Mini-Reviews in Medicinal Chemistry, **2010**, *10,* **126-137**

Polycationic Nanoparticles as Nonviral Vectors Employed for Gene Therapy *in vivo*

Jinfeng Xing[1], Liandong Deng[1], Shutao Guo[1, 2], Anjie Dong[*,1] and Xing-Jie Liang[*,2]

[1]*Department of Polymer Science and Engineering, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, P.R. China*

[2]*CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, Zhongguancun, Beijing, 100190, P.R. China*

Abstract: Currently, polycationic nanoparticles (polycation) as nonviral vectors are promising to overcome the defects of traditional viral vectors. This review focuses on the recent progress and challenges to improve the transfection efficacy and circulation time of polyplexes (complexes between polycation and pDNA or oligonucleotides such as siRNA) *in vivo*.

Key Words: Gene therapy, polycation, nanoparticles, non-viral vectors, transfection efficiency, PEGylation, gene delivery, circulation time.

INTRODUCTION

Gene therapy is defined as the transfer of genetic information into specific cells to modulate protein expression for treatment of inherited or acquired diseases [1,2]. Generally, recombinant plasmid DNA (pDNA) (encoding therapeutic proteins) and oligonucleotides (relatively small fragments of synthetic DNA or RNA) have been used as a “drug” to modulate gene expression associating with a particular disease. Since it is difficult for negative charged pDNA and oligonucleotides to be taken up by targeted cells, the clinic use of naked pDNA and oligonucleotides is severely limited due to rapid degradation by nucleases in serum and quick clearance by immune-system. Therefore, successful gene therapy largely depends on the development of efficient delivery vectors [3].

Two classes of primary gene vehicles including recombinant viruses and non-viral vectors have been developed [4]. Viral vectors were widely applied in gene delivery studies because of its evolutionarily rooted efficiency as gene delivery vehicle [5,6]. However, safety concerns on the random recombination immunogenicity, limited nucleic acid loading capacity and high cost hinder the wide clinical application of recombinant viruses [7-9]. Clinical trials have underscored the safety risks of viral vectors because of concomitant development of cancer [10] and even death [11] with viral gene therapy. Therefore, new attention has been focused on non-viral vectors due to their potential advantages such as low immunogenic response, easy modification, capability to carry large inserts and facile manufacturing even though the transfection efficiency of non-viral vectors is lower than that of viral vectors [12,13]. The flexibility in formulation design provides a great promising potential in improving the transfection efficiency of non-viral vectors to the level comparable to that of viral vectors.

There are many potential hurdles as shown in Fig. (**1**) to be overcome for successful gene delivery, a process broadly defined as transfection. The delivery system has to survive in the serum for sufficient lifetime instead of rapid clearance resulting from interaction with serum proteins, and the delivered pDNA or oligonucleotides need to be encapsulated into particles, which could provide excellent protection of pDNA or oligonucleotides from degradation and further clearance. Moreover, the particles containing pDNA or oligonucleotides have to be accessible to targeted cells and internalized by cellular uptake through mechanisms such as electrostatic interactions, ligand-receptor interaction or both. Following uptake, gene-containing particles need escape the endocytosis of endosomal/phagosomal compartments and release their DNA cargo into the cytoplasm. Functional DNA finally translocates into the nucleus and subsequently modulate the expression of targeted protein [1,12-15]. In this review, we will focus on state of the art developments of the widely studied polycations as non-viral vectors. The relationship of polycation structure and function in the efficiency and mechanism of transfection are discussed, and different modification mainly including the PEGylation of polycations is explained on the purpose of overcoming barriers in the process of gene delivery.

1. POLYCATIONS EMPLOYED AS NON-VIRAL VECTORS

Cationic polymers (usually directly designated as polycations) as gene carriers have been widely used. Besides their potential advantages such as lower safety risks and greater flexibility, polycations have advantage for carrying large DNA molecules, facile manufacturing and easy modi-

*Address correspondence to these authors at the [1]Department of Polymer Science and Engineering, School of Chemical Engineering and Technology, Tianjin University, E-mail: ajdong@tju.edu.cn; and [2]Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, Beijing 100190, China; Tel: +86-10-82545569(O); Fax: +86-10-62656765; E-mail: liangxj@nanoctr.cn

1389-5575/10 $55.00+.00 © 2010 Bentham Science Publishers Ltd.

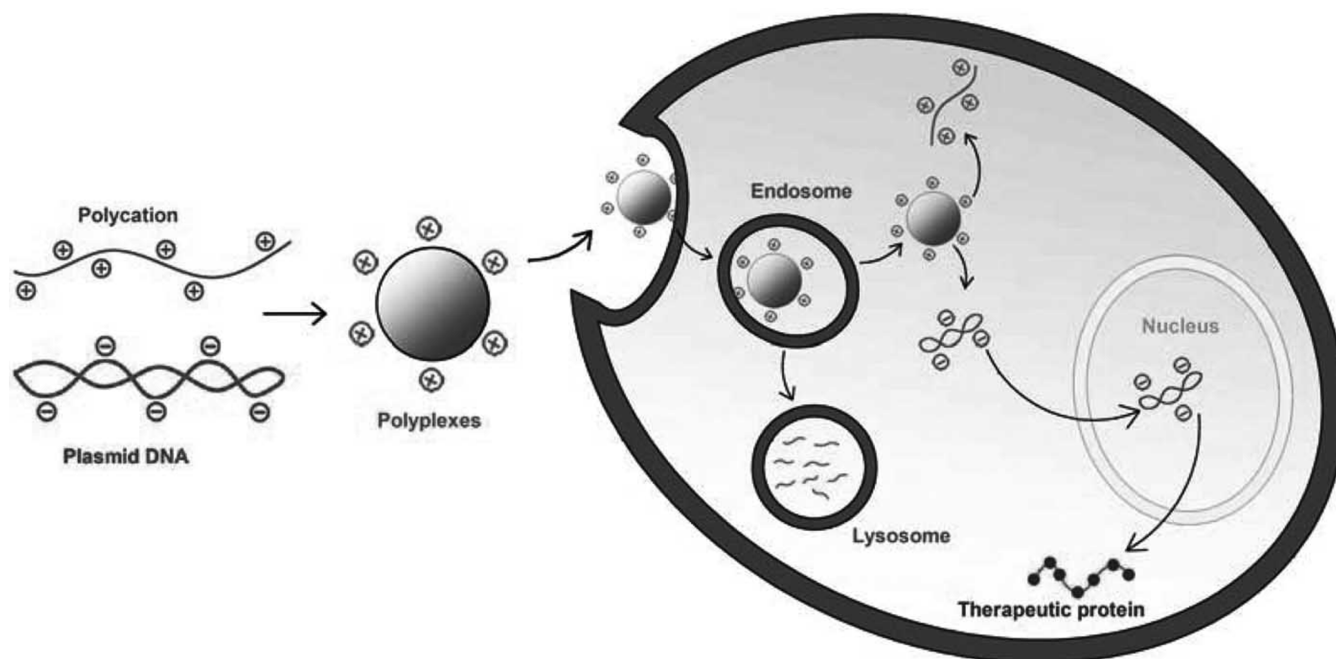

Fig. (1). Scheme of mechanism employed by polyplexes for efficient gene delivery. Positive charged polyplexes firstly adsorb on the plasma membrane of targeted cells, then pass through plasma membrane and enter into cells by receptor-mediated endocytosis. During it trafficks in cytoplasm, it is necessary for polyplexes to free DNA cargo in endosomal vesicles just before the participation of lysosome. Functional DNA is released into cytosol and modulates the expression of therapeutic targeted protein, which is critical in inherited or acquired diseases.

fication compared to viral vectors [12-14]. When complexed with DNA, excessive polycation are required to effectively encapsulate pDNA or oligonucleotides. The residual positive charges on polyplexes surface are beneficial to polyplexes for adsorptive endocytosis, because it is easy for the positively charged polyplexes to interact with negatively charged glycoproteins, proteoglycans and glycerophosphates on the cell membrane for activated endocytosis. However, residual positive surface charges could also result in toxicity at the cellular and/or the systemic level. In particular, the *in vivo* applicability of such polyplexes seems to be potentially hazardous, since residual surface charge leads to non-specific interaction with components of circulation system. It often causes a rapid removal of polyplexes from the blood stream as well as unwanted events such as organ embolism due to aggregate formation with erythrocytes [13]. Many methods were utilized to solve the non-specific interactions and aggregation, which will be discussed in section 2.

1.1. Poly(L-Lysine)

Poly(L-lysine) (PLL) as shown in Fig. (**2**) is one of the polymers used in non-viral gene delivery [13,15]. Although PLL with high molecular weight exhibits relatively high cytotoxicity, PLL is still broadly used due to its peptide structure, which is biodegradable in *in vivo* [16,17]. It is known that PLL/DNA polyplexes can be internalized in a way comparable to other polymer/DNA complexes, but it is disappointed to their low transfection efficiency. Currently, it is thought probably due to lack of amino groups with a pK between 5 and 7 that are helpful for acidic endo-lysosomal escape [13]. The average environmental pH surrounding PLL was found to be 4.0 to 4.5 after cellular uptake, it indicates that most of the PLL polyplexes are trafficked into the lysosome instead of being released into the cytoplasm [18].

Extensive researches have been done on PLL structure modification for improving its ability to escape endosome in time. Typical methods of promoting endosomal lysis were known to modulate PLL with histidine or other imidazole containing structures. Those modifications provide PLL with buffering capacity in the endosome lumen where vesicular escape is mediated by a ‘proton sponge’ mechanism [17,19-21]. In an effort to reduce cytotoxicity of PLL and improve DNA release from the PLL polyplexes after endocytosis, various biodegradable PLL conjugates have been synthesized by introducing susceptible chemical bonds such as disulfide bonds [22-24] and ester bonds [25] inside PLL polyplexes.

1.2. Polyethylenimine

Polyethylenimine (PEI) is a versatile polymer which has a privileged place in the domain of non-viral gene delivery and often considered as the gold standard of gene transfection that is ascribed to its superior transfection efficiency in a broad range of cell types compared to other polymer systems [9,17]. The transfection efficiency of PEI has been shown to be due, at least in part, to the “proton sponge” properties of the polymer [26], which originates from PEIs high density of near-neutral pKa groups buffering the acidic environment of the endosome. The “proton sponge” function of PEI polymer guarantees PEI-based polyplexes to escape the late endosome into the cytoplasm just before endocytic vesicles encounter the lysosome. Thereby, the nucleotides payload by PEI polymers could avoid to be degraded by lysosomal nucleases [27,28].

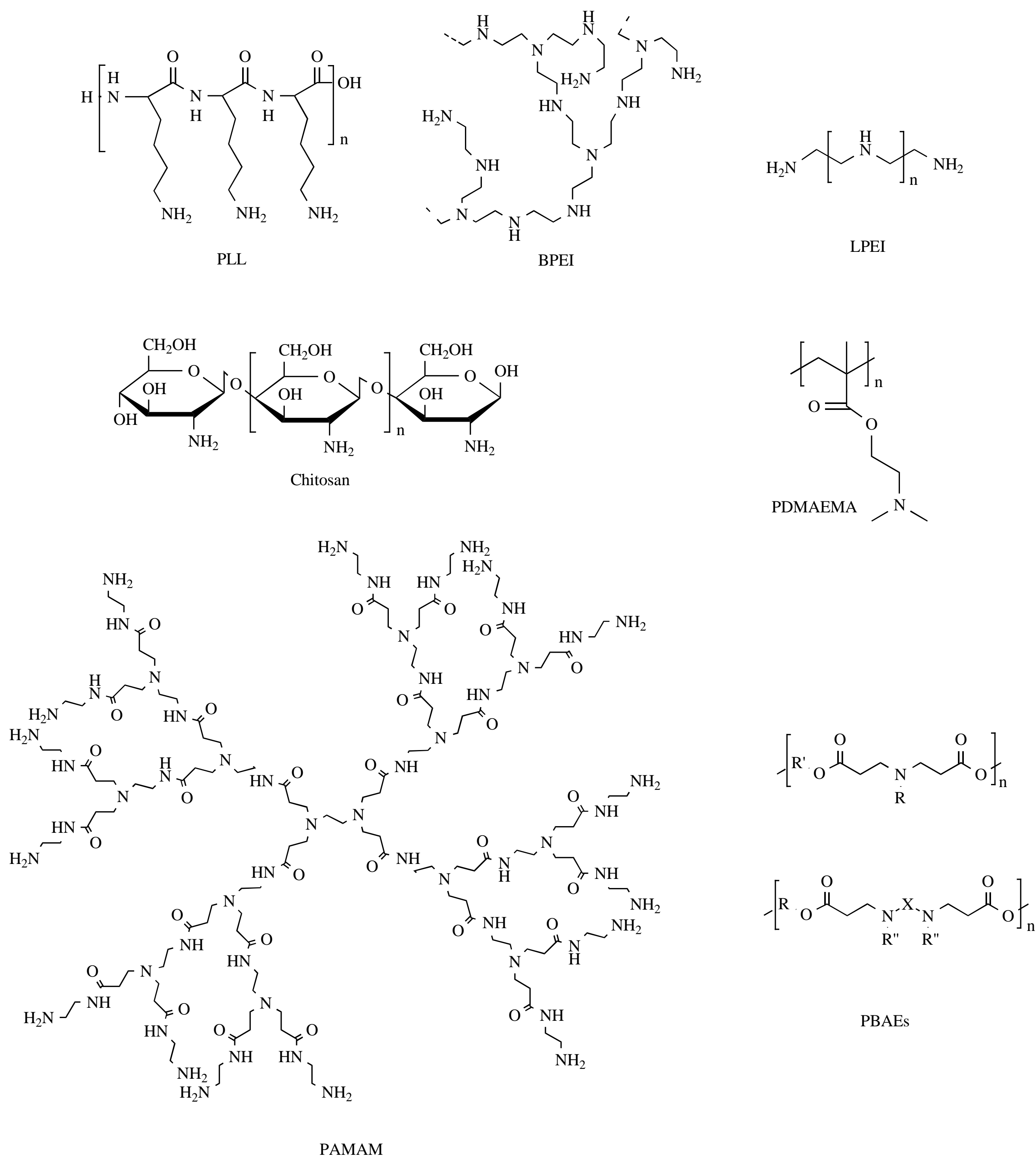

Fig. (2). Structures of current cationic polymers used in gene therapy. PLL = Poly(L-lysine), BPEI = Branched poly(ethyleneimine), LPEI = Linear poly(ethyleneimine), PEMAEMA = Poly[2-(dimethylamino) ethyl methacrylate], PBAE = Poly(β-amino esters), PAMAM = polyamidoamine.

After the first PEI-mediated oligonucleotide transfer successfully conducted by Behr *et al.* in 1995 [29], different PEI derivatives have been synthesized to improve the physicochemical and biological properties of PEI polyplexes [30]. In general, PEI can be categorized into branched (BPEI) and linear (LPEI) subtypes according to the structure (BPEI and LPEI) as shown in Fig. (**2**). BPEI and LPEI can be synthesized via acid-catalyzed polymerization of aziridine [31] and ring opening polymerization of 2-ethyl-2-oxazoline followed by hydrolysis [32], respectively.

The transfection efficiency of PEI polyplexes strongly depends on its molecular weight and its amine/DNA phosphate (N/P) ratio. Godbey *et al.* reported that transfection efficiency of PEI polyplexes was improved with increasing PEI molecular weight ranging from 0.6 to 70 kDa [33], how-

ever, higher cytotoxicity was also concomitant with high molecular weight PEI polymers due to their stronger aggregation and adherence on the cell surface, which resulted in significant necrosis [34,35]. The optimal molecular weight for PEI polyplex was typically ranged from 5 to 25 kDa [30]. Low molecular weight PEI can transfect cell lines more effectively compared to the high molecular weight counterparts only under the condition when higher N/P ratio are used [17,36].

Not only the molecular weight but also the branching degree plays an important role in affecting DNA complex formation and stability [13, 17]. Linear PEIs were demonstrated to display excellent transfection efficiency with a rather low toxicity, especially *in vivo* studies, probably due to their unique topology [37-39]. However, linear PEI was confirmed to be less effective for condensing DNA compared to the branched form with similar molecular weights [40]. Moreover, the stability of PEI polyplex is better for complexes with more primary amines, it proposes branched PEI to be a seemingly more suitable transfection vector compared to liner PEI [41].

Various modifications of PEI structure have been mainly focused on reducing their fairly high cytotoxicity *in vivo*, which results from the high amount of positive charges and their non-biodegradability. To prepare biodegradable PEI-based gene vectors, the low molecular weight PEI and biocompatible molecules were conjugated with reducible disulfide linkages [42-46], hydrolyzable ester linkages [47-50], hydrolyzable ester amine linkages [51-57], hydrolyzable amide and imine linkages [58-69]. The cleaving mechanism of disulfide linkages is totally different from those of the hydrolyzable ester linkages, ester amine linkages, hydrolyzable amide and imine linkages. Disulfide linkages can be cleaved rapidly by glutathione and thioredoxin reductases in cytoplasm instead of hydrolytic reaction. Therefore, the PEI polycations with disulfide bonds can be used to prepare stable complexes with plasmid DNA in the extracellular environment. Hydrolyzable ester linkages, ester amine linkages, hydrolyzable amide and imine linkages are comparatively stable in extracellular environment, but prone to be cleaved in acid endosomal environment due to the hydrolytic reaction. The cytotoxicity of modified PEI derivatives is dramatically reduced compared to parental PEI polymer, although the transfection efficiency is also hampered in some cases.

1.3. Poly(Amino-Ester)

Poly(β-amino esters) (PBAEs) is one particularly interesting class of polymers for gene delivery, since it was first developed through classical Michael addition reaction involving the conjugation of either a primary amine or a bis(secondary amine) monomer with a diacrylate ester as shown in Fig. (**2** and **3**) [70]. PBAEs are more promising polymers compared to PEI due to their biodegradability via hydrolytically degradable ester groups as well as reduced cytotoxicity, capability for triggering DNA release within the cell, and flexibility for their structural modification [71]. PBAEs can successfully modulate the physical environment of endosomal compartment and escape endosome through the "proton sponge" mechanism similarly to PEI polymers [72]. A 140 PBAEs library composed of 7 diacrylate monomers and 20 amine monomers was synthesized and screened for gene delivery efficacy in parallel, it was found that the polyplexes with effective diameters smaller than 250 nm and positive ζ in 10 mM HEPES buffer to show the best performance, which presented with high uptake and mediated gene delivery 4-8 fold higher than PEI, comparable to the efficacy of classical lipofectamine 2000 reagent [71-73]. Unfortunately, the two PBAEs polyplexes with best performance also demonstrated with high cytotoxicity. Their cytotoxicity might be reduced by further modification of these polyplexes with different molecular weight, end group and optimized ratio of polymer to DNA weight [74].

Previous studies indicated a better way to identify novel polymer with good quality of gene delivery is to synchronously screen the synthesized polymer library. In 2003, Anderson *et al.* synthesized a library with 2350 structurally unique PBAEs by using the revolutionary method, automation and high-throughput combinatorial chemistry. This large polymers library was screened to identify polymers with efficient DNA binding and COS-7 cells transfection in serum-free conditions. The structure-activity connection demonstrated that transfection efficiency of these polymers was dependent on the molecular weight, end groups and

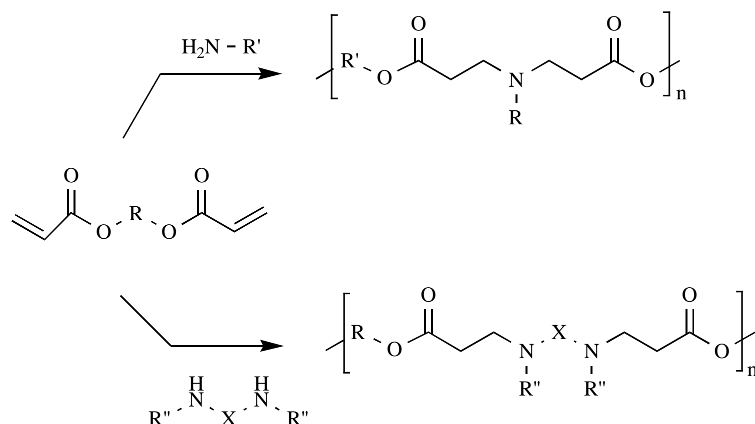

Fig. (3). Synthesis of PBAEs by conjugating addition of amines to diacrylates.

optimal polymer/DNA ratio [17,73-75]. Interestingly, the properties of polymer could be significantly enhanced just by simple modification of the polymer ends. Polymers synthesized with end groups containing terminal primary amines were found to bind DNA more tightly, form smaller polymer/DNA nanoparticles, facilitate higher DNA cellular uptake and mediate higher protein expression [76-78].

Inspired by the works of Dr. Langer and co-workers, many groups have synthesized hyperbranched polymer structures with significant efficiency for gene transfer [79-83]. For example, Park *et al.* synthesized hyperbranched Poly(amino esters) (PAEs) polymers for effective gene transfer. The cytotoxicity of these polymers was much lower than that of PEI or PAMAM, however, the transfection efficiency was higher than that of Poly[α-(4-aminobutyl)-L-glycolic acid] [80].

In general, PAEs could be completely degraded to monomeric units with negligible side effects on cell viability in physiological conditions within 5 h [70]. Longer degradation time is sometimes required for polyplexes during transfection in dendritic cell [84]. The network-type PAEs synthesized by Park *et al.* can prolong the degradation time of the polyplex and exhibit transfection efficiency rivaling that of PEI, even in the presence of serum [85-88]. The degradation of the network-type PAEs was found to be relatively slow in that the complex with DNA lasted 5-10 days in physiological buffer conditions [88].

1.4. Poly[2-(Dimethylamino) Ethyl Methacrylate]

Poly[2-(dimethylamino) ethyl methacrylate] (PDMAEMA) as shown in Fig. (**2**) is another effective gene transfer agent due to its inherent cationic charge. The high *in vitro* transfection efficiency of PDMAEMA polyplexes is attributed to the ability of the polymer capability of destabilizing endosome as well as dissociating easily with the plasmid payload once the polyplexes were delivered into the cytosol [89]. Unlike PEI polymer, many researches indicate that the transfection mechanism of PDMAEMA polyplexes does not accord with “proton sponge” hypothesis [90-92]. For example, Hennink *et al.* attempted to improve the endosome lysis capability of PDMAEMA by incorporating an additional tertiary amino group in each monomeric unit for promoting the “proton sponge” effect, however, the transfection efficiency of modified polymeric structure was significantly lower than that of the unmodified polymer. This result suggests that the “proton sponge” hypothesis may not be universally applicable to all polymers that can mediate the acidic endosomal compartments [90].

Similar with PEI, the transfection efficiency of PDMAEMA could be improved by increasing the molecular weight, but employment of high molecular weight polymers is often concomitant with higher cytotoxicity. Only PDMAEMA with higher molecular weight (MW > 300 kDa) was found to form small and stable polyplexes with DNA in neutral media, while the polymers with relative low molecular weight (MW < 27 kDa) could condense DNA to form large polyplexes [14]. It is known that nonbiodegradable polycations with molecular weight more than 30 kDa can not be eliminated by the kidney [48]. PDMAEMA based polyplexes were mostly designed to be used as biodegradable gene carriers. You *et al.* firstly synthesized oligomers of DMAEMA containing terminal thiol groups by reversible addition-fragmentation chain transfer polymerization using difunctional chain transfer agent. The reducible PDMAEMA (rPDMAEMA) was synthesized by oxidation of the terminal thiol groups, forming a polymer with disulfide bonds in the backbone. Transfection activity of rPDMAEMA was measured in B16F10 mouse melanoma and six human pancreatic cancer cell lines, the results indicated that rPDMAEMA polyplexes present a comparable or better activity than PDMAEMA polyplexes [93]. Jiang *et al.* worked on brushed polymers composed of a backbone of poly(hydroxyethyl methacrylate) onto which PDMAEMA was grafted via a hydrolyzable linker. Under physiological conditions, the brushed polymer was degraded by carbonate ester hydrolysis with a half-life of 96 h. The molecular weights of the formed degradation products was very close to that of the starting PDMAEMA, which is likely below the renal excretion limit (<30 kDa) [94].

1.5. Chitosan

Chitosan (CS) is a biodegradable and biocompatible linear cationic amine-containing polysaccharide (see Fig. (**2**)). Chitosan can be used to condense DNA into 100-200 nm nanoparticles, although they have variable stability in the presence of serum proteins [95]. Chitosan demonstrates protection against DNase degradation that is comparable to PEI polymer, and displays significantly better biocompatibility. Its cationic polyelectrolyte property provides strong electrostatic interaction with negatively charged mucosal surfaces and other macromolecules such as DNA [17]. Numerous modifications to this polymer structure have been made to improve transfection efficiency of chitosan polyplexes [96-101]. For example, N-quaternization of chitosan terminal amines resulted in increased transfection efficiency despite of higher cytotoxicity [100]. The chitosan-g-PLL polymer synthesized by grafting chitosan with polylysine exhibited better DNA-binding ability due to enhanced charge density, reduced cytotoxicity and increased transfection efficiency as compared to both polylysine and 25 kDa PEI polyplexes [101].

1.6. Dendrimers

Dendrimers are spherical, highly branched polymers prepared either by divergent (starting from central core molecule) or convergent (starting where will become the periphery of the molecule building inwards) synthesis strategies [13]. The most currently used dendrimers are polyamines, polyamides and/or polyesters, but the most commonly encounter is polyamidoamine (PAMAM) as shown in Fig. (**2**) because of its high transfection efficiency [102-103]. Dendrimers bear primary amine groups on their surface and tertiary amine groups inside. The primary amine groups participate in DNA binding, compact it into nanoscale particles and promote its cellular uptake, while the buried tertiary amino groups act as a proton-sponge in endosome and enhance the release of DNA into the cytoplasm [9]. Dendrimers are not required to function in their perfect spherical shape [104]. Actually, transfection efficacy can be improved by effectively pruning the branches of the dendrimer or by using the branches themselves. Partially

degraded or fractured PAMAM dendrimers with more flexible structures than intact dendrimers have been shown to have more than 50-fold enhancement for gene delivery as compared to complete PAMAM [105].

2. STRATEGIES TO IMPROVE CELL UPTAKE EFFICIENCY AND CIRCULATION TIME OF POLYPLEXES *IN VIVO*

Polycation/plasmid DNA complexes are usually prepared in the presence of an excess amount of polycation to effectively condense DNA for preventing the access of nucleases to plasmid DNA and improving resistance of plasmid DNA against enzymatic degradation. Therefore, the finally formed complex has a net positive charge [13,106]. Although the positively charged complexes can interact with negatively charged cell membranes and facilitate cellular uptake of the polymer/plasmid DNA complexes *via* endocytosis, the net positive charge of complexes makes them prone to bind with negative charged serum proteins in circulation system, which results in rapid clearance by macrophages and reduction of delivery efficiency. The nonspecific binding may also be the cause for cytotoxicity [107]. However, the ability of polyplexes to circulate *in vivo* for a prolonged period of time is often a prerequisite for successful gene therapy.

To increase biocompatibility and transfection efficiency of polyplexes, it is necessary to mask the net positive charge to enable them circulating *in vivo*. Poly(ethylene glycol) (PEG) was widely used to conjugate the cationic polymers to prevent the inter-particle aggregation of the complexes and to increase complex stability in the presence of serum proteins [9, 107]. PEG-conjugated copolymers have advantages for gene delivery: 1) The PEG-conjugated copolymers show low cytotoxicity to cells *in vitro* and *in vivo*, 2) PEG increases water-solubility of the polymer/DNA complex, 3) PEG reduces the interaction of the polymer/DNA complex with serum proteins and increases circulation time of the complex, 4) PEG can be used as a spacer between a targeting ligand and a cationic polymer, 5) PEG can prevent the capture and clearance by macrophages [106].

2.1. PEGylated Cationic Polymer-Based Gene Delivery Systems

So far, numerous of PEGylated PLL [16,108-117], PEI [68,118-129] and PDMAEMA polymers [14,130-133] have been synthesized in order to increase the polyplexes circulation time and reduce their cytotoxicity *in vivo*. Studies indicate that the properties of the PEGylated PLL/PEI conjugates are strongly dependent on the degree of PEGylation and the molecular weight of PEG [111,120].

Besides increased cytotoxicity, high molecular weight PLL/DNA complexes have shown a tendency to aggregate and precipitate depending on the ionic strength of the solution. The block copolymers of PLL with PEG (PLL-b-PEG) were prepared to prohibit the formation of insoluble precipitates [109]. The polyplexes of PLL-b-PEG and DNA or oligodeoxynucleotides show reduced sizes regardless of the NaCl concentration in the buffer and are resistant to deoxyribonuclease I (DNase I) digestion [109,110]. Comb-shaped PEG grafted PLL (PEG-g-PLL) was first synthesized with different PEG-grafted ratios (PEG: 550; PLL: 25,000) by Choi *et al.* [16]. PEG-g-PLL formed a complex with plasmid DNA at or above a 1:1 weight ratio. DNase I protection assay indicated that 10 mol% PEG-g-PLL could completely protect plasmid DNA for more than 60 min. In addition, PEG-g-PLL had dramatically low toxicity to HepG2 cells. Recently, the PEGylated dendritic PLL was found to enhance blood residence and reduce hepatic accumulation compared to dendritic PLL [134].

Various synthetic strategies have adopted to conjugate PEI with PEG. The primary amino groups of PEI molecules can react with N-hydroxysuccinimide activated PEG to form PEGylated PEI [135]. Alternatively, in a two-step procedure, PEG can be activated with either epoxide [122] or isocyanate groups [120] followed by reaction with the amino groups of PEI. Two series of branched PEI-grafted-PEG (BPEI-g-PEG) was synthesized by Petersen *et al.* [120]. PEI (25 kDa) was grafted to PEG (5 kDa) with different degrees of substitution in first BPEI-g-PEG. The second branched PEI-grafted-PEG contains PEG with molecular weight (MW) from 550 Da to 20kDa. 5 kDa PEG significantly reduced the diameter of spherical complexes from 142 ± 59 to 61 ± 28 nm. With increasing of the grafted PEG, complexation with DNA was impeded and complexes lost their spherical shape. Copolymers with PEG 20 kDa yielded small and compact complexes with DNA (51 ± 23 nm) whereas copolymers with PEG 550 Da resulted in large and diffuse structures (130 ± 60 nm). The zeta potential of complexes was reduced with increasing PEG grafted with molecular weight more than 5 kDa [120]. The synthesized PEG-block-linear PEI (PEG-LPEI) with linear 22 kDa polymer can form complex with DNA and allow the preparation of higher concentrated polyplexes compared to unmodified PEI. High transgene expression was accessed by PEI–PEG/DNA complexes administrated by nasal instillation in mice [119]. Recently, a new biodegradable chitosan-g-PEI-g-PEG-OH with a hydroxyl group at the PEG chain end was prepared for constructing amphiphilic copolymers by grafting reaction of chitosan, polyethylenimine (PEI) and heterobifunctional PEG. The amphiphilic and end-functionalized copolymer may have a potential utility in conjugating with targeting moieties for targetable gene or drug delivery [63].

An acid-labile block copolymer was recently synthesized using a PEG as macroinitiator with an acid-cleavable end group by atom transfer radical polymerization of DMAEMA. PEG and PDMAEMA segments were connected through a cyclic ortho ester linkage (PEG-a-PDMAEMA) at pH 7.4, PEG-a-PDMAEMA polyplexes exhibited smaller particle size, lower surface charge, reduced interaction with erythrocytes, and less cytotoxicity compared to PDMAEMA-derived polyplexes [14]. The strong hydrophilicity of the PEG segments could dissociate PEGylated polyplexes under the physiological environment and attenuate polycation carrying with target genes. To circumvent this issue, Won *et al.* incorporated the hydrophobic middle block of poly(n-butyl acrylate) (PnBA) into the PEG-PDMAEMA and achieved a triblock PEG-b-PnBA-b-PDMAEMA polymer [133]. The properties of the triblock copolymer/DNA complexes are better than that of PDMAEMA homopolymer and PEG-PDMAEMA diblock copolymer, which had comparable molecular weights for individual blocks.

In addition, polyanion was also utilized to assembly with polyplexes or polycation to screen the positive charge [84, 136,137], which may be the next hotspot in polymeric gene vectors. The unique advantage of this strategy is that polyanion conjugated with PEG can assembly with different polyplexes to dissimulate the positive charge. Therefore, intricate PEGylation synthetic steps of different polycation can be avoided. Moreover, the PEG density on the polyplexes surface can be easily controlled by tuning the amount of added polyanion. Polymethacrylic acid-b-polyethylene oxide (PMAA2100-b-POE5000) was used to complex poly-L-lysine. Micelles showed a hydrodynamic diameter of 30 nm with a peculiar core organized with hydrogen bonds as well as hydrophobic domains. The micelles proved high stability in physiological conditions (pH and ionic strength) and were also able to disassemble under acidic conditions mimicking acidic endolysosome [84]. Ni *et al.* designed a novel polyanion named MePEG2000-block-poly(methacrylic acid) carring partial thiol groups (MePEG2000-b-PMAASH). MePEG2000-b-PMAASH was used to coat the poly[(dimethylamino)ethyl methacrylate] end-capped with cholesterol moiety (Chol-PDMAEMA30)/DNA complexes via electrostatic interaction between PDMAEMA and PMAASH. Hydrogen peroxide solution was then added to oxidize the mercapto groups to form bridging disulfide linkages between the MePEG2000-b-PMAASH chains and to stabilize the complexes. This system can provide a better stability during systemic circulation, disintegrate and release DNA once the disulfide linkages are destroyed in cell reducing environment [138].

2.2. Solutions to the limitations of PEGylation

While PEGylation can increase circulation time of polyplexes *in vivo* and reduce their toxicity, gene expression usually concomitantly decreases. The reduced gene expression originates from completely or partially dissimulation of positive charge on the polyplexes surface, which results in decreasing interaction with cell membrane and thus dramatically hampering cellular internalization of polyplexes.

To improve the cell uptake efficiency of polyplexes, various cell-specific targeting ligands have currently been attached to polypexes. Ligand-mediated uptake not only increases cell-specific delivery of pDNA, but also avoids the problem of non-specific interaction to a certain extent [3]. These ligands include but are not limited to small molecules such as folate [139-142] and galactose [143-145] or macromolecules such as transferrin [146-148] and RGD [137,149, 150]. The folate receptor is known to be overexpressed in a large fraction of human tumors, but it is only minimally distributed in normal tissues. Therefore, the folate has been widely used as a tumor-targeting ligand for gene delivery [3]. Lectins are abundantly expressed in the lung, therefore, galactose is selected as a ligand to improve the binding and uptake of the modified polyplexes into lung cells [144]. Transferrin, an iron-binding glycoprotein, is a ligand for tumor targeting. Iron-loaded transferrin is recognized and binds to transferrin receptors on the cell surface. The expression of transferrin receptors is increased in rapidly dividing cells due to a need for iron. Therefore, transferrin receptors are often upregulated on the surface of malignant cells. Transferrin has been used as a tumor-targeting ligand for several drug delivery systems [3]. The RGD peptide as a targeting ligand is known to selectively recognize and bind $\alpha v\beta_3$ and $\alpha v\beta_5$ integrins that are expre-ssed on cell surfaces of certain cell types such as endothelial cells, osteoclast, macrophage, platelets, and melanomas. It was recognized that the integrins are a class of transmem-brane glycoproteins that interact with the extracellular matrix and mediate endocytosis. The receptor-mediated endocytosis is a representative internalization pathway and cellular uptake for targeting gene delivery systems [150]. The localization of ligand in PEGylated polycation can affect the targeting effect. It seems crucial to place it several nano-meters away from the surface of the polyplexes to provide an effective binding with cell surface receptors [9,151].

The reduced transfection efficiency observed with PEGylated PEI has mainly been attributed to the difficulty of gene transfer that occurs after cellular uptake instead of reduced interactions with the cell membrane. This viewpoint is confirmed by the phenomenon that PEI-PEG with cell-targeting ligands is still internalized less effectively than unmodified PEI. While the neutral PEGylated polycation exhibits reduced aggregation, endosomal escape is attributed to cationic charge of the vector. It is necessary that the coating of PEG could be shed as soon as possible after polyplexes enter into endosome, which can be fulfilled by conjugating PEG and polycation with chemical bond hydrolyzing quickly in the endosome environment (pH=5.0). Knorr *et al.* synthesized an acetal-based PEGylation reagent with a maleimide terminus that could be coupled to mercaptan-modified PEI. The hydrolysis of acetal-conjugated PEG chains had a half-life of 2 h at physiological conditions but was reduced to approximately 3 min at pH 5.5. *In vitro* results from this study showed the transfection efficiency of the acetal-based PEGylated PEI was comparable to unmodified PEI, suggesting a potential for improved efficiency *in vivo* [17,127]. Li *et al.* reported an acid-labile block copolymer named PEG-a-PDMAEMA consisting of PEG and PDMAEMA segments connected through a cyclic ortho ester linkage mentioned above. At pH 7.4, PEG-a-PDMAEMA polyplexes exhibited smaller particle size, lower surface charge, reduced interaction with erythrocytes and less cytotoxicity compared to PDMAEMA-derived polyplexes. At pH 5.0, zeta potential of PEG-a-PDMAEMA polyplexes increased up after 2 h incubation and gradual aggregation occurred in the presence of bovine serum albumin (BSA) [14].

3. CONCLUSIONS AND PERSPECTIVES

Undoubtedly, PEI is still the most widely used and effective non-viral gene vector. The modification of its structure is required further study to not only reduce its cytotoxicity but also improve or at least not impair its transfection efficiency. PAEs are a promising series of polycations whose transfection efficiency can rival PEIs, and their biodegradation performance is an overwhelming advantage compared to PEIs. Design and synthesis of new PAEs is an important direction in non-viral vectors for gene therapy. PDMAEMA seems one unique non-viral vector independent of “proton sponge” mechanism. Although PDMAEMA does not show exceptionally high transfection efficiency compared to other cationic polymers, PDMAEMA serves as an excellent model

for structure-transfection studies. Moreover, it is known that PDMAEMA is a thermosensitive macromolecule. Some interesting phenomena will be found with the progress of research for non-viral vectors based on PDMAEMA.

PEGylation of polycations is an effective approach to improve the polyplexes circulation performance *in vivo* and reduce their cytotoxicity. The introduction of targeting ligands in the terminal of PEG can compensate the decrease of cell uptake efficiency of PEGylated polyplexes. After internalization, however, the shielding PEG chains are required to be removed at least partially or completely, re-exposing the surface charge of the polyplexes at endosomal pH condition, which would promote membrane disruption and endosomal escape of the polyplexes and thus enhance gene transfection efficiency. It owns a great significance to develop intelligent delivery systems based on physiologically triggered, reversible shielding technology.

ACKNOWLEDGEMENTS

Authors thank the support of National Grand Program on Key Infectious Disease Control (2008ZX10001-015-10), National Natural Science Foundation of China (30772007 and 30970784), Tianjin Natural Science Foundation (09JCYBJC13800) and Tianjin University Youth Teacher Culture Foundation (TJU-YFF-08B15). This work is also financially supported by the Collaborating Program of China-Finland Nanotechnology (2008DFA01510) and the National Key Basic Research Program of China (2009CB930200).

REFERENCES

[1] Putnam, D. Polymers for gene delivery across length scales. *Nat. Mater*., **2006**, *5*, 439-51.

[2] Luten, J.; Van Nostrum, C.F.; De Smedt, S.C.; Hennink, W.E. Biodegradable polymers as non-viral carriers for plasmid DNA delivery. *J. Control. Release*., **2008**, *126*, 97-110.

[3] Kawakami, S.; Higuchi, Y.; Hashida, M. Nonviral approaches for targeted delivery of plasmid DNA and oligonucleotide. *J. Pharm. Sci*., **2008**, *97*, 726-45.

[4] Nguyen, D.N.; Green, J.J.; Chan, J.M.; Langer, R.; Anderson, D.G. Polymeric materials for gene delivery and DNA vaccination. *Adv. Mater*., **2008**, *20*, 1–21.

[5] Edelstein, M.L.; Abedi, M.R.; Wixon, J. Gene therapy clinical trials worldwide to 2007-an update. *J. Gene. Med*., **2007**, *9*, 833-42.

[6] Jang, J.H.; Lim, K.I.; Schaffer, D.V. Library selection and directed evolution approaches to engineering targeted viral vectors. *Biotechnol. Bioeng*., **2007**, *98*, 515-24.

[7] Verma, I.M.; Somia, N. Gene therapy-promises, problems and prospects. *Nature*, **1997**, *389*, 239-42.

[8] Kodama, K.; Katayama, Y.; Shoji, Y.; Nakashima, H. The features and shortcomings for gene delivery of current non-viral carriers. *Curr. Med. Chem*., **2006**, *13*, 2155-61.

[9] Morille, M.; Passirani, C.; Vonarbourg, A.; Clavreul, A.; Benoit, J.P. Progress in developing cationic vectors for non-viral systemic gene therapy against cancer. *Biomaterials*, **2008**, *29*, 3477-96.

[10] Check, E. Gene therapy put on hold as third child develops cancer. *Nature*, **2005**, *433*, 561.

[11] Hollon, T. Researchers and regulators reflect on first gene therapy death. *Nat. Med*., **2000**, *6*, 6.

[12] Pack, D.W.; Hoffman, A.S.; Pun, S.; Stayton, P.S. Design and development of polymers for gene delivery. *Nat. Rev. Drug. Discov*., **2005**, *4*, 581-93.

[13] Merdan, T.; Kopeček, J.; Kissel, T. Prospects for cationic polymers in gene and oligonucleotide therapy against cancer. *Adv. Drug. Deliv. Rev*., **2002**, *54*, 715-58.

[14] Lin, S.; Du, F.S.; Wang, Y.; Ji, S.P.; Liang, D.H.; Yu, L.; Li, Z.C. An acid-labile block copolymer of PDMAEMA and PEG as potential carrier for intelligent gene delivery systems. *Biomacromolecules*, **2008**, *9*, 109-15.

[15] Wolfert, M.A.; Dash, P.R.; Nazarova, O.; Oupický, D.; Seymour, L.W.; Smart, S.; Strohalm, J.; Ulbrich, K. Polyelectrolyte vectors for gene delivery: influence of cationic polymer on biophysical properties of complexes formed with DNA. *Bioconjug. Chem*. **1999**, *10*, 993-1004.

[16] Choi, Y.H.; Liu, F.; Kim, J.S.; Choi, Y.K.; Park, J.S.; Kim, S.W. Polyethylene glycol-grafted poly-L-lysine as polymeric gene carrier. *J. Control. Release*., **1998**, *54*, 39-48.

[17] Mintzer, M.A.; Simanek, E.E. Nonviral vectors for gene delivery. *Chem. Rev*. **2009**, *109*, 259-302.

[18] Akinc, A.; Langer, R. Measuring the pH environment of DNA delivered using nonviral vectors: implications for lysosomal trafficking. *Biotechnol. Bioeng*., **2002**, *78*, 503-8.

[19] Midoux, P.; Monsigny, M. Efficient gene transfer by histidylated polylysine/pDNA complexes. *Bioconjug. Chem*., **1999**, *10*, 406.

[20] Benns, J.M.; Choi, J.S.; Mahato, R.I.; Park, J.S.; Kim, S.W. pH-sensitive cationic polymer gene delivery vehicle: N-Ac-poly(L-histidine)-graft-poly(L-lysine) comb shaped polymer. *Bioconjug. Chem*., **2000**, *11*, 637-45.

[21] Fajac, I.; Allo, J.C.; Souil, E.; Merten, M.; Pichon, C.; Figarella, C.; Monsigny, M.; Briand, P.; Midoux, P. Histidylated polylysine as a synthetic vector for gene transfer into immortalized cystic fibrosis airway surface and airway gland serous cells. *J. Gene. Med*., **2000**, *2*, 368-78.

[22] McKenzie, D.L.; Smiley, E.; Kwok, K.Y.; Rice, K.G. Low molecular weight disulfide cross-linking peptides as nonviral gene delivery carriers. *Bioconjug. Chem*., **2000**, *11*, 901-9.

[23] Kakizawa, Y.; Harada, A.; Kataoka, K. Glutathione-sensitive stabilization of block copolymer micelles composed of antisense DNA and thiolated poly(ethylene glycol)-block-poly(L-lysine): a potential carrier for systemic delivery of antisense DNA. *Biomacromolecules*, **2001**, *2*, 491-7.

[24] Miyata, K.; Kakizawa, Y.; Nishiyama, N.; Harada, A.; Yamasaki, Y.; Koyama, H.; Kataoka, K. Block catiomer polyplexes with regulated densities of charge and disulfide cross-linking directed to enhance gene expression. *J. Am. Chem. Soc*., **2004**, *126*, 2355-61.

[25] Park, S.; Healy, K. E. Nanoparticulate DNA packaging using terpolymers of poly(lysine-g-(lactide-b-ethylene glycol)). *Bioconjug. Chem*., **2003**, *14*, 311-9.

[26] Putnam, D.; Gentry, C.A.; Pack, D.W.; Langer, R. Polymer-based gene delivery with low cytotoxicity by a unique balance of side-chain termini. *Proc. Natl. Acad. Sci. USA*., **2001**, *98*, 1200-5.

[27] Sonawane, N.D.; Szoka, F.C.; Verkman, A.S. Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. *J. Biol. Chem*., **2003**, *278*, 44826-31.

[28] Akinc, A.; Thomas, M.; Klibanov, A.M.; Langer, R. Exploring polyethylenimine-mediated DNA transfection and the proton sponge hypothesis. *J. Gene. Med*., **2005**, *7*, 657-63.

[29] Boussif, O.; Lezoualćh, F.; Zanta, M.A.; Mergny, M.D.; Scherman, D.; Demeneix, B.; Behr, J.P. A versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*: Polyethylenimine. *Proc. Natl. Acad. Sci. USA*., **1995**, *92*, 7297-301.

[30] Neu, M.; Fischer, D.; Kissel, T. Recent advances in rational gene transfer vector design based on poly(ethylene imine) and its derivatives. *J. Gene. Med*., **2005**, 7, 992-1009.

[31] Jones, G.D.; Langsjoen, A.; Neumann, M.M.C.; Zomlefer, J. The polymerization of ethylenimine. *J. Org. Chem*., **1944**, *9*, 125-47.

[32] Brissault, B.; Kichler, A.; Guis, C.; Leborgne, C.; Danos, O.; Cheradame, H. Synthesis of linear polyethylenimine derivatives for DNA transfection. *Bioconjug. Chem*., **2003**, *14*, 581-87.

[33] Godbey, W.T.; Wu, K.K.; Mikos, A.G. Size matters: Molecular weight affects the efficiency of poly(ethylenimine) as a gene delivery vehicle. *J. Biomed. Mater. Res*., **1999**, *45*, 268-75.

[34] Fischer, D.; Li, Y.; Ahlemeyer, B.; Krieglstein, J.; Kissel, T. *In vitro* cytotoxicity testing of polycations: influence of polymer structure on cell viability andhemolysis. *Biomaterials*, **2003**, *24*, 1121-31.

[35] Fischer, D.; Bieber, T.; Li, Y.; Elsasser, H.P.; Kissel, T. A novel non-viral vector for DNA delivery based on low molecular weight, branched polyethylenimine: effect of molecular weight on transfection efficiency and cytotoxicity. *Pharm. Res*., **1999**, *16*, 1273-79.

[36] Kunath, K.; von Harpe, A.; Fischer, D.; Petersen, H.; Bickel, U.; Voigt, K.; Kissel, T. Low-molecular-weight polyethylenimine as a non-viral vector for DNA delivery: comparison of physicochemical properties, transfection efficiency and *in vivo* distribution with high-molecular-weight polyethylenimine. *J. Control. Release*., **2003**, *89*, 113-25.

[37] Goula, D.; Benoist, C.; Mantero, S.; Merlo, G.; Levi, G.; Demeneix, B.A. Polyethylenimine-based intravenous delivery of transgenes to mouse lung. *Gene. Ther*., **1998**, *5*, 1291-95.

[38] Coll, J.L.; Chollet, P.; Brambilla, E.; Desplanques, D. Behr, J.P. Favrot, M. *In vivo* delivery to tumors of DNA complexed with linear polyethylenimine. *Hum. Gene. Ther*., **1999**, *10*, 1659-66.

[39] Zou, S.M.; Erbacher, P.; Remy, J.S.; Behr, J.P. Systemic linear polyethylenimine (L-PEI)-mediated gene delivery in the mouse. *J. Gene Med*., **2000**, *2*, 128-34.

[40] Wightman, L.; Kircheis, R.; Rossler, V.; Carotta, S.; Ruzicka, R.; Kursa, M.; Wagner, E. Different behavior of branched and linear polyethylenimine for gene delivery *in vitro* and *in vivo*. *J. Gene. Med*., **2001**, *3*, 362-72.

[41] Dunlap, D.D.; Maggi, A.; Soria, M.R.; Monaco, L. Nanoscopic structure of DNA condensed for gene delivery. *Nucleic. Acids. Res*., **1997**, *25*, 3095-101.

[42] Gosselin, M. A.; Guo, W.; Lee, R.J. Efficient gene transfer using reversibly cross-linked low molecular weight polyethylenimine. *Bioconjug. Chem*., **2001**, *12*, 989-94.

[43] Lee, Y.; Mo, H.; Koo, H.; Park, J.Y.; Cho, M.Y.; Jin, G.W.; Park, J.S. Visualization of the degradation of a disulfide Polymer, linear poly(ethylenimine sulfide), for gene delivery. *Bioconjug. Chem*. **2007**, *18*, 13-18.

[44] Peng, Q.; Zhong, Z.L.; Zhuo, R.X. Disulfide cross-linked polyethylenimines (PEI) prepared via thiolation of low molecular weight PEI as highly efficient gene vectors. *Bioconjug. Chem*., **2008**, *19*, 499-506.

[45] Choi, S.; Lee, K.D. Enhanced gene delivery using disulfide-crosslinked low molecular weight polyethylenimine with listeriolysin o-polyethylenimine disulfide conjugate. *J. Control. Release*., **2008**, *131*, 70-76.

[46] Ou, M.; Wang, X.L.; Xu, R.Z.; Chang, C.W.; Bull, D.A.; Kim, S.W. Novel biodegradable poly(disulfide amine)s for gene delivery with high efficiency and low cytotoxicity. *Bioconjug. Chem*., **2008**, *19*, 626-33.

[47] Ahn, C.H.; Chae, S.Y.; Bae, Y.H.; Kim, S.W. Biodegradable poly(ethylenimine) for plasmid DNA delivery. *J. Control. Release*., **2002**, *80*, 273-82.

[48] Shuai, X.; Merdan, T.; Unger, F.; Wittmar, M.; Kissel, T. Novel biodegradable ternary copolymers hy-PEI-g-PCL-b-PEG: synthesis, characterization, and potential as efficient nonviral gene delivery vectors. *Macromolecules*, **2003**, *36*, 5751-59.

[49] Shuai, X.; Merdan, T.; Unger, F.; Kissel, T. Supramolecular gene delivery vectors showing enhanced transgene expression and good biocompatibility. *Bioconjug. Chem*., **2005**, *16*, 322-29.

[50] Petersen, H.; Merdan, T.; Kunath, K.; Fischer, D.; Kissel, T. Poly(ethylenimine-co-L-lactamide-co-succinamide): a biodegradable polyethylenimine derivative with an advantageous pH-dependent hydrolytic degradation for gene delivery. *Bioconjug. Chem*., **2002**, *13*, 812-21.

[51] Arote, R.; Kim, T. H.; Kim, Y. K.; Hwang, S. K.; Jiang, H.L.; Song, H.H.; Nah, J.W.; Cho, M.H.; Cho, C.S. A biodegradable poly(ester amine) based on polycaprolactone and polyethylenimine as a gene carrier. *Biomaterials*, **2007**, *28*, 735-44.

[52] Forrest, M.L.; Koerber, J.T.; Pack, D.W. A degradable polyethylenimine derivative with low toxicity for highly efficient gene delivery. *Bioconjug. Chem*., **2003**, *14*, 934-40.

[53] Kloeckner, J.; Bruzzano, S.; Ogris, M.; Wagner, E. Gene carriers based on hexanediol diacrylate linked oligoethylenimine: effect of chemical structure of polymer on biological properties. *Bioconjug. Chem*., **2006**, *17*, 1339-45.

[54] Park, M.R.; Han, K.O.; Han, I.K.; Cho, M.H.; Nah, J.W.; Choi, Y. J.; Cho, C.S. Degradable polyethylenimine-alt-poly(ethylene glycol) copolymers as novel gene carriers. *J. Control. Release*., **2005**, *105*, 367-80.

[55] Li, L.; Zheng, S.X. Poly(ethylene imine)-graft-poly(ethylene oxide) brush-like copolymers: preparation, thermal properties, and selective supramolecular inclusion complexation with a-Cyclodextrin. *J. Polym. Sci. Part B: Polym. Phys*., **2008**, *46*, 2296-306.

[56] Park, M.R.; Kim, H.W.; Hwang, C.S.; Han, K.O.; Choi, Y.J.; Song, S.C.; Cho, M.H.; Cho, C.Su. Highly efficient gene transfer with degradable poly(ester amine) based on poly(ethylene glycol) diacrylate and polyethylenimine *in vitro* and *in vivo*. *J. Gene. Med*., **2008**, *10*, 198-207.

[57] Piest, M.; Lin, C.; Mateos-Timoneda, M.A.; Lok, M.C.; Hennink, W.E. Feijen, J.; Engbersen, J.F.J. Novel poly(amido amine)s with bioreducible disulfide linkages in their diamino-units: structure effects and *in vitro* gene transfer properties. *J. Control. Release*., **2008**, *130*, 38-45.

[58] Kim, Y.H.; Park, J.H.; Lee, M.; Kim, Y.H.; Park, T.G.; Kim, S.W. Polyethylenimine with acid-labile linkages as a biodegradable gene carrier. *J. Control. Release*., **2005**, *103*, 209-19.

[59] Jiang, G.; Park, K.; Kim, J.; Kim, K.S.; Oh, E.J.; Kang, H.; Han, S.E.; Oh, Y.K.; Park, T.G.; Hahn, S.K. Hyaluronic acid-polyethyleneimine conjugate for target specific intracellular delivery of siRNA. *Biopolymers*, **2008**, *89*, 635-42.

[60] Zintchenko, A.; Philipp, A.; Dehshahri, A.; Wagner, E. Simple modifications of branched PEI lead to highly efficient siRNA carriers with low toxicity. *Bioconjug. Chem*., **2008**, *19*, 1448-55.

[61] Saraf, A.; Hacker, M.C.; Sitharaman, B.; Grande-Allen, K.J.; Barry, M.A.; Mikos, A.G. Synthesis and conformational evaluation of a novel gene delivery vector for human mesenchymal stem cells. *Biomacromolecules*, **2008**, *9*, 818-27.

[62] Sun, Y.X.; Zhang, X.Z.; Cheng, H.; Cheng, S.X.; Zhuo, R.X. A low-toxic and efficient gene vector: carboxymethyl dextran-graft-polyethylenimine. *J. Biomed. Mater. Res. Part A*., **2008**, *84A*, 1102-10.

[63] Wu, Y.D. Liu, C.B. Zhao, X.Y.; Xiang, J.N. A new biodegradable polymer: PEGylated chitosan-g-PEI possessing a hydroxyl group at the PEG end. *J. Polym. Res*., **2008**, *15*,181-5.

[64] Lu, B.; Xu, X.D.; Zhang, X.Z.; Cheng, S.X.; Zhuo, R.X. Low molecular weight polyethylenimine grafted N-maleated chitosan for gene delivery: properties and *In vitro* transfection studies. *Biomacromolecules*, **2008**, *9*, 2594-600.

[65] Jeon, O. Yang, H.S.; Lee, T.J.; Kim, B.S. Heparin-conjugated polyethylenimine for gene delivery. *J. Control. Release*., **2008**, *132*, 236-42.

[66] Bajaj, A.; Kondaiah, P.; Bhattacharya, S. Synthesis and gene transfection efficacies of PEI-cholesterol-based lipopolymers. *Bioconjug. Chem*., **2008**, *19*, 1640-51.

[67] Xu, S.L.; Chen, M.Y.; Yao, Yi.; Zhang, Z.Q.; Jin, T.; Huang, Y.; Zhu, H. Novel poly(ethylene imine) biscarbamate conjugate as an efficient and nontoxic gene delivery system. *J. Control. Release*., **2008**, *130*, 64-68.

[68] Zhang, X.; Pan, S.R.; Hu, H.M.; Wu, G.F.; Feng, M.; Zhang, W.; Luo, X. Poly(ethylene glycol)-block-polyethylenimine copolymers as carriers for gene delivery: effects of PEG molecular weight and PEGylation degree *J. Biomed. Mater. Res. Part A*., **2008**, *84A*, 795-804.

[69] Sun, Y.X.; Xiao, W.; Cheng, S.X.; Zhang, X.Z.; Zhuo. R.X. Synthesis of (Dex-HMDI)-g-PEIs as effective and low cytotoxic nonviral gene vectors. *J. Control. Release*., **2008**, *128*, 171-8.

[70] Lynn, D.M.; Langer, R. Degradable Poly(β-amino esters): Synthesis, characterization, and self-assembly with plasmid DNA. *J. Am. Chem. Soc*., **2000**, *122*, 10761-8.

[71] Lynn, D.M.; Anderson, D.G.; Putnam, D.; Langer, R. Accelerated discovery of synthetic transfection vectors: parallel synthesis and screening of a degradable polymer library. *J. Am. Chem. Soc*., **2001**, *123*, 8155-6.

[72] Akinc, A.; Lynn, D.M.; Anderson, D.G.; Langer, R. Parallel synthesis and biophysical characterization of a degradable polymer library for gene delivery. *J. Am. Chem. Soc*., **2003**, *125*, 5316-23.

[73] Green, J.J.; Langer, R.; Anderson, D.G. A combinatorial polymer library approach yields insight into nonviral gene delivery. *Acc. Chem. Res*., **2008**, *41*, 749-59.

[74] Akinc, A.; Anderson, D.G.; Lynn, D.M.; Langer, R. Synthesis of poly(β-amino ester)s optimized for highly effective gene delivery. *Bioconjug. Chem*., **2003**, *14*, 979-88.

[75] Anderson, D.G.; Lynn, D.M.; Langer, R. Semi-automated synthesis and screening of a large library of degradable cationic polymers for gene delivery. *Angew. Chem., Int. Ed*., **2003**, *42*, 3153-58.

[76] Green, J.J.; Zugates, G.T.; Tedford, N.C.; Huang, Y. Griffith, L.G.; Lauffenburger, D.A.; Sawicki, J.A.; Langer, R.; Anderson, D.G. Combinatorial modification of degradable polymers enables transfection of human cells comparable to adenovirus. *Adv. Mater*., **2007**, *19*, 2836-42.

[77] Zugates, G.T.; Peng, W.; Zumbuehl, A.; Jhunjhunwala, S.; Huang, Y.H.; Langer, R.; Sawicki, J.A.; Anderson, D.G. Rapid optimization of gene delivery by parallel end-modification of poly(*β*-amino ester)s. *Mol. Ther*., **2007**, *15*, 1306-12.

[78] Zugates, G.T.; Tedford, N.C.; Zumbuehl, A.; Jhunjhunwala, S.; Kang, C.S.; Griffith, L.G.; Lauffenburger, D.A.; Langer, R.; Anderson, D.G. Gene delivery properties of end-modified poly(*β*-amino ester)s. *Bioconjug. Chem*., **2007**, *18*, 1887-96.

[79] Wu, D.; Liu, Y.; Jiang, X.; Chen, L.; He, C.; Goh, S.H.; Leong, K.W. Evaluation of hyperbranched poly(amino ester)s of amine constitutions similar to polyethylenimine for DNA delivery. *Biomacromolecules*, **2005**, *6*, 3166-73.

[80] Lim, Y.B.; Kim, S.M.; Lee, Y.; Lee, W.K.; Yang, T.G.; Lee, M.J.; Suh, H.; Park, J.S. Cationic hyperbranched poly(amino ester): a novel class of DNA condensing molecule with cationic surface, biodegradable three-dimensional structure, and tertiary amine groups in the interior. *J. Am. Chem. Soc*., **2001**, *123*, 2460-61.

[81] Zhong, Z.; Song, Y.; Engbersen, J.F.J.; Lok, M.C.; Hennink, W.E.; Feijen, J. A versatile family of degradable non-viral gene carriers based on hyperbranched poly(ester amine)s. *J. Control. Release*., **2005**, *109*, 317-29.

[82] Li, X.; Su, Y.; Chen, Q.; Lin, Y.; Tong, Y.; Li, Y. Synthesis and characterization of biodegradable hyperbranched poly(ester-amide)s based on natural material. *Biomacromolecules*, **2005**, *6*, 3181-88.

[83] Wu, D.; Liu, Y.; Jiang, X.; He, C.; Goh, S.H.; Leong, K.W. Hyperbranched poly(amino ester)s with different terminal amine groups for DNA delivery. *Biomacromolecules*, **2006**, *7*, 1879-83.

[84] Boudier, A.; Aubert-Pouëssel, A.; Louis-Plence, P.; Gérardin, C.; Jorgensen, C.; Devoisselle, J.M.; Bégu, S. The control of dendritic cell maturation by pH-sensitive polyion complex micelles. *Biomaterials*, **2009**, *30*, 233-41.

[85] Lim, Y.B.; Kim, S.M.; Suh, H.; Park, J.S. Biodegradable, endosome disruptive, and cationic network-type polymer as a highly efficient and nontoxic gene delivery carrier. *Bioconjug. Chem*., **2002**, *13*, 952-57.

[86] Kim, T.I.; Seo, H.J.; Choi, J.S.; Yoon, J.K.; Baek, J.; Kim, K.; Park, J.S. Synthesis of biodegradable cross-linked poly(β-amino ester) for gene delivery and its modification, inducing enhanced transfection efficiency and stepwise degradation. *Bioconjug. Chem*., **2005**, *16*, 1140-48.

[87] Kim, H.J.; Kwon, M.S.; Choi, J.S.; Yang, S.M.; Yoon, J.K.; Kim, K.; Park, J.S. Highly effective and slow-biodegradable network-type cationic gene delivery polymer: Small library-like approach synthesis and characterization. *Biomaterials*, **2006**, *27*, 2292-301.

[88] Kim, H.J.; Kwon, M.S.; Choi, J.S.; Kim, B.H.; Yoon, J.K.; Kim, K.; Park, J.S. Synthesis and characterization of poly(amino ester) for slow biodegradable gene delivery vector *Bioorg. Med. Chem*., **2007**, *15*, 1708-15.

[89] Cherng, J.Y.; van de Wetering, P.; Talsma, H.; Crommelin, D.J.A.; Hennink, W.E. Effect of size and serum proteins on transfection efficiency of poly((2-dimethylamino)ethyl methacrylate)-plasmid nanoparticles. *Pharm. Res*., **1996**, *13*, 1038-42.

[90] Funhoff, A.M.; van Nostrum, C.F.; Koning, G.A.; Schuurmans-Nieuwenbroek, N.M.E.; Crommelin, D.J.A.; Hennink, W.E. Endosomal escape of polymeric gene delivery complexes is not always enhanced by polymers buffering at Low pH. *Biomacromolecules*, **2004**, *5*, 32-9.

[91] Dubruel, P.; Christiaens, B.; Vanloo, B.; Bracke, K.; Rosseneu, M.; Vandekerckhove, J.; Schacht, E. Physicochemical and biological evaluation of cationic polymethacrylates as vectors for gene delivery. *Eur. J. Pharm. Sci*., **2003**, *18*, 211-20.

[92] Funhoff, A.M.; Van Nostrum, C.F.; Lok, M.C.; Fretz, M.M.; Crommelin, D.J.A.; Hennink, W. E. Poly(3-guanidinopropyl methacrylate): a novel cationic polymer for gene delivery. *Bioconjug. Chem*., **2004**, *15*, 1212-20.

[93] You, Y.Z.; Manickam, D.S.; Zhou, Q.H.; Oupický, D. Reducible poly(2-dimethylaminoethyl methacrylate): synthesis, cytotoxicity, and gene delivery activity. *J. Control. Release*., **2007**, *122*, 217-25.

[94] Jiang, X.; Lok, M.C.; Hennink, W.E. Degradable-brushed pHEMA–pDMAEMA synthesized *via* ATRP and click chemistry for gene delivery. *Bioconjug. Chem*., **2007**, *18*, 2077-84.

[95] Illum L, Jabbal-Gill I, Hinchcliffe M, Fisher AN, Davis SS. Chitosan as a novel nasal delivery system for vaccines. *Adv. Drug. Deliv. Rev*., **2001**, *51*, 81-96.

[96] Thanou, M.; Florea, B.I.; Geldof, M.; Junginger, H.E.; Borchard, G. Quaternized chitosan oligomers as novel gene delivery vectors in epithelial cell lines. *Biomaterials*, **2002**, *23*,153-9.

[97] Li, F.; Liu, W.G.; Yao, K.D. Preparation of oxidized glucose-crosslinked N-alkylated chitosan membrane and *in vitro* studies of pH-sensitive drug delivery behaviour. *Biomaterials*, **2002**, *23*, 343-7.

[98] Kim, Y.H.; Gihm, S.H.; Park, C.R.; Lee, K.Y.; Kim, T.W.; Kwon, I.C. Structural characteristics of size-controlled self-aggregates of deoxycholic acid-modified chitosan and their application as a DNA delivery carrier. *Bioconjug. Chem*., **2001**, *12*, 932-8.

[99] Sato, T.; Ishii. T.; Okahata, Y. *In vitro* gene delivery mediated by chitosan. Effect of pH, serum, and molecular mass of chitosan on the transfection efficiency. *Biomaterials*., **2001**, *22*, 2075-80.

[100] Kean, T.; Roth, S.; Thanou, M. Trimethylated chitosans as non-viral gene delivery vectors: cytotoxicity and transfection efficiency. *J. Control. Release*., **2005**, *103*, 643-53.

[101] Yu, H.; Chen, X.; Lu, T.; Sun, J.; Tian, H.; Hu, J.; Wang, Y.; Zhang, P.; Jing, X. Poly(L-lysine)-graft-chitosan copolymers: synthesis, characterization, and gene transfection effect. *Biomacromolecules*, **2007**, *8*, 1425-35.

[102] Bielinska. A.; Kukowska-Latallo, J.F.; Johnson, J.; Tomalia, D.A.; Baker Jr, J.R. Regulation of *in vitro* gene expression using antisense oligonucleotides or antisense expression plasmids transfected using starburst PAMAM dendrimers. *Nucleic Acids Res*., **1996**, *24*, 2176-82.

[103] Dennig, J.; Duncan, E. Gene transfer into eukaryotic cells using activated polyamidoamine dendrimers. *Rev. Mol. Biotechnol*., **2002**, *90*, 339-47.

[104] Lee, C.C.; MacKay, J.A.; Frechet, J.M.; Szoka, F.C. Designing dendrimers for biological applications. *Nat. Biotechnol*., **2005**, *23*, 1517-26.

[105] Tang, M.X.; Redemann, C.T.; Szoka Jr, F.C. *In vitro* gene delivery by degraded polyamidoamine dendrimers. *Bioconjug. Chem*., **1996**, *7*, 703-14.

[106] Lee, M.; Kim, S.W. Polyethylene glycol-conjugated copolymers for plasmid DNA delivery. *Pharm. Res*., **2005**, *22*, 1-9.

[107] Park, T.G.; Jeong, J.H.; Kim, S.W. Current status of polymeric gene delivery systems. *Adv. Drug Deliv. Rev*., **2006**, *58*, 467-86.

[108] Wolfert, M.A.; Schacht, E.H.; Toncheva, V.; Ulbrich, K.; Nazarova, O.; Seymour. L.W. Characterization of vectors for gene therapy formed by self-assembly of DNA with synthetic block co-polymers. *Hum. Gene Ther*., **1996**, *7*, 2123-33.

[109] Katayose, S.; Kataoka, K. Water-soluble polyion complex associates of DNA and poly(ethylene glycol)-poly(L-lysine) block copolymer. *Bioconjug. Chem*., **1997**, *8*, 702-7.

[110] Katayose, S.; Kataoka, K. Remarkable increase in nuclease resistance of plasmid DNA through supramolecular assembly with poly(ethylene glycol)-poly(L-lysine) block copolymer. *J. Pharm. Sci*., **1998**, *87*, 160-3.

[111] Choi, J.S.; Lee, E.J.; Choi, Y.H.; Jeong, Y.J.; Park, J.S. Poly(ethylene glycol)-block-poly(L-lysine) dendrimer: novel linear polymer/dendrimer block copolymer forming a spherical water-soluble polyionic complex with DNA. *Bioconjug. Chem*. **1999**, *10*, 62-5.

[112] Choi, J.S.; Joo, D.K.; Kim, C.H. Kim, K. Park, J.S. Synthesis of a barbell-like triblock copolymer, poly(L-lysine) dendrimer-block-poly(ethylene glycol)-block-poly(L-lysine) dendrimer, and its self-assembly with plasmid DNA. *J. Am. Chem. Soc*., **2000**, *122*, 474-80.

[113] Lee, M.; Han, S.O.; Ko, K.S.; Koh, J.J.; Park, J.S.; Yoon, J.W.; Kim, S.W. Repression of GAD autoantigen expression in pancreas β-cells by delivery of antisense plasmid/PEG-g-PLL complex. *Mol. Ther*., **2001**, *4*, 339-46.

[114] Harada, A.; Togawa, H.; Kataoka, K. Physicochemical properties and nuclease resistance of antisenseoligodeoxynucleotides entrapped in the core of polyion complex micelles composed of poly(ethylene glycol)–poly(L-Lysine) block copolymers. *Eur. J. Pharm. Sci*., **2001**, *13*, 35-42.

[115] Harada-Shiba, M.; Yamauchi, K.; Harada, A.; Takamisawa, I.; Shimokado, K.; Kataoka, K. Polyion complex micelles as vectors in gene therapy –pharmacokinetics and *in vivo* gene transfer *Gene Ther*., **2002**, *9*, 407-14.

[116] Bikram, M.; Ahn, C.H.; Chae, S.Y.; Lee, M.; Yockman, J.W.; Kim, S.W. Biodegradable poly(ethylene glycol)-co-poly(L-lysine)-g-histidine multiblock copolymers for nonviral gene delivery. *Macromolecules*, **2004**, *37*, 1903-16.

[117] Fukushima, S.; Miyata, K.; Nishiyama, N.; Kanayama, N.; Yamasaki, Y.; Kataoka, K. PEGylated polyplex micelles from triblock catiomers with spatially ordered layering of condensed pDNA and buffering units for enhanced intracellular gene delivery. *J. Am. Chem. Soc*., **2005**, *127*, 2810-11.

[118] Petersen, H.; Kunath, K.; Martin, A.L.; Stolnik, S.; Roberts, C.J.; Davies, M.C.; Kissel, T. Star-shaped poly(ethylene glycol)-block-polyethylenimine copolymers enhance DNA condensation of low molecular weight polyethylenimines. *Biomacromolecules*, **2002**, *3*, 926-36.

[119] Kichler, A.; Chillon, M.; Leborgne, C.; Danos, O.; Frisch, B. Intranasal gene delivery with a polyethylenimine–PEG conjugate. *J. Control. Release*., **2002**, *81*, 379-88.

[120] Petersen, H.; Fechner, P.M.; Martin, A.L.; Kunath, K.; Stolnik, S.; Roberts, C.J.; Fischer, D.; Davies, M.C.; Kissel, T. Polyethylenimine-graft-poly(ethylene glycol) copolymers: influence of copolymer block structure on DNA complexation and biological activities as gene delivery system. *Bioconjug. Chem*., **2002**, *13*, 845-54.

[121] Shi, L.; Tang, G.P.; Gao, S.J.; Ma, Y.X.; Liu, B.H.; Li, Y.; Zeng, J.M.; Ng, Y.K.; Leong, K.W.; Wang. S. Repeated intrathecal administration of plasmid DNA complexed with polyethylene glycol-grafted polyethylenimine led to prolonged transgene expression in the spinal cord *Gene Ther*., **2003**, *10*, 1179-88.

[122] Sung, S.J.; Min, S.H.; Cho, K.Y.; Lee, S.; Min, Y.J.; Yeom, Y.; Park, J.K. Effect of polyethylene glycol on gene delivery of polyethylenimine. *Biol. Pharm. Bull*., **2003**, *26*, 492-500.

[123] Brus, C.; Petersen, H.; Aigner, A.; Czubayko, F.; Kissel, T. Physicochemical and biological characterization of polyethylenimine-graft-poly(ethylene glycol) block copolymers as a delivery system for oligonucleotides and ribozymes. *Bioconjug. Chem.*, **2004**, *15*, 677-84.

[124] Merdan, T.; Kunath, K.; Petersen, H.; Bakowsky, U.; Voigt, K.H.; Kopecek, J.; Kissel, T. PEGylation of poly(ethylene imine) affects stability of complexes with plasmid DNA under *in vivo* conditions in a dose-dependent manner after intravenous Injection into mice. *Bioconjug. Chem*., **2005**, *16*, 785-92.

[125] Walker, G.F.; Fella, C.; Pelisek, J.; Fahrmeir, J.; Boeckle, S.; Ogris, M.; Wagner, E. Toward synthetic viruses: endosomal pH-triggered deshielding of targeted polyplexes greatly enhances gene transfer *in vitro* and *in vivo*. *Mol. Ther*., **2005**, *11*, 418-25.

[126] Mao, S.; Neu, M.; Germershaus, O.; Merkel, O.; Sitterberg, J.; Bakowsky, U.; Kissel, T. Influence of polyethylene glycol chain length on the physicochemical and biological properties of poly(ethylene imine)-graft-poly(ethylene glycol) block copolymer/SiRNA polyplexes. *Bioconjug. Chem*., **2006**, *17*, 1209-18.

[127] Knorr, V.; Allmendinger, L.; Walker, G.F.; Paintner, F.F.; Wagner, E. An acetal-based PEGylation reagent for pH-sensitive shielding of DNA polyplexes. *Bioconjug. Chem*., **2007**, *18*, 1218-25.

[128] Xiong, M.P.; Forrest, M.L.; Karls, A.L.; Kwon, G.S. Biotin-triggered release of poly(ethylene glycol)-avidin from biotinylated polyethylenimine enhances *in vitro* gene expression. *Bioconjug. Chem*., **2007**, *18*, 746-53.

[129] Lungwitz, U., Breunig, M.; Liebl, R.; Blunk, T.; Goepferich, A. Methoxy poly(ethylene glycol)- Low molecular weight linear polyethylenimine-derived copolymers enable polyplex shieldin. *Eur. J. Pharm. Biopharm*., **2008**, *69*, 134-48.

[130] Xu, F.J.; Li, H.Z.; Li, J.; Zhang, Z.X.; Kang, E.T.; Neoh, K.G. Pentablock copolymers of poly(ethylene glycol), poly((2-dimethyl amino) ethyl methacrylate) and poly(2-hydroxyethyl methacrylate) from consecutive atom transfer radical polymerizations for non-viral gene delivery *Biomaterials.*, **2008**, *29*, 3023-33.

[131] Verbaan, F.J.; Oussoren, C.; Snel, C.J.; Crommelin, D.J.A.; Hennink, W.E.; Storm, G. Steric stabilization of poly(2-(dimethylamino)ethyl methacrylate)-based polyplexes mediates prolonged circulation and tumor targeting in mice. *J. Gene. Med*., **2004**, *6*, 64-75.

[132] Tan, J.F.; Too, H.P.; Hatton, T.A.; Tam, K.C. Aggregation behavior and thermodynamics of binding between poly(ethylene oxide)-block-poly(2-(diethylamino)ethyl methacrylate) and plasmid DNA. *Langmuir*, **2006**, *22*, 3744-50.

[133] Sharma, R.; Lee, J.S.; Bettencourt, R.C.; Xiao, C.; Konieczny, S.F.; Won, Y.Y. Effects of the incorporation of a hydrophobic middle block into a PEG-Polycation diblock copolymer on the physicochemical and cell interaction properties of the polymer-DNA complexes. *Biomacromolecules*, **2008**, *9*, 3294-307.

[134] Okuda T, Kawakami S, Akimoto N, Niidome T, Yamashita F, Hashida M. PEGylated lysine dendrimers for tumor-selective targeting after intravenous injection in tumor-bearing mice. *J. Control. Release*., **2006**, *116*, 330-6.

[135] Ogris, M.; Brunner, S.; Schuller, S.; Kircheis, R.; Wagner, E. PEGylated DNA/transferrin–PEI complexes: reduced interaction with blood components, extended circulation in blood and potential for systemic gene delivery. *Gene. Ther*., **1999**, *6*, 595-605.

[136] Ren, K.F.; Ji, J.; Shen, J.C. Construction of polycation-based non-viral DNA nanoparticles and polyanion multilayers via layer-by-layer self-assembly. *Macromol. Rapid. Commun*., **2005**, *26*, 1633-8.

[137] Green, J.J.; Chiu, E.; Leshchiner, E.S.; Shi, J.L.; Langer, R.; Anderson, D. G. Electrostatic ligand coatings of nanoparticles enable ligand-specific gene delivery to human primary cells. *Nano. Lett*., **2007**, *7*, 874-9.

[138] Gu, Z.X.; Yuan, Y.; He, J.L.; Zhang, M.Z.; Ni, P.H. Facile approach for DNA encapsulation in functional polyion complex for triggered intracellular gene delivery: design, synthesis, and mechanism. *Langmuir*, **2009**, *25*, 5199-208.

[139] Leamon, C.P.; Weigl, D.; Hendren, R.W. Folate copolymer-mediated transfection of cultured cells. *Bioconjug. Chem*., **1999**, *10*, 947-57.

[140] Benns, J.M.; Maheshwari, A.; Furgeson, D.Y.; Mahato, R.I.; Kim, S.W. Folate-PEG-folate-graft-polyethylenimine-based gene delivery. *J. Drug Target*., **2001**, *9*, 123-39.

[141] Ward, C.M.; Pechar, M.; Oupicky, D.; Ulbrich, K.; Seymour, L.W. Modification of pLL/DNA complexes with a multivalent hydrophilic polymer permits folate-mediated targeting *in vitro* and prolonged plasma circulation *in vivo*. *J. Gene Med*., **2002**, *4*, 536-47.

[142] Cheng, H.; Zhu, J.L.; Zeng, X.; Jing, Y.; Zhang, X.Z.; Zhuo, R.X. Targeted gene delivery mediated by folate-polyethylenimine-block-poly(ethylene glycol) with receptor selectivity. *Bioconjug. Chem*., **2009**, *20*, 481-7.

[143] Choi, Y.H.; Liu, F.; Choi, J.S. Kim, S.W.; Park, J.S. Characterization of a targeted gene carrier, lactose–polyethylene glycol-grafted poly-L-lysine, and its complex with plasmid DNA. *Hum. Gene. Ther*., **1999**, *10*, 2657-65.

[144] Chen, J.; Gao, X.L.; Hu, K.L.; Pang, Z.Q.; Cai, J.; Li, J.W.; Wu, H.B.; Jiang, X.G. Galactose-poly(ethylene glycol)-polyethylenimine for improved lung gene transfer. *Biochem. Biophys. Res. Commun*., **2008**, *375*, 378-83.

[145] Jiang, H.L.; Kwon, J.T.; Kim, E.M.; Kim, Y.K.; Arote, R.; Jere, D.; Jeong, H.J.; Jang, M.K.; Nah, J.W.; Xu, C.X.; Park, I.K.; Cho, M.H.; Cho, C.S. Galactosylated poly(ethylene glycol)-chitosan-graft-polyethylenimine as a gene carrier for hepatocyte-targeting. *J. Control. Release*., **2008**, *131*, 150-7.

[146] Kircheis, R.; Blessing, T.; Brunner, S.; Wightman, L.; Wagner, E. Tumor targeting with surface-shielded ligand–polycation DNA complexes. *J. Control. Release*., **2001**, *72*, 165-70.

[147] Kursa, M.; Walker, G.F.; Roessler, V.; Ogris, M.; Roedl, W.; Kircheis, R.; Wagner, E. Novel shielded transferrin-polyethylene glycol-polyethylenimine/DNA complexes for systemic tumor-targeted gene transfer. *Bioconjug. Chem*., **2003**, *14*, 222-31.

[148] Heidel, J.D.; Yu, Z.; Liu, J. Y.C.; Rele, S.M.; Liang, Y.; Zeidan, R.K.; Kornbrust, D.J.; Davis, M.E. Administration in non-human primates of escalating intravenous doses of targeted nanoparticles containing ribonucleotide reductase subunit M2 siRNA. *Proc. Natl. Acad. Sci. USA*., **2007**, *104*, 5715-21.

[149] Schiffelers, R.M.; Ansari, A.; Xu, J.; Zhou, Q.; Tang, Q.; Storm, G. Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticle *Nucleic Acids Res*., **2004**, *32*, e149.

[150] Sun, Y.X.; Zeng, X.; Meng, Q.F.; Zhang, X.Z.; Cheng, S.X.; Zhuo, R.X. The influence of RGD addition on the gene transfer characteristics of disulfide-containing polyethyleneimine/DNA complexes *Biomaterials*, **2008**, *29*, 4356-435.

[151] Blessing, T.; Kursa, M.; Holzhauser. R.; Kircheis, R.; Wagner, E. Different strategies for formation of PEGylated EGF-conjugated PEI/DNA complexes for targeted gene delivery. *Bioconjug. Chem*., **2001**, *12*, 529-37.

Received: October 16, 2009 Revised: December 11, 2009 Accepted: December 12, 2009