Expert Opinion

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Degradable polyethylenimines as DNA and small interfering RNA carriers

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Gene therapy is a powerful approach in the treatment of a wide range of both inherited and acquired diseases. Nonviral delivery systems have been proposed as safer alternatives to viral vectors because they avoid the inherent immunogenicity and production problems that are seen when viral systems are used. Many cationic polymers, including high-molecular-weight polyethylenimine (PEI) have been widely studied as gene-delivery carriers, both, *in vitro* and *in vivo*. However, interest has recently developed in degradable polymeric systems. The advantage of degradable polymer is its low *in-vivo* cytotoxicity, which is a result of its easy elimination from the cells and body. Degradable polymer also enhances transfection of DNA or small interfering RNA (siRNA) for efficient gene expression or silencing, respectively. This review paper summarizes and discusses the recent advances with degradable PEIs, such as cross-linked and grafted PEIs for DNA and siRNA delivery.

Keywords: degradable polyethylenimine, DNA delivery, gene therapy, siRNA delivery

Expert Opin. Drug Deliv. (2009) 6(8):827-834

1. Introduction

Gene therapy is a powerful tool in the treatment of a wide range of inborn and acquired diseases because it can prevent, treat, and even cure such diseases by regulating the expression of bioactive proteins in the cells [1]. Although the gene-therapy approach is promising, success has been limited in clinics by the lack of efficient and safe delivery systems [2]. To date, viral vectors have been tried in the majority of clinical trials because of their high *in-vitro* and *in-vivo* transfection capacity, Unfortunately, their application to human gene therapy is limited by several drawbacks; for example, high immunogenicity, activation of viral components, and complex and expensive engineering [3].

Nonviral delivery systems have been proposed as safer alternatives to viral vectors for gene delivery. These systems have substantial advantages over their viral counterparts because of greater control of their molecular composition and analysis, flexibility in the size of the transgene to be delivered, and relatively lower immunogenicity. Also, nonviral systems are stable, cell targetable, economic, and easy to produce on a large scale. For these reasons, much research has been invested in this area. The greatest disadvantages of these systems are their relatively inefficient transfection compared with the viral vectors, and their cytotoxicity [4].

Among the nonviral vectors, cationic polymers are promising carriers with many unique advantages for efficient gene or small interfering (siRNA) delivery. They have been widely investigated as transfection vectors due to the facility with which they condense and protect negatively charged DNA. Cationic polymers are cheap and easily available commercially. They can be specifically tailored for the

10.1517/17425240903029183 © 2009 Informa UK Ltd ISSN 1742-5247

proposed application with desirable physicochemical or physiological properties, such as degradation under certain conditions or cell-specific targeting. A wide variety of cationic polymers has been characterized for their transfection potential. These polymers include diethylaminoethyl dextran, poly(L-lysine) (PLL), polyethylenimine (PEI), gelatin, polyamidoamine dendrimer, polybrene, poly(vinyl imidazole), poly(L-histidine-g-poly(L-lysine)), poly(β-amino ester), and chitosan. Their gene-binding and condensation capacities, as well as their *in-vitro* and *in-vivo* transfection properties, have been elaborated in the recent literature [5-10].

High-molecular-weight PEI (25 kDa) is one of the most potent polymeric vectors because of its high pH-buffering capacity for endosomal escape; it has been widely explored for gene and siRNA delivery [11]. However, it lacks degradable linkages and is too toxic for therapeutic applications. Hence, low-molecular-weight (LMW) PEI, an alternative to PEI (25 kDa), has been explored. LMW PEI has low cytotoxicity and also poor transfection efficiency [12]. To overcome this limitation, multiple researchers have reported a number of degradable PEIs consisting of LMW PEIs and degradable cross-linkers for intracellular degradation, such as simple hydrolysis, hydrolysis at low endosomal pH, enzymatic degradation, and cytosol-specific reductive degradation by glutathione. These PEIs displayed high transfection efficiency and low cytotoxicity as a result of the rapid *in-situ* degradation of the polymer into small-molecular-weight water-soluble fragments, which are processed easily and removed by the cells.

This review covers recent progress in the development of degradable PEIs as DNA and siRNA carriers to reduce cytotoxicity. It also summarizes transfection activity or silencing efficiency based on the structure of degradable PEIs prepared from linear and branched PEI.

2. Classification of degradable polyethylenimines

Developments in polymeric research originated the concept of degradable PEIs modified with either hydrolysable backbones or reducible linkages. Degradable PEIs can be viewed mainly as cross-linked PEIs or grafted PEIs, depending on the structural differences and the methods of synthesis.

2.1 Degradable cross-linked polyethylenimines

Degradable cross-linked PEI was first described for highly efficient gene delivery purposes by Gosselin *et al.*, who cross-linked LMW PEI using dithiobis succinimidylpropionate (DSP) and dimethyl 3,3′-dithiobispropionimidate (DTBP) as the cross-linking agents [13]. The cross-linked PEI showed efficient gene transfection in Chinese hamster ovary (CHO) cells and reduced toxicity, because disulfide bonds introduced by cross-linking are reduced by the intracellular reducing agent glutathione. The polymers mediated variable levels of transfection depending on the nature cross-linking agent, the extent of conjugation, and the charge ratio N/P (Nitrogen/Phosphate) [13].

Forrest *et al.* cross-linked LMW PEI (800 Da) with 1,3-butanediol (or 1,6-hexanediol) diacrylate as cross-linking agents to generate the ester-cross-linked polymer [14]. They observed that the acrylate groups reacted with both primary and secondary amines, resulting in highly branched, cross-linked, degradable PEI with a final molecular weight (MW) of 14 kDa. The half-life of the cross-linked PEI synthesized from the 1,3-butanediol was 4 h, due to the rapid hydrolysis of ester bonds in the polymeric structure at physiological conditions to produce the diol linkers and amino acids [14]. The degradable polymers exhibited similar size, structure, and DNA-binding properties as commercially available PEI (25 kDa), but mediated gene expression between 2- and 16-fold higher than that of PEI (25 kDa) in MDA-MB-231 cells with lower cytotoxicity.

Thomas *et al.* cross-linked LMW PEI with disuccinimidyl suberate (DSS) and ethylene glycol bis[succinimidylsuccinate] (EGS) as cross-linking agents [15]. The cross-linked PEI-mediated, *in-vitro* gene expression was 550-fold higher than that of LMW PEI and also exceeded – by an order of magnitude – the branched PEI (25 kDa), with lower cytotoxicity. Moreover, *in vivo*, these cross-linked PEIs exhibited 17- to 80-fold higher transfection than the unmodified ones with their efficiencies 2-fold higher than that of PEI (25 kDa) without increasing cytotoxicity [15].

Kloeckner *et al.* synthesized degradable carriers based on oligoamines and cross-linkers DTBP, DSP, and hexanediol diacrylate using the polymer library technique [16]. Degradable polymer using LMW PEI (800 Da) and hexanediol diacrylate showed higher gene-transfer efficiency than linear PEI (22 kDa), with hemocompatibility due to the reductive cleavage of disulfide bonds and ester hydrolysis. Kloeckner *et al.* also reported that the temperature of a Michael addition reaction is very important for the degradation property of the synthesized polymer. A high reaction temperature (60°C) leads to higher amide/ester ratios than a lower temperature (20°C) and thus results into slow degradation half-lives [17].

The degradable, branched PEIs have number of advantages over linear ones due to their high amine density. As the amine density in linear PEI is limited, it may not be enough to condense DNA efficiently. Branched PEIs are therefore more popular for the synthesis of degradable PEIs, although they need more stringent control over the reaction conditions due to the involvement of primary, secondary, and tertiary amines. Linear degradable PEI exhibits a short half-life, as even a few cleavages can reduce chain length rapidly, with quick drop in molecular weight [18], whereas the branched PEI degrades slowly due to the lesser water accessibility of the ester linkages in the branched structures [19].

Petersen *et al.* synthesized degradable branched PEIs by cross-linking LMW PEI (1200 Da) with oligo (L-lactic-co-succinic acid) [20]. The polymer degraded very slowly due to

the amide cross-links in the polymeric backbone with reduction to half of its initial MW after 1.5 months at physiological pH. This polymer mediated gene expression 10-fold higher than the starting PEI (8 kDa) without significant toxicity [20].

Ahn *et al.* cross-linked LMW PEIs (600, 1200, and 1800 Da) with bifunctional PEG to form degradable polymer with ester linkages [21]. Due to the introduction of PEG with degradable ester linkages, the polymer showed three times higher transfection efficiency in 293T cells than the starting PEI (1800 Da), with 80% cell survival; however, the transfection efficiency was lower than that of PEI (25 kDa) [21].

Kim *et al.* synthesized degradable, branched PEI with acid-labile imine linkages using simple reaction conditions [22]. The polymer was rapidly degraded in acidic conditions with a half-life of 2.5 h, and showed transfection efficiency similar to that of PEI (25 kDa) with minimal toxicity.

Cho and co-workers recently synthesized degradable, branched PEIs by means of a simple Michael-type addition reaction of LMW PEI and several cross-linkers. Degradable, hyperbranched PEI was synthesized by means of a Michael-type addition reaction of poloxamer diacrylate (2500 Da) and LMW PEI (1800 Da) [23]. The polymer showed good DNA-binding ability and the sizes of complexes under physiological condition were below 150 nm. The polymer showed much higher transfection efficiencies in A549, 293T, and HepG2 cells than with PEI (25 kDa), with low cytotoxicity resulting from the presence of hydrophobic segments in the polymers [24]. In another study, Cho *et al.* also synthesized degradable branched PEI from LMW PEIs (600, 1200 and 1800 Da) and polycaprolactone (PCL) diacrylate as the hydrophobic cross-linker by a Michael addition reaction, as shown in **Figure 1A**. These polymers degraded in a controlled manner, with a half-life of 4.5 – 5 days at physiological conditions. The polymers showed effective and stable DNA condensation, with particle sizes below 200 nm, and showed low cytotoxicity in three different cells (293T, HepG2, and HeLa). The highest gene expression was obtained for PCL/PEI-1.2 (MW 1200) complexes, which had an *in-vitro* transfection efficiency 15- to 25-fold higher than PEI (25 kDa). Also, these complexes successfully transfected cells *in vivo* after pulmonary administration in the aerosol form [25]. Further, the same group synthesized a novel, degradable, branched PEI based on glycerol dimethacrylate and LMW PEI (1200 Da), as shown in **Figure 1B**. The polymer condensed DNA into nanosized particles below 150 nm with zeta potential in the range of 30 – 55 mV at physiological pH, and degraded slowly with a half-life of 9 – 10 days due to the hyperbranched structure of the polymer. The polymer showed significantly lower cytotoxicity in three different cells (HeLa, HepG2, and 293T cells). The polymer also demonstrated much higher transfection efficiency than PEI (25 kDa) and Lipofectamine™ in three different cells due to the synergistic effect arising from the hyperosmotic glycerol and proton sponge active PEI in the polymer. Furthermore, this polymer was able to transfect cells *in vivo* after aerosol administration [26].

Sun *et al.* recently prepared disulfide-containing PEI based on LMW PEI (800 Da) with cystamine bisacrylamide as the cross-linker [27]. The cross-linked PEI exhibited comparable transfection efficiency, but lower cytotoxicity, with PEI (25 kDa) due to the degradability of the polymer by means of reversible disulfide bonds.

Peng *et al.* also prepared disulfide cross-linked PEIs by ring-opening reaction of LMW PEI (800 Da) with methylthiirane as a thiolation and cross-linking agent [28]. The polymer showed higher gene-transfection efficiency compared with PEI (25 kDa), with low cytotoxicity as a result of the reductively degradable disulfide linkages in the polymer.

Kim and Diamond prepared LMW PEI (600 Da)-modified-poly(ethylene oxide) (PEO) beads with ortho-nitrobenzyl group as a photolabile linker to control the release of DNA spatially and temporally by photoirradiation of a solid phase [29]. Although the PEI-modified solid phase condensed DNA efficiently and released DNA after irradiation, the transfection efficiency and the conformational stability of the released DNA on irradiation were major concerns in the report.

2.2 Degradable grafted polyethylenimines

Several investigators synthesized graft copolymers with either linear or hyperbranched LMW PEIs to overcome cytotoxicity of high MW PEIs. Most studies utilized nonionic hydrophilic cyclodextrin (CD), degradable polymers such as PCL, chitosan and dextran, and polypropylenimine dendrimers.

The first example of high MW PEI grafted with CD for gene delivery was reported by Davis and co-workers [30]. Further, CD-containing PEIs were modified by inclusion complex formation [31], although, the polymers did not degrade at physiological condition.

Tang *et al.* synthesized degradable PEI based on LMW PEI (600 Da) and β-CD-carbonate benzotriazole by means of a polycondensation reaction. The MW of polymer decreased from 61 to 30 kDa within 1 month in PBS buffer (pH 7.0) at 37°C due to the hydrolysis of carbamate carbaryl in the polymer [32], whereas the degradation of the polymer in the Tris buffer was not obvious [33]. The *in-vitro* transfection efficiency of the polymer was higher than that of PEI (25 kDa) in neuronal cells. Also, a level of gene expression was close to the PEI (25 kDa) after intrathecal injection of the complexes into the rat spinal cord [33].

Yang *et al.* synthesized cationic star polymers consisting of an α-CD core and LMW PEI arms. The polymers were stable and resistant to hydrolysis under physiological conditions because of the urethane linkages in the backbone; they complexed DNA stably. The polymers with longer and branched LMW PEIs showed higher transfection efficiency and safety than that of PEI (25 kDa) [34].

Park *et al.* grafted LMW PEI (423 Da) and three different molecular weights of polyethylene glycol (PEG) diacrylates

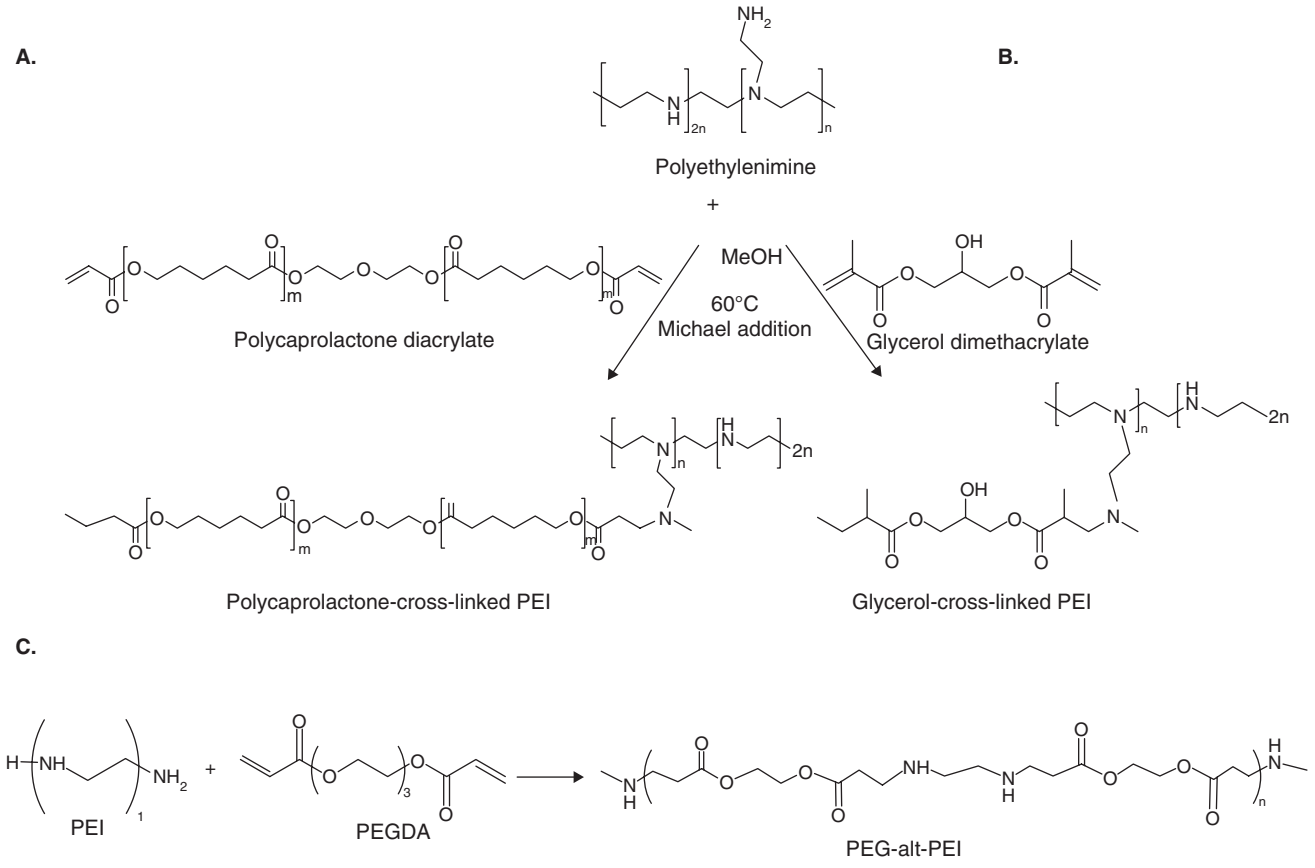

Figure 1. Michael addition reaction schemes. A. For the synthesis of polycaprolactone diacryalte cross-linked polyethylenimine. **B.** For the synthesis of glycerol diacrylate cross-linked polyethylenimine. **C.** For the synthesis of poly(ethylene glycol) diacryalte-alt-polyethylenimine.

by means of a Michael addition reaction (**Figure 1C**) [35]. The MWs of the prepared polymers were between 7 and 12 kDa, with a composition of PEG to PEI of around 1. The polymers were degraded rapidly at 37°C in PBS and the polymer of PEG with MW 575 Da exhibited a half-life of 8 h. Interestingly, the MW of PEG diacrylate drastically affected the transfection efficiency of the copolymer in three cell lines. As the MW of PEG diacrylate increased, the shielding effect of PEG also increased and decreased the uptake of polyplexes by masking the surface charges. These polymers showed enhanced gene transfer efficiency in HepG2 and MG63 cells as compared with PEI (25 kDa) with low cytotoxicity [35]. Park *et al.* have also evaluated the gene expression in various organs after intravenous (IV) and aerosol administration using this polymeric system. The results indicate that the cross-linked LMW PEI efficiently transfects in the lungs and liver by IV and aerosol administrations. This polymer showed higher transfection efficiency than PEI (25 kDa) by both routes of administration as a result of the degradability and low toxicity of the used polymer. Interestingly, higher gene expression by means of aerosol administration was observed in all of the organs when compared with the IV method. In particular, the gene expression of the polymer/DNA complexes at an N/P ratio of 27 in lung after inhalation was about 1500-fold greater than that after IV administration, despite the fact that the delivered aerosol dose was one-tenth of the IV dose [36].

Chitosan and chitosan derivatives have been studied as nonviral carriers due to biocompatibility, biodegradability, and low toxicity. However, they have a significant limitation as a result of their low transfection efficiency. Wong *et al.* synthesized PEI-graft-chitosan by cationic polymerization of aziridine in the presence of water-soluble chitosan (3400 Da) [37]. The polymer showed higher transfection efficiency and safety than that of PEI (25 kDa) in the different cells (HepG2, HeLa, and hepatocyte) because of the proton sponge effect of PEI in the polymer. The polymer also showed 58-fold higher transfection efficiency in liver than in PEI (25 kDa) after administration into the common bile duct in rat liver.

Jiang *et al.* synthesized another chitosan-graft-PEI by an imine reaction between periodate-oxidized chitosan and LMW PEI (1800 Da), as shown in **Figure 2** [38]. The polymer showed good DNA binding and protection from nuclease attack.

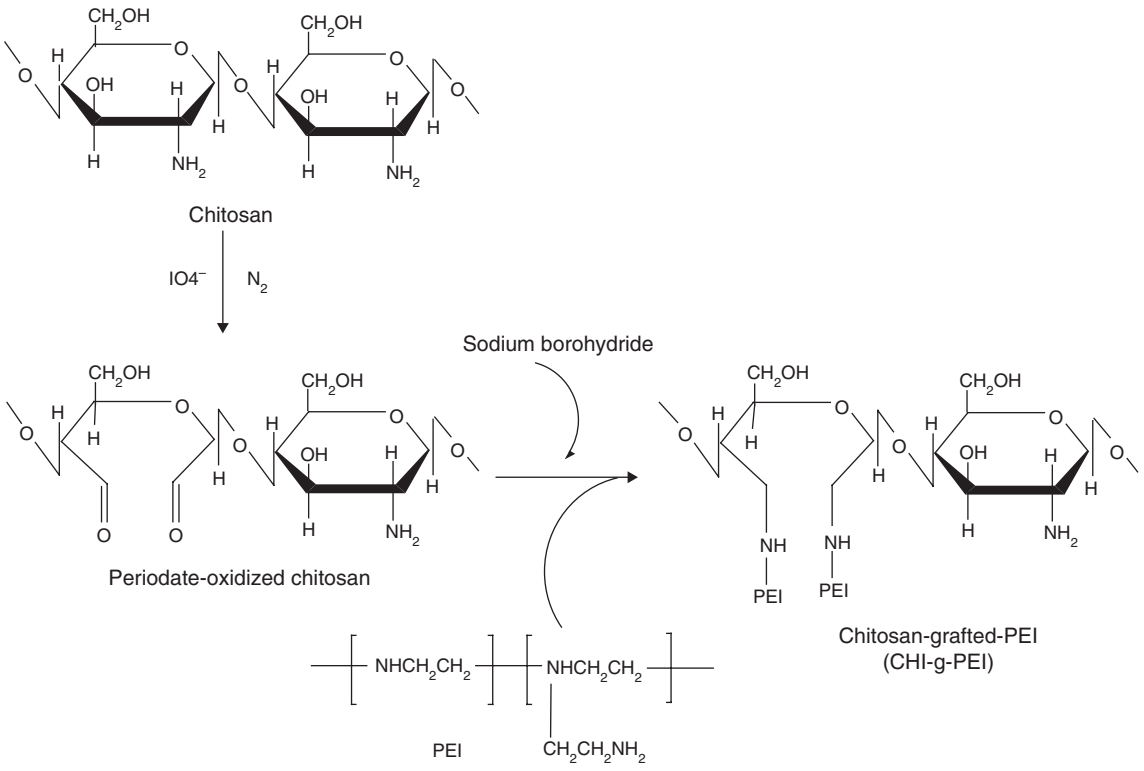

Figure 2. Reaction scheme for the synthesis of chitosan-graft-polyethylenimine [38].

The particle sizes of the polymer/DNA complexes decreased with an increase in N/P ratio and were < 250 nm. At high N/P ratios, the polymer mediated higher transfection efficiency in three different cell lines (HeLa, 293T, and HepG2) than PEI (25 kDa), with lower cytotoxicity. Jiang *et al*. also induced galactose moiety into chitosan-graft-PEI [39] or PEG-chitosan-graft-PEI to obtain better hepatocyte specificity [40]. The galactosylated-chitosan-graft-PEI more efficiently transfected HepG2 cells possessing asialoglycoprotein receptors (ASGPRs) than HeLa cells that are without the receptors, indicating the receptor-mediated endocytosis. The polymer also displayed higher *in-vivo* transfection efficiency in liver than the PEI (25 kDa) carrier after intraperitoneal administration. Generally, PEG facilitates the formation of polyplexes with improved solubility, decreased aggregation, lower cytotoxicity, and decreased opsonization with serum proteins in the bloodstream [41]. Therefore, ^{99m}Tc-Gal-PEG-chitosan-g-PEI/DNA complexes accumulated mainly in the liver, lungs, and heart, whereas ^{99m}Tc-PEI/DNA complexes accumulated rapidly in the lung because the Gal-PEG-chitosan-g-PEI/DNA complexes had increased circulation time after IV injection of the complexes into mice due to the hydrophilic nature of PEG [40]. Also, more ^{99m}Tc-Gal-PEG-chitosan-g-PEI/DNA complexes accumulated in the liver over the time than ^{99m}Tc-PEI/DNA or ^{99m}Tc-chitosan-g-PEI/DNA complexes, due to the specific interaction of galactose moieties with ASGPRs in the hepatocytes.

In another study, Lou *et al*. synthesized PEI-graft-chitosan by grafting LMW PEI (600 Da) into the chitosan through a short PEG linker (440 Da) with terminal epoxide rings to decrease the inherent cytotoxicity of PEI-graft-chitosan [42]. The polymer showed higher cell viability in 293T cells than chitosan, and also mediated higher gene expression in 293T cells than chitosan.

Lu *et al*. synthesized PEI-graft-*N*-maleated chitosan through grafting of LMW PEI (800 Da) to *N*-maleated chitosan by a Michael addition reaction [43]. The polymer showed low cytotoxicity and good transfection efficiency in both 293T and HeLa cells, although high MW polymer showed higher cytotoxicity and lower transfection efficiency than the LMW polymer.

Dextran is also one of the typical natural biodegradable polysaccharides that is digested enzymatically in human body [44] and would be favorable to decrease the cytotoxicity if derivatized for the gene delivery application. Sun *et al*. synthesized dextran-graft-PEIs through grafting LMW PEI (800 Da) to hexamethylenediisocynate modified dextran [45]. The polymer showed lower cytotoxicity than PEI (25 kDa). The gene transfection efficiency of dextran-graft-PEI/DNA complexes in 293T cells was higher than or comparable to

PEI (25 kDa)/DNA complexes. Dextran-graft-PEI synthesized from the LMW dextran demonstrated lower cytotoxicity and higher transfection efficiency than the dextran-graft-PEI with a high MW dextran. Sun *et al*. also synthesized another dextran derivative of carboxymethyl dextran-graft-PEI by grafting LMW PEI (800 Da) to carboxymethyl dextran. The polymer showed lower cytotoxicity than PEI (25 kDa) and the exhibited higher gene expression in HEK293 cells owing to the endosomal disruption capacity of the polymer [46].

Russ *et al*. synthesized degradable PEI-graft-polypropylenimine (PPI) dendrimers through ester-degradable branches with either LMW PEI (800 Da) or PPI dendrimer G2 [47]. The polymer showed similar or even higher transfection efficiency in B16F10 and neuro 2A cells than PEI (25 kDa) without polymer-induced erythrocyte aggregation. Also, on IV injection of PEI-grafted dendrimer polyplexes into tumor-bearing transgenic mice, the expression was predominantly observed in subcutaneous tumors.

3. Small interfering RNA delivery by degradable polyethylenimines

The discovery of RNA interference (RNAi)-mediated gene silencing has recently increased our knowledge of the molecular mechanisms involved in the development of a number of diseases. Being highly target specific, RNAi has wide therapeutic potential, including in cancer. Many researchers have already explored this strategy for silencing overexpressed cancer proteins in cancer therapy [48,49]. However, efficient delivery of siRNA is still a major bottleneck in their success, besides their nonuniform and transient silencing, which necessitate multiple deliveries. Therefore, delivery of siRNA with an efficient and safe polymeric carrier may provide an alternative strategy for RNAi-based research. Although degradable PEI-mediated siRNA delivery is at an initial stage, it has immense therapeutic potential.

The first report of degradable PEI based on chemically condensed LMW PEI containing beta-aminopropionamide was performed for siRNA delivery by Tarcha *et al*. [50]. The polymer was obtained by *N*-acylation of degradable PEI made by means of the Michael reaction of LMW PEI (800 Da) and hexanediol diacrylate to improve chemical stability relative to ester-containing polymers, but in comparison to PEI (25 kDa), better degradability through the amide linkages. The polymer showed significant *in-vitro* knockdown of the luciferase gene, up to 80%, in comparison to nontargeting siRNA in stably transfected HUH7 cells [50].

Breunig *et al*. synthesized degradable PEI by introducing disulfide bonds into the LMW PEI and studied the relationship between cellular uptake and RNAi activity among linear PEI (5 kDa), cross-linked PEI and branched PEI (25 kDa). The results indicated that the cellular uptake of siRNA was more efficient with increasing branching of the polymer, whereas the siRNA release was promoted by cross-linked PEI, suggesting that a combination of a high branching density and reductively cleavable bonds within the PEI is promising towards improving siRNA delivery [51].

Jere *et al*. evaluated degradable PEI based on LMW PEI (423 Da) and PEG diacrylate (258 Da) for small interfering/small hairpin (si/sh) RNA delivery in A549 cells [52]. The Polymer successfully delivered siRNA targeting enhanced green fluorescence protein (EGFP) and silenced EGFP expression. The silencing achieved with the polymer was 1.5-fold higher and safer than PEI (25 kDa). The polymer also exhibited superior protein kinase Akt1 shRNA delivery, and thereby efficiently silenced oncoprotein Akt1. Furthermore, polymer shAkt-mediated Akt1 knockdown hindered cancer-cell growth in A549 cells in an Akt1-specific manner due to the degradability of the polymer [52]. Akt (protein kinase B) is an important regulator of cell survival [53] and plays a key role in cancer by stimulating cell proliferation, inhibiting apoptosis, and modulating the protein translation [54]. Hence, the same group studied the Akt1 gene silencing after aerosol delivery of degradable PEI/Akt1 siRNA complexes into *K-ras* and urethane-induced lung-cancer-model mice [55]. The aerosol-delivered Akt1 siRNA suppressed the mRNA and protein expression of Akt1 specifically without affecting the Akt2 and Akt3 in the lungs of *K-ras* mice. Also, the number of tumors and the mean of tumor diameter were significantly decreased by Akt1 siRNA treatment [55].

4. Conclusions

Degradable PEIs comprise a class of degradable cationic polymers with many desirable properties in the perspective of gene and siRNA delivery. Their synthesis is straightforward and economical. Various structural variants can be generated from a broad array of commercially available monomers. Degradable PEIs can be cross-linked or grafted, depending mainly on the MW and type of PEI monomer and on reaction conditions. The branched, degradable PEIs are more stable than linear ones; however, linear degradable PEIs have displayed similar or slightly higher transfection efficiency than branched ones. Linear as well as branched degradable PEIs have been proved efficient both *in vitro* and *in vivo*. Moreover, degradable PEIs demonstrated high potential in siRNA-based noninvasive cancer therapies. Overall, degradable PEIs are nonviral vectors with high potential in gene- and siRNA-based therapies.

5. Expert opinion

Degradable polymers for gene delivery have been increasingly investigated over the past 5 – 10 years because the high molecular weight of PEI limits the use of gene carriers *in vitro* and *in vivo* because of the cytotoxicity of the polymers and accumulation *in vivo*. Degradation of the polymers as gene carriers enables a reduction in cytotoxicity owing to small molecular weights by degradation and easy elimination by the *in-vivo* excretion pathway. It might also

enhance transfection of DNA or gene silencing of siRNA by unpackaging the polymer/DNA or polymer/siRNA complexes and release of DNA (or siRNA). Degradable PEI-based polymers such as linear, branched, and grafted PEIs with degradable or reducible linkers are very promising because these polymers mediate higher transfection or higher gene silencing than PEI (25 kDa). Moreover, they are safe and biocompatible with potential properties such as prolonged circulation half-life, bioresponsiveness, and target-specific degradability. Degradable PEIs also opt for spatial and temporal delivery of nucleic acids as they are proved superior to PEI (25 kDa). However, the structure–transfection activity relationship of polymer should be studied in detail to find leading gene-carrier candidates to elucidate the influence of chemical structure, charge density, hydrophobicity, degradable linkages, and molecular weight on transfection efficiency or gene silencing. The pharmacokinetic and pharmacodynamic aspects including nonspecific uptake by immune system also need special attention. Moreover, comprehensive *in-vivo* studies are needed in a variety of animal models because the majority of studies carried out so far are either *in vitro* or in the mouse model. Ultimately, the optimum carrier will expand the traditional applications of genetic therapy with the arms of gene and RNAi therapy within the clinical trials, and will provide an effective technology for genetic manipulations of diseases and disorders to offer a better and healthy human life.

Declaration of interest

This work was supported from National Research Laboratory of Korean Science and Engineering Foundation (ROA-2008-000-20024-0). Authors D Jere and HL Jiang are supported by the Brain Korea (BK) Fellowship.

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