroblasts and RAW264.7 macrophage cells (Arima, et al., 2001).

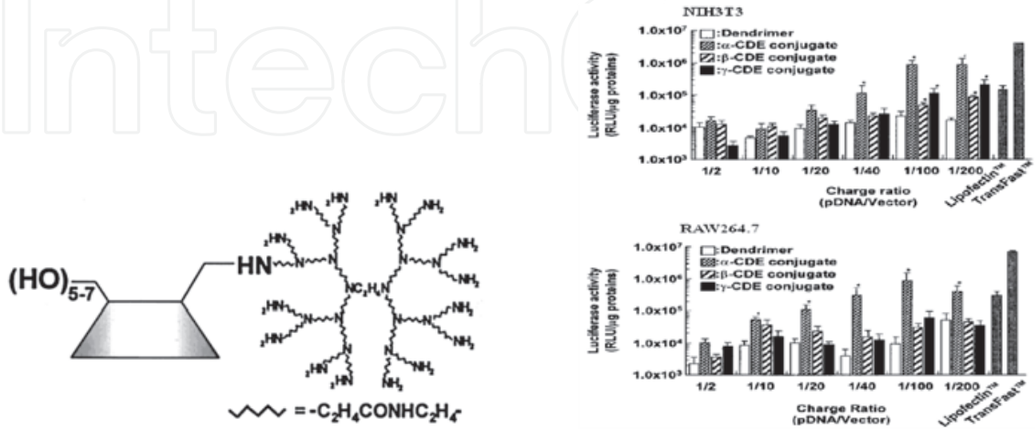


Fig. 1. Chemical structure of dendrimer-conjugated CD (left) and transfection efficiency of the complexes of pDNA/dendrimer or pDNA/CDE conjugates complexes at various charge ratios (right). [Source from Ref. (Arima, et al., 2001)].

They also studied the effect of dendrimer structure on gene transfection efficiency by preparation of CDEs with different dendrimer generations (Kihara, et al., 2002). The generation3 (G3) CDE showed the highest gene expression levels. More recently, the same group developed a lactose moiety-bearing CDE (Lac-α-CDE) for hepatocyte targeting (Arima, et al., 2010). Lac-α-CDE provided higher gene transfer activity than jetPEI™-Hepatocyte to hepatocytes with significantly fewer changes of blood chemistry values 12 h after intravenous administration in mice.

As shown in Fig. 2, Pun et al. synthesized linear and branched poly(ethylenimines) (PEIs) grafted with β-CD (CD-lPEI and CD-bPEI, respectively) by reaction of a mono-tosylated cyclodextrin with PEI amines and evaluated gene delivery ability as non-viral gene delivery agents *in vitro* and *in vivo* (Pun, et al., 2004).

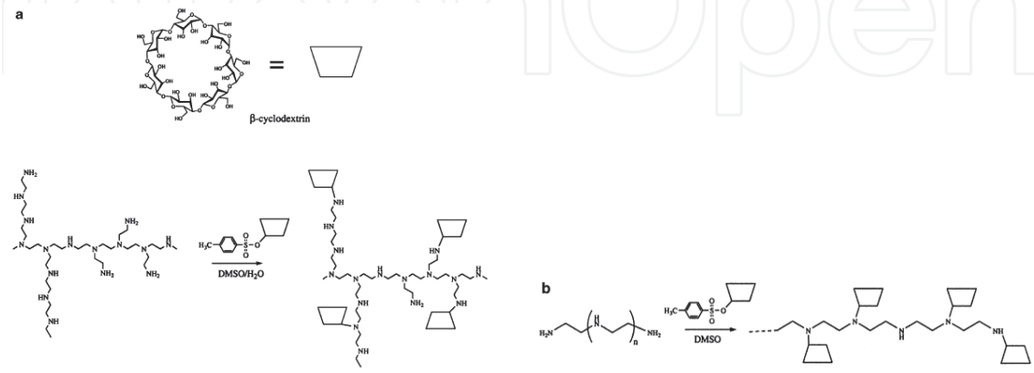


Fig. 2. (a) Synthesis of β-CD-bPEI. (b) Synthesis of β-CD-lPEI. [Source from Ref. (Pun, et al., 2004)].

Transfection efficiency of the polymers was impaired as cyclodextrin grafting increased, and toxicity was affected by cyclodextrin grafting due to the increasing polymer solubility, by capping primary amines, or by reducing polycation binding affinity [Fig. 3].

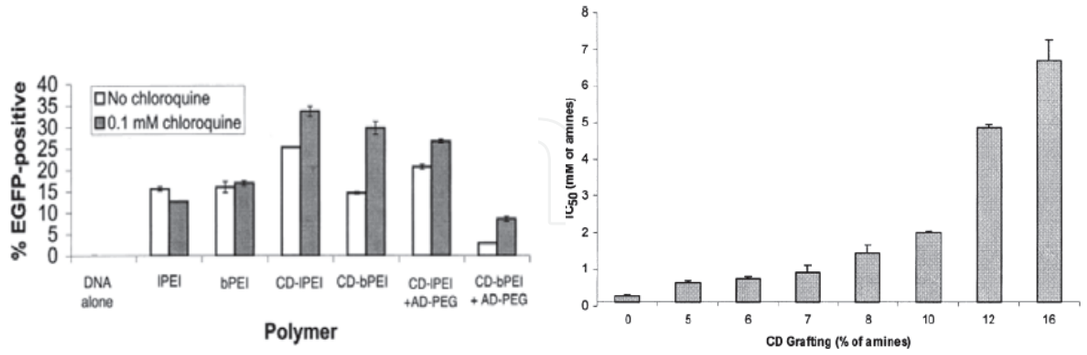


Fig. 3. Comparison of transgene expression from PEI and CD-PEI polymers in the presence or absence of 0.1 mM chloroquine (left) and effect of cyclodextrin grafting on CD-bPEI toxicity to PC3 cells (right). [Source from Ref. (Pun, et al., 2004)].

Recently, Huang et al. also used CDs for crosslinking of low MW branched PEI (MW 600) in order to form high MW cationic polymers (average MW 61K), which displayed lower cytotoxicity and high gene transfection in cultured cells (Huang, et al., 2006). As shown in Fig. 4, a series of new cationic star polymers were also synthesized by conjugation of multiple oligoethylenimine (OEI) arms onto an α-CD core as non-viral gene delivery vectors (Yang, et al., 2007).

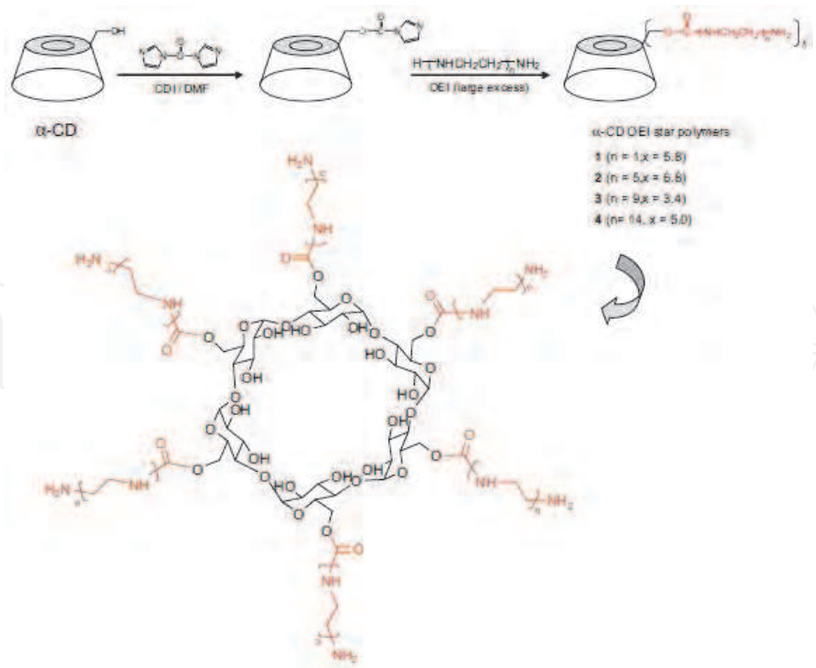


Fig. 4. Synthesis procedures and the structures of α-CD-OEI star polymers. [Source from Ref. (Yang, et al., 2007)].

All of the α-CD-OEI star polymers inhibited migration of pDNA on agarose gel through formation of complexes with pDNA, and the complexes formed nanoparticles with sizes ranging from 100-200 nm at N/P ratios of 8 or higher. Star polymers displayed much lower *in vitro* cytotoxicity than that of branched PEI 25 kD. α-CD-OEI star polymers showed excellent gene transfection efficiency in HEK293 and Cos7 cells. In general, transfection efficiency increased with an increase in OEI arm length. Star polymers with longer and branched OEI arms showed higher transfection efficiency. α-CD-OEI star polymers with different OEI arms have shown promise as new non-viral gene delivery vectors with low cytotoxicity and high gene transfection efficiency for use in future gene therapy applications.

In summary, CD-conjugated polymeric gene carriers showed enhanced transfection efficiency and reduced cytotoxicity, suggesting that CD is a material of potential interest for use in non-viral gene therapy, because these CD-conjugated polymeric gene delivery systems have been evaluated extensively in animal studies as well as clinical trials.

2.2 Chitosan

Chitosan [Fig. 5], a (1→4) 2-amino-2-deoxy-β-D-glucan, is a linear cationic polysaccharide derived by partial alkaline deacetylation of chitin, a polymer abundant in nature. The backbone of chitosan consists of two subunits, D-glucosamine and N-acetyl-D-glucosamine (Muzzarelli, 1997). It is a biocompatible, biodegradable polycationic polymer, which has minimum immunogenicity and low cytotoxicity (Mansouri, et al., 2004). Therefore, chitosan and chitosan derivatives may represent potentially safe cationic carriers for use in gene delivery.

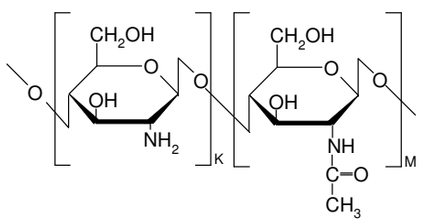


Fig. 5. Chemical structure of chitosan.

Factors including the degree of deacetylation, molecular weight, and charge of chitosan, and the media pH are important in determination of the transfection efficiency of polyplexes containing chitosan and DNA (Huang, et al., 2005, Ishii, et al., 2001, Lavertu, et al., 2006). The increased degree of deacetylation resulted in an increased level of DNA binding ability, and high transgene expression due to higher charge density along the chain (Kiang, et al., 2004, Lavertu, et al., 2006, Saranya, et al., 2011). The effect of the molecular weight of chitosan on complex formulation with DNA can be attributed to the chain entanglement effect (Kiang, et al., 2004). Chain entanglement contributes less to complex formulation as the molecular weight of chitosan decreases. The high molecular weight of chitosan resulted in easier entanglement of free DNA once the initial electrostatic interaction had occurred (Kiang, et al., 2004). Huang et al. reported that low molecular weight chitosan was less efficient at retaining DNA upon dilution, and, consequentially, less capable of protecting condensed DNA from degradation by DNase and serum components, and resulted in low transfection efficiency (Huang, et al., 2005). At acidic pH, below 5.5 or so, the primary

amines in chitosan become positively charged due to the pKa value of chitosan around 6.3-6.4 (Li, et al., 1996). At this acidic pH, the primary amine groups are protonated, resulting in a cationic polymer of high charge density, which can form stable complexes with plasmid DNA, protecting DNA from nuclease degradation (Mao, et al., 2001).

N,N,N-trimethyl chitosan chloride (TMC) was synthesized in order to induce an increase of charge density and solubility of chitosan at physiological pH. TMC induced more effective condensation of DNA at physiological pH, compared with chitosan, and the transfection efficiency of TMC/DNA complex showed a 30-fold increase over that of chitosan/DNA (Thanou, et al., 2002). Of particular interest, the presence of fetal calf serum (FCS) did not affect the transfection efficiency of the chitoplexes, whereas the transfection efficiency of DOTAP–DNA complexes was decreased. Cells remained approximately 100% viable in the presence of chitosan oligomers, whereas viability of DOTAP treated cells decreased to about 50% in both cell lines (Thanou, et al., 2002). In addition, folate conjugated TMC (folate-TMC) was recently studied as a target gene delivery carrier (Zheng, et al., 2009). Transfection efficiency of folate-TMC/pDNA complexes in KB cells and SKOV3 cells (folate receptor over-expressing cell lines) increased with increasing N/P ratio and was enhanced up to 1.6-fold and 1.4-fold, compared with that of TMC/pDNA complexes; however, no significant difference was observed between transfection efficiencies of the two complexes in A549 cells and NIH/3T3 cells (folate receptor deficient cell lines), indicating that the increase in transfection efficiencies of folate-TMC/pDNA complexes were attributed to folate receptor mediated endocytosis (Zheng, et al., 2009).

PEGylation of proteins, drugs, and liposomes has been proven to be an effective approach in extending circulation in the blood stream (Patel, 1992). Therefore, in order to reduce the aggregation of complexes and increase circulation time, Jiang et al. synthesized and characterized chitosan-g-PEG [Fig. 6] as a gene carrier (Jiang, et al., 2006).

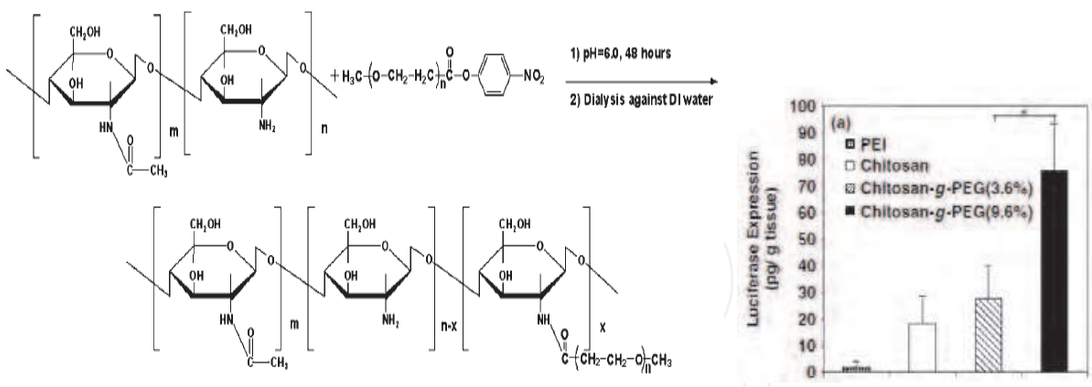


Fig. 6. Sythesis of chitosan-g-PEG polymers (left) and luciferase expression in rat liver after infusion of the complexes from common bile duct (right). [Source from Ref. (Jiang, et al., 2006)].

PEG grafting to chitosan efficiently shields the positive charge on the surface of chitosan/DNA complexes, improving particle stability in bile and serum; therefore, higher transfection efficiency was observed after infusion of the complexes through the bile duct [Fig. 6]. Chitosan-g-PEG mediated 3-fold higher luciferase expression in the liver than unmodified chitosan following intrabiliary infusion. Chitosan-g-PEG also exhibited slightly lower acute toxicity to the liver than chitosan.

Although chitosan showed good properties as a non-viral gene carrier, low transfection efficiency and low cell specificity of chitosan need to be overcome for clinical trials. Many research studies have been conducted for enhancement of transfection efficiency, such as pH-sensitive modification (Jiang, et al., 2007, Jones, et al., 2003, Kim, et al., 2003, Wong, et al., 2006), temperature-sensitive modification (Cho, et al., 2004, Dang, et al., 2006, Sun, et al., 2005), specific target ligand modification (Hashimoto, et al., 2006, Kim, et al., 2004, Kim, et al., 2006, Mansouri, et al., 2006, Wu & Wu, 1998, Zhang, et al., 2006) and so on. Among the chemical modifications of chitosan, PEI grafted chitosan showed some benefit due to high transfection efficiency. Wong et al. prepared PEI-graft-chitosan [Fig. 7] through cationic polymerization of aziridine in the presence of water-soluble oligo-chitosan (Wong, et al., 2006).

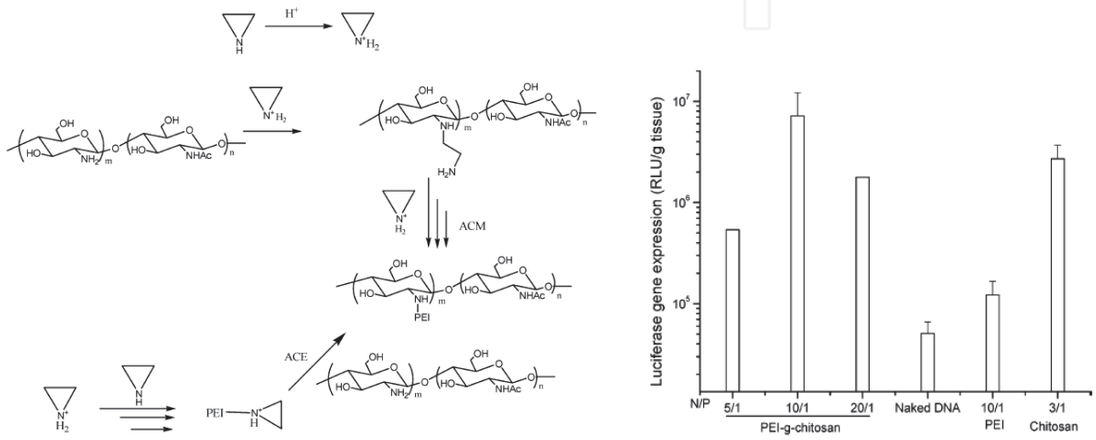


Fig. 7. Preparation of PEI-g-chitosan (left) and in vivo transfection efficiency of the complexes of PEI-g-chitosan/DNA in comparison with that of PEI (25 kDa) and chitosan after administration into common bile duct in rat liver (right). [Source from Ref. (Wong, et al., 2006)].

Results indicated that PEI-g-chitosan had a lower cytotoxicity than PEI 25K and PEI-g-chitosan showed higher transfection efficiency than PEI 25K both *in vitro* and *in vivo*. In addition, improved biocompatibility and long-term safety will be expected for PEI-g-chitosan due to the degradable chitosan main chain and short PEI side chains.

Wong et al. synthesized PEI-graft-chitosan using water-soluble chitosan; however, commercial chitosan is insoluble at neutral and alkaline pH values due to a weak base with a pKa value of the D-glucosamine residue of about 6.2-7.0. Using commercial chitosan, Cho's group synthesized a chitosan-g-PEI copolymer [Fig. 8] by an imine reaction between periodate-oxidized chitosan and an amine group of PEI (Jiang, et al., 2007).

In addition, the same group developed specific ligand-conjugated chitosan-g-PEI, such as galactosylated- (Jiang, et al., 2007), mannosylated- (Jiang, et al., 2009), and folate-conjugated (Jiang, et al., 2009). The specific ligand-conjugated chitosan-g-PEI showed low cytotoxicity and high transfection efficiency with specific cell targeting.

In summary, the transfection efficiency was dependent on the degree of deacetylation, molecular weight of the chitosan, and medium pH. Also, specific ligand-conjugation will increase the transfection efficiency depending on the targeting ability of the ligands. A number of *in vitro* and *in vivo* studies have shown that modified-chitosan is a suitable material for use in efficient non-viral gene therapy.

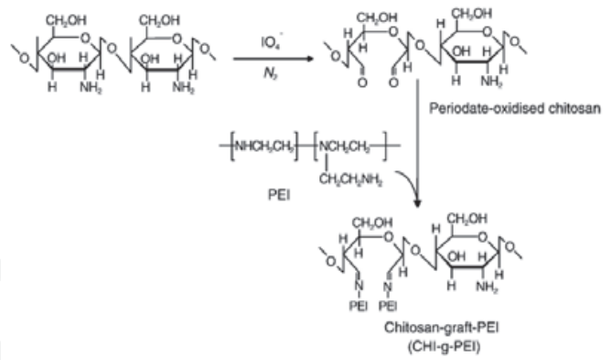


Fig. 8. Proposed reaction scheme for synthesis of CHI-g-PEI. [Source from Ref. (Jiang, et al., 2007)].

Similarly, according to Wong's results, the chitosan-g-PEI copolymer showed higher transfection efficiency and lower cytotoxicity than PEI 25K due to the buffering capacity of low moleculer weight PEI and biocompatible chitosan [Fig. 9].

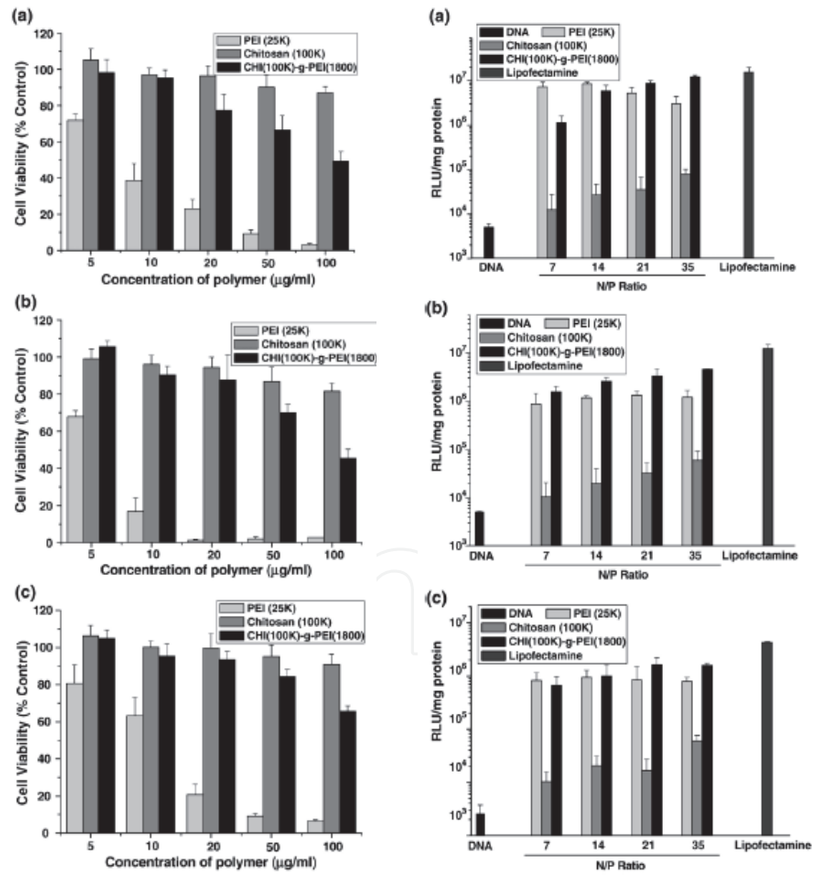


Fig. 9. Cytotoxicity of copolymer at various concentrations in different cell lines. (a) 293T, (b) HeLa and (c) HepG2 (left) and transfection efficiency of copolymer/DNA (pGL3-control) complex at various N/P ratios and in various cell lines. (a) 293T, (b) HeLa and (c) HepG2 (right). [Source from Ref. (Jiang, et al., 2007)].

2.3 Polyethylenimine (PEI)

PEI has received much attention due to its high transfection efficiency. In 1995, Behr's group made the first use of this polymer for delivery of DNA and oligonucleotides (Boussif, et al., 1995). As shown in Fig. 10, PEI exists in two principal forms, branched and linear, with a wide range of molecular weights (Lungwitz, et al., 2005).

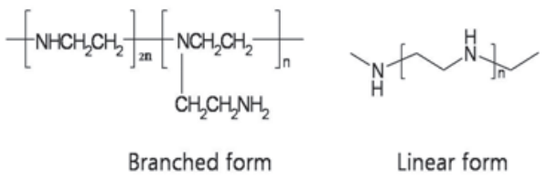


Fig. 10. Chemical structures of branched and linear PEI.

It is widely accepted that the high transfection ability of PEI is due to its high buffering capacity over a broad pH, which is called "the proton sponge effect" (Akinc, et al., 2005). In addition, the high content of primary amino groups enables chemical coupling of targeting moieties or intracellular active components; high density of positive charges in the molecule allows for a tight compaction of nucleic acids. However, high molecular weight of PEI shows high cytotoxicity, and when further decreasing the molecular weight, both cellular toxicity and transfection efficiency are decreased (Godbey, et al., 2001, Kunath, et al., 2003). One way to reduce toxicity of PEI is to reduce or mask the surface charge by attachment of vesicles with hydrophilic molecules, such as PEG. PEG chains of different length were used for modification of low-molecular weight PEIs (2 kDa), as well as high-molecular weight PEIs, such as the branched PEI (b-PEI) of 25 kDa [Fig. 11] (Petersen, et al., 2002) and the linear PEI (L-PEI) of 22 kDa (Kichler, et al., 2002). One beneficial effect of PEGylation is that PEG-PEI conjugates are less cytotoxic than non-modified polymers.

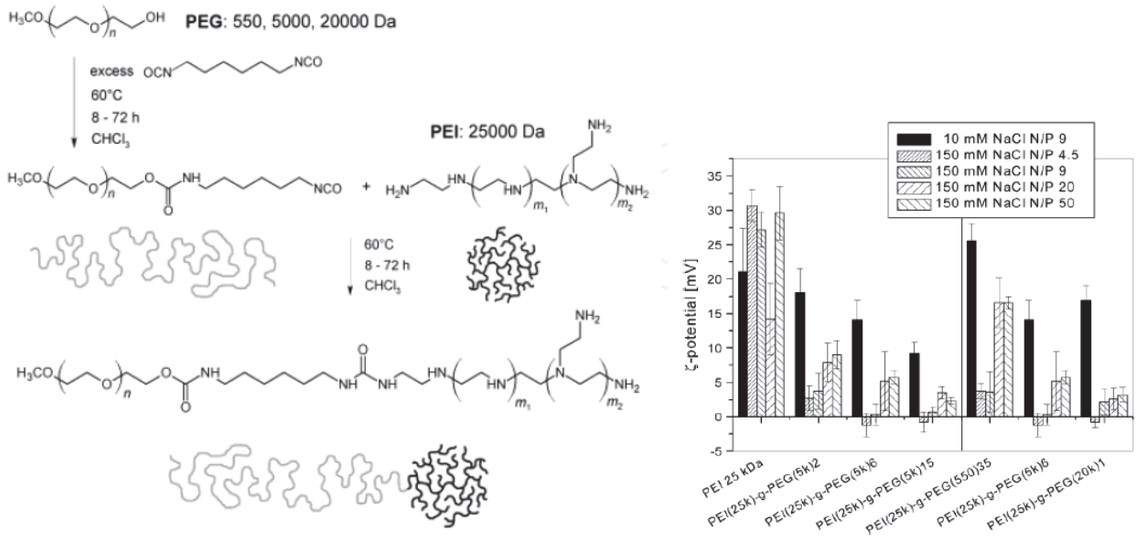


Fig. 11. Synthesis of bPEI-g-PEG copolymers (left) and zeta-potential of plasmid DNA complexes with PEI 25 kDa and bPEI-g-PEG block copolymers at different ionic strength and at different N/P ratios (right). [Source from Ref. (Petersen, et al., 2002)].

In all of the studies, covalent modification of PEI with PEG reduced the positive surface charge (zeta-potential) of the polyplexes, whereas it only marginally affected their size. However, PEGylation also reduces the DNA-binding capacity of the polymer and sterically hinders interactions of the polyplexes with the target cells. Therefore, in order to increase its usefulness, stealth technology must be combined with the use of ligands that allow specific cell targeting. Different types of ligands, such as sugar residues, peptides, proteins, and antibodies have been used for targeting of PEGylated PEI/DNA complexes [Table 2].

PEGylated PEI	
Ligand	**References**
Galactose	(Sagara & Kim, 2002)
Folate	(Benns, et al., 2002)
Transferrin	(Kursa, et al., 2003)
Epidermal growth factor	(Blessing, et al., 2001)

Table 2. Specific cell-targeting ligands conjugated with PEG-PEI.

Specific cell-targeting ligand-conjugated PEG-PEI showed low cytotoxicity and high transfection efficiency with specific cell targeting ability.

In summary, PEI is one of the successful and widely used gene delivery polymers, which has become the gold standard of non-viral gene delivery due to its high transfection efficiency. However, concerns over the cytotoxicity of PEI have to be solved for clinical trials. Cytotoxicity of PEI is dependent on its molecular weight; a lower molecular weight PEI has a lower cytotoxicity. Therefore, it is an attractive strategy by combination of lower molecular weight of PEI and biocompatible polymers as gene vectors for reduction of the toxicity of PEI. Also, similar to other cationic gene carriers, specific ligand-conjugation will be a way to increase transfection efficiency with specific cell-targeting.

2.4 Poly(β-amino ester)s (PAEs)

PAEs are one of the biodegradable cationic gene carriers. Biodegradable cationic PAEs are of interest both from the standpoint of mitigating the toxicity of conventional materials as well as a potential means through which to effect the timely release of DNA inside transfected cells (Lim, et al., 2000, Lim, et al., 2002, Luo & Saltzman, 2000). The Langer group has been particularly interested in PAEs as gene carriers, as they are easily synthesized via conjugate addition of either primary or bis(secondary) amine to diacrylate compounds, as shown in Fig. 12.

The Langer group reported a parallel approach suitable for synthesis of hundreds to thousands of structurally unique PAEs and application of these libraries to rapid and high throughput identification of new gene delivery agents and structure-function trends (Lynn, et al., 2001). The advantage of combinatorial chemistry and automated highthroughput synthesis is that it has revolutionized modern drug discovery by rapid synthesis and evaluation with greater precision. As shown in Fig. 13, 140 different PAEs (the set of 7 diacrylate monomers and 20 amine-based monomers) were synthesized as a screening library. Most of the PAEs showed low transfection efficiencies, compared with Lipofectamine 2000, a commercially available lipid-based vector system. However, B14 and G5 yielded higher gene transfection efficiencies. In particular, B14 showed higer transfection efficiency, compared with

Lipofectamine 2000, due to the high endosomal pH buffering capacity, similar to that of other imidazole-substituted polymers (Benns, et al., 2000, Pack, et al., 2000), suggesting that polymer B14 may be the more promising polymer as a gene delivery carrier.

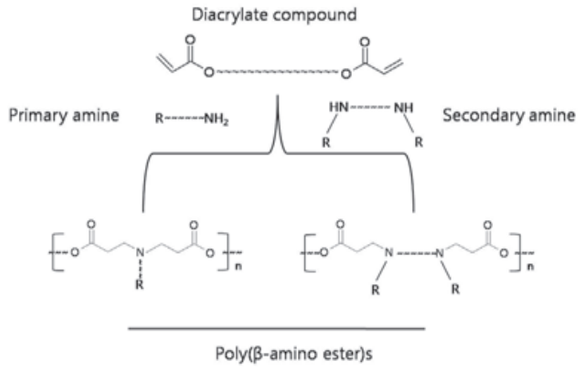
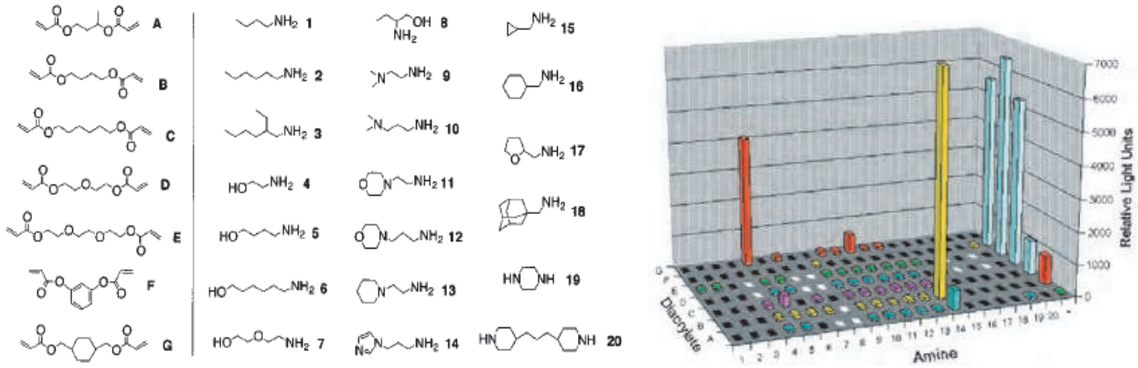


Fig. 12. Synthesis scheme of PAEs.



[Source from Ref. (Zugates, et al., 2006)].

Fig. 13. Diacrylate (A-G) and amine (1-20) monomers chosen for the synthesis of an initial screening library (left) and transfection data as a function of structure for an assay employing pCMV-Luc (600 ng/well, DNA/polymer = 1:20, right). [Source from Ref. (Lynn, et al., 2001)].

As shown in Fig. 14, using high throughput methods, over 2,350 PAEs were synthesized (Anderson, et al., 2003). Biodegradable PAEs demonstrated efficient transfection of cells and 26 of these polymers showed higher gene expression, compared with Lipofectamine 2000. Response to intracellular stimuli, such as pH, is a major advantage of a gene delivery system (Stayton, et al., 2005). Zugates et al synthesized new PAEs using a primary amine monomer, 2-(pyridyldithio)-ethylamine (PDA), speculating that pyridyldithio groups in these side chains display fast and selective reactivity with thiols without alteration of the charge density of the polymer backbone, as shown in Fig. 15 (Zugates, et al., 2006). This property of PDA-based PAEs further led to conjugation of cell-targeting peptides or ligands for targeted and site-specific delivery. As one potential application, they conjugated the mercaptoethylamine (MEA) and the RGDC peptide to PDA PAEs. MEA-based PAE has an advantage that it is sensitive to glutathione. The MEA-based polymer delivery system has demonstrated relative stability in the extracellular space; however, it is responsive to intracellular conditions in which partial unpacking is triggered.

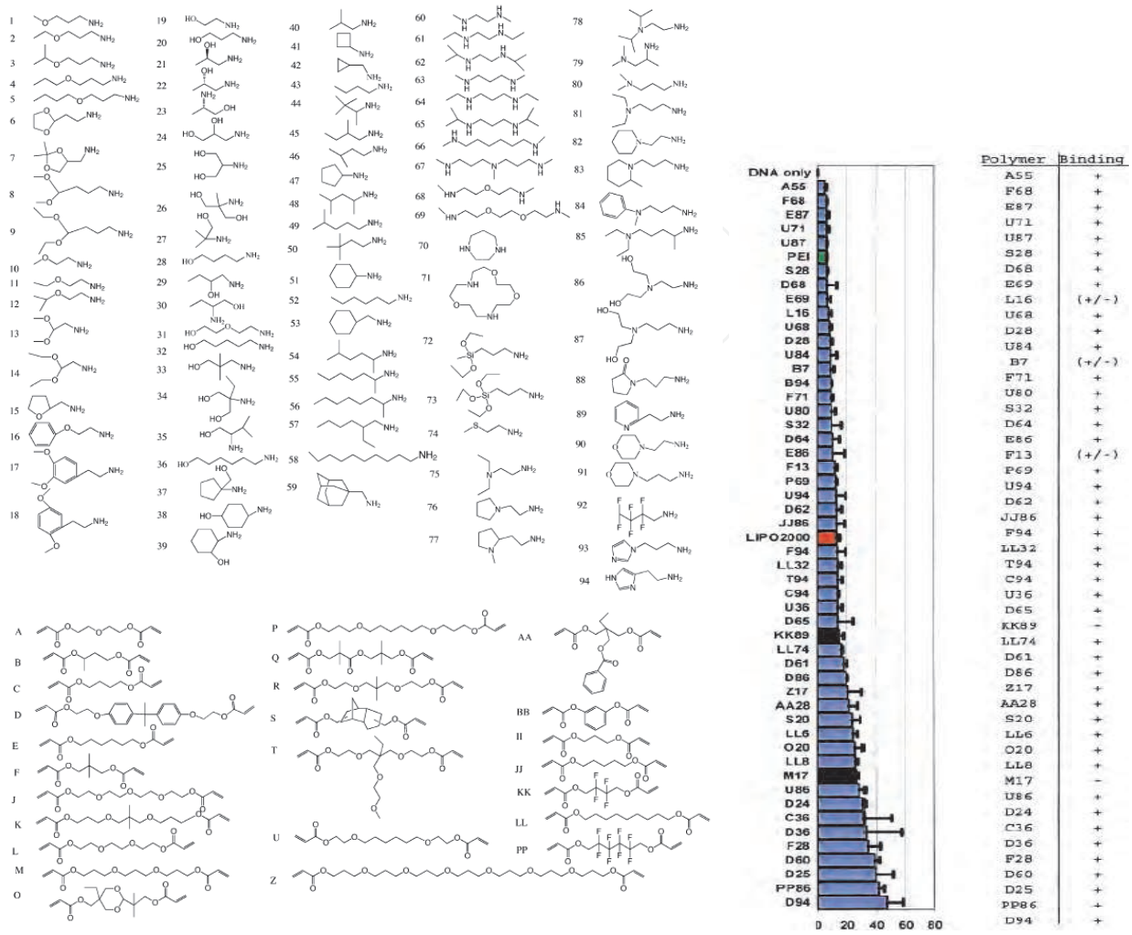


Fig. 14. Amino (numbers) and diacrylate (letters) monomers (left) and optimized transfection efficiency of the top 50 polymers relative to PEI and Lipofectamine 2000. [Source from Ref. (Anderson, et al., 2003)].

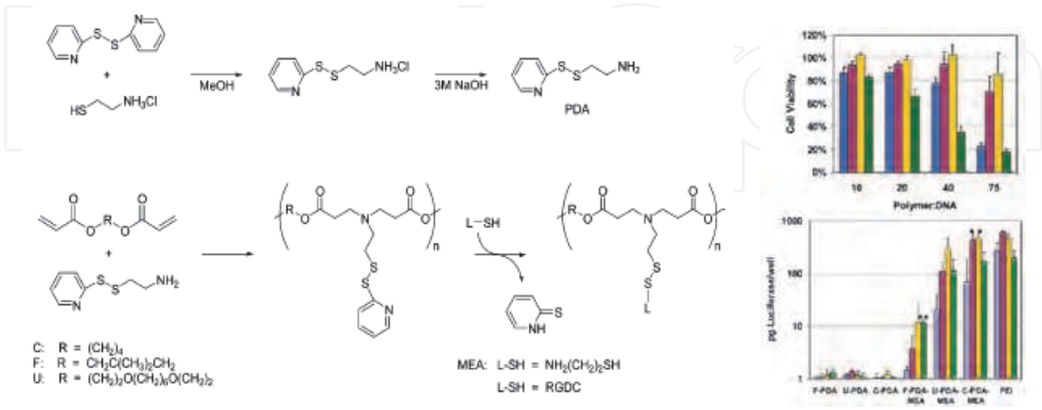


Fig. 15. Synthesis scheme of MEA (left) and cytotoxicity [C-PDA (blue), C-PDA-MEA (Redenti, et al.), 2-mercaptopyridine (2-MP, yellow), and PEI (green)] and transfection studies (right). [Source from Ref. (Zugates, et al., 2006)].

As shown in Fig. 16, Cho's group synthesized novel biodegradable PAEs composed of gamma-aminopropyl-triethoxysilane (APES) and poly (ethylene glycol) diacrylate (PEGDA) for gene delivery (Jere, et al., 2008).

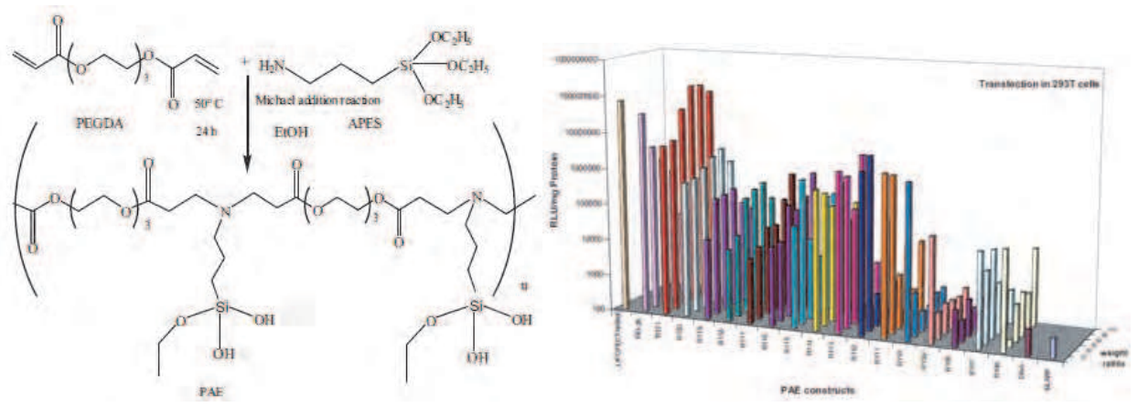


Fig. 16. Proposed reaction scheme for PAE copolymer (left) and transfection efficiency of PAE/DNA complexes in serum free-media at various mass ratios in a 293T cells (right). [Source from Ref. (Jere, et al., 2008)].

They reported that addition of PEGDA over APES resulted in a novel PAE, which shows high safety and transfection efficiency, especially in R121. PAE obtained from R121 showed good DNA binding and condensation with average particle sizes of 133 nm. In addition, PAE-mediated gene expression in the lung and liver was higher than that of the conventional PEI carrier. Of particular interest, non-invasive aerosol delivery induced higher gene expression in all organs, compared with an intravenous method, in an *in vivo* mice study (Park, et al., 2008). The same group developed a new PAE based on hydrophobic polycaprolactone (PCL) and low molecular weight branched PEI following the Michael addition reaction (Arote, et al., 2007). The synthesized PAE showed controlled degradation and was essentially non-toxic in all three cells (293T, HepG2 and HeLa) in contrast with PEI 25K. PAEs revealed much higher transfection efficiencies in three cell lines, compared with PEI 25K, and were also successfully transfected *in vivo*, compared with PEI 25K after aerosol administration. Targeting confers another important criterion in gene delivery. Recently, Arote et al. coupled folic acid moiety for a folate receptor targeting the PAE backbone using PEG (MW: 5000 Da) as a linker (Arote, et al., 2010). At the initial stage, folate-conjugated PAE revealed folate receptor-mediated endocytosis with elevated levels of luciferase expression in folate receptor positive cancer cell lines, suggesting application of specific ligand-modified PAE. They also developed folate-PEG-PAE (FP-PAE) as a gene carrier, which mediated high level folate receptor mediated endocytosis *in vitro* as well as *in vivo* [Fig. 17]. FP-PAE showed marked anti-tumor activity against folate receptor-positive human KB tumors in nude mice with no evidence of toxicity during and after therapy using the TAM67 gene. Anti-tumor activity with PAE without folic acid moiety (PEG-PAE, P-PAE) proved ineffective against a xenograft mice model with KB cells when administered at the same dose as that of FP-PAE, suggesting that FP-PAE is a highly effective gene carrier capable of producing a therapeutic benefit in a xenograft mice model without any signs of toxicity.

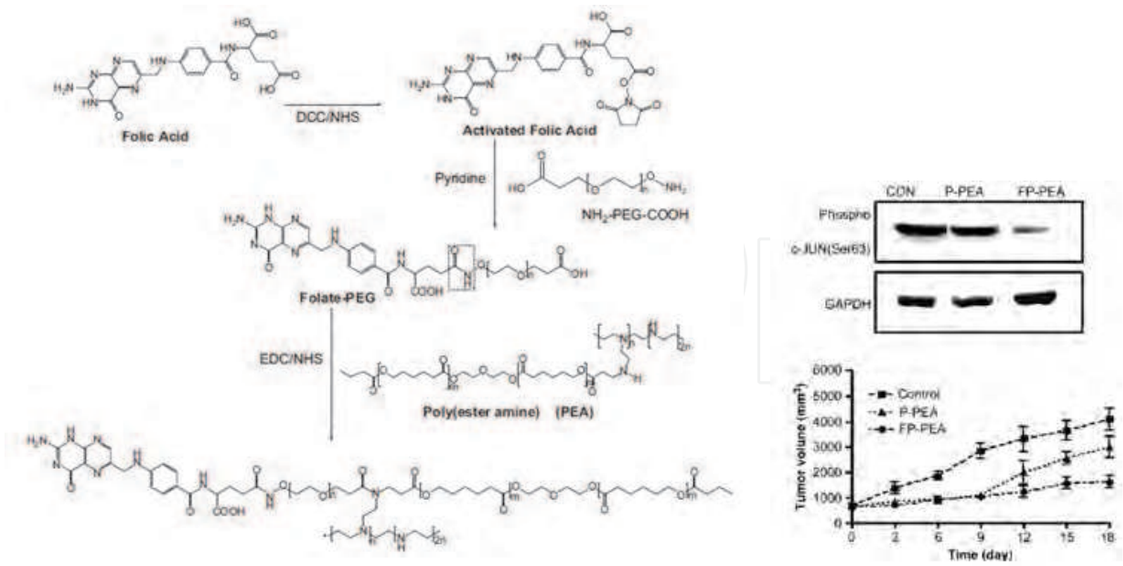


Fig. 17. Synthesis scheme of FP-PEA (left) and effect of FP-PEA/TAM67 complexes on tumor growth (right). Expression level of phospho-c-Jun and suppression of tumor growth by FP-PEA/TAM67 complexes. [Source from Ref. (Arote, et al., 2010)].

In summary, PAEs have excellent characteristics as gene carriers. PAEs comprise a class of degradable cationic polymers with many desirable properties in the context of gene delivery, including condensation of DNA into nanoscale-size particles, which facilitates cellular uptake of DNA and protects DNA from endogenous nucleases as well as efficient delivery of DNA with low toxicity. Tissue targeting, endosome disruption, and nuclear transport should be combined for development of an effective PAE for use in gene therapy. Also, extensive *in vitro* and *in vivo* evaluation and optimization of PAEs will provide valuable information for safe and efficient gene therapy applications.

3. Conclusion

Gene therapy shows tremendous promise for a broad spectrum of clinical applications. Development of a safe and efficient gene delivery system is one of the main challenges to be solved before this strategy can be adopted for routine use in clinical trails. In this chapter, medical polymers, including CD, chitosan, PEI, PAEs, and their derivatives as non-viral vectors in the area of gene therapy have been described. Although more development of structure-function relationships and fundamental research into cellular processes *in vitro* and *in vivo* should be performed for future direction of medical polymer based gene carriers, combination of these polymers will be a way to reduce toxicity and enhance tranfection efficiency. Also, selective tissue or cell targeting ligand conjugation will provide cell-specificity or improve transfection efficiency. Nowadays, multiple targeting gene therapy with multiple-functionalized genes and delivery system are possible. Suitable formulations of these polyplexes with low toxicity and high transfection efficiency must be chosen for *in vivo* use, which will allow for multiple applications of therapeutic genes; however, for this idea to be realized, much work lies ahead.

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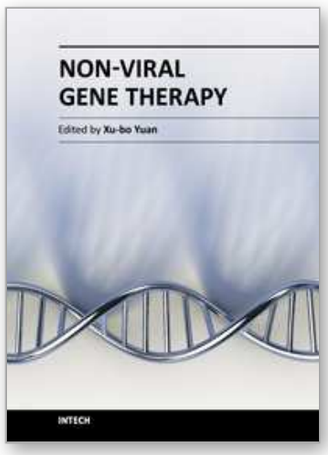

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This book focuses on recent advancement of gene delivery systems research. With the multidisciplinary contribution in gene delivery, the book covers several aspects in the gene therapy development: various gene delivery systems, methods to enhance delivery, materials with modification and multifunction for the tumor or tissue targeting. This book will help molecular biologists gain a basic knowledge of gene delivery vehicles, while drug delivery scientist will better understand DNA, molecular biology, and DNA manipulation.

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