Expert Opinion



# Degradable poly(amido amine)s as gene delivery carriers

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***Introduction:*** In recent years, there has been a great deal of interest in the development of vectors which are being developed based on the capacity of polymers to mediate appropriate interactions with the cellular environment, or to interface with specific cellular processes. Several such vectors have been synthesized, resulting in biomacromolecules with low cytotoxicity and higher gene delivery ability.

***Areas covered:*** This review briefly describes the recent success of poly(amido amine)s (PAAs) as non-viral vectors, and highlights their promising future in the development of nucleic acid-based therapy. It also provides an overview on the synthesis, characterization and application of PAAs as gene carriers, which will be useful for various biological motifs. This review helps the readers to better understand the emergence of non-viral vectors for gene therapy, especially PAAs, their properties, their advantages and disadvantages and the gene therapy based on them.

***Expert opinion:*** The future of gene-based therapy needs to identify approaches to develop new carriers, depending on the properties of the biological membranes they face, and their physicochemical properties, in order to successfully deliver the genes to the target sites. With the emergence of a variety of non-viral vectors, such as biodegradable polymers, it may not take long before non-viral vectors are observed that are not just safe and tissue-specific, but even more efficient than viral vectors.

**Keywords:** gene delivery, poly(amido amine)s, siRNA delivery, transfection efficiency

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## 1. Introduction

Gene therapy is one of the most promising therapies of inherited and acquired diseases because it can treat, cure and prevent such diseases by controlling the expression of bioactive proteins in the cells [1]. However, the lack of safety and efficient delivery systems limits the success of gene therapy. Viral vectors have been tried in the majority of clinical trials due to their specific characters to enter the cells and to deliver the gene into cells and results in high transfection efficiency [2]. Unfortunately, their application to human trials is limited by severe immune responses, activation of viral components and limited insert size. Among non-viral vectors, polyethylenimine (PEI) has been widely used as a successful gene carrier owing to its high pH-buffering capacity for endosomal escape [3]. However, high molecular weight (HMW) PEI is too toxic for therapeutic applications due to lack of biodegradability and interference with intracellular membranes. Low molecular weight (LMW) PEI has much lower cytotoxicity, but polyplexes of it lack stability, resulting in poor transfection. Improvement could be achieved with LMW PEI that was cross-linked with biodegradable linkers. [4]. Therefore, many researchers have reported a number of degradable PEIs consisting of LMW PEIs and degradable cross-linkers. These degradable PEIs showed low cytotoxicity and high transfection efficiency due to the degradation of the polymers [5,6].

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**Article highlights.**

- Understanding of the correlations between polymeric functionalities and gene delivery properties is important for the rational design of efficient cationic polymeric vectors.
- Poly(amido amine)s (PAAs) is a group of synthetic functional polymers with a significant potential for variety of biomedical applications such a gene/drug delivery.
- The polymerization reaction for PAAs is tolerable with a large variation in structural fragments in the amine and bisacrylamide monomers, thus allowing large variation of chemical functionalities in the main chain and side chain of the polymers.
- Physicochemical properties such as degradability, surface charge, particle size and crystallinity are useful for rational design of PAAs for stability of the DNA nanocomplexes, and their intracellular trafficking in the gene transfer process.
- The introduction of disulfide bonds into the backbone of polymers enables fast intracellular fragmentation of the polymers, leading to facilitated gene unpacking and improved transfection efficiency.
- PAAs with various structural characteristics such as charge density, acid–base properties, buffer capacity, hydrophilicity/hydrophobicity, type and number of amino groups, backbone degradability and specific interaction groups are recognized to influence gene delivery properties such as DNA binding capability, colloidal stability, endosomal escape (buffer capacity), vector unpacking and cytotoxicity.
- PAAs with minor fractions of protonatable fragments in the main chain are promising carriers for delivery of siRNA. Thus, the positive charge density in the gene carriers has to be delicately tuned to obtain maximum transfection results.

This box summarizes key points contained in the article.

Development and characterization of non-viral vectors, especially poly(amido amine)s (PAAs) have been highlighted along with their transfection activity which is based on the structure of degradable PAAs prepared from linear, branched and reducible linkages. However, PAA dendrimers will not be discussed due to the page limitation although several references can be cited for the readers [7-9]. The PAAs have better water solubility with low cytotoxicity and are hydrolytically more stable than poly(ester amine)s (PEAs) although amide hydrolysis is too slow to completely free the DNA from polyplexes in the cytoplasm [10].

This review focuses the non-viral vectors which are characterized by an interaction by means of either hydrophobicity or surface charge between cationic polymers and nucleic acids. These non-viral vectors with various biodegradable linkages show great ease of application as gene carriers owing to their safety and higher transfection efficiency. **Figure 1** shows the overview of basic biodegradable linkages which shows promising gene delivery capability. Any carrier system developed normally characterized *in vitro* first and then followed by *in vivo*. Interestingly, the correlation between *in vitro* and *in vivo* rarely exists [11] and experimental conditions such as various concentrations of solutions used and variety of buffers, which are optimized for the given delivery vehicle, always vary among the reports. Taken together, though numerous studies have been done in this field, very little is known about the characteristics and properties of successful *in vivo* gene delivery vectors and among them only few guiding principles are generally applicable. The combination of extensive chemical possibilities and the complexity of living organism lead to such circumstances; therefore, developing new drug/gene carrier is always a long and extensive process.

For the past few years progress in the field of pharmacy has increased remarkably with the new techniques so that severe diseases can be cured with new advances. Providing more information on common principles and general guidelines is equally important for significant progress in the field. Several important parameters which are considered to play an important role in pharmacokinetics as well as pharmacodynamics are (i) physicochemical parameters such as structure, composition, solubility and degradation of polymers and (ii) size and surface charges of polyplexes. At the same time, pH values, buffer composition and temperature are likely to have effect on solubility, size and surface charges of particles. For successful gene delivery, intracellular barriers have to be addressed and considered carefully while designing of the formulation. Cell internalization plays an important role during the travelling of polyplexes and it was found that carrier properties affect this process drastically [12]. At the same time carrier characteristics affects endosomal escape [13,14] and nuclear uptake [15-17]. The importance of carrier characteristics and how those affects the formulation parameters as well as cell internalization, endosomal escape and diffusion are finding the prime importance during the successful gene delivery. So, some of the recent reports of carrier development which constitutes PAAs, their physicochemical properties, *in vitro* and *in vivo* gene delivery ability are discussed. Specifically, the composition of the nanoparticles concerning size, surface charge, PEGylation and functionalization is discussed.

## 2. Physicochemical properties of PAAs

### 2.1 Degradation

Solubility of polymers generally depends on the presence of functional groups in their side chain and PAAs containing amide bond in their side chain are degradable in aqueous solution [18]. Degradation rate gets affected by the structure–activity relationship and the structure of both amine and amide bond [19]. Several reports show that the presence of additional tertiary amino group boosts the degradation rate. However, no clear evidence has been found where degradation rate of PAA is affected by the basicity of the amino groups in the backbone. No apparent effect of



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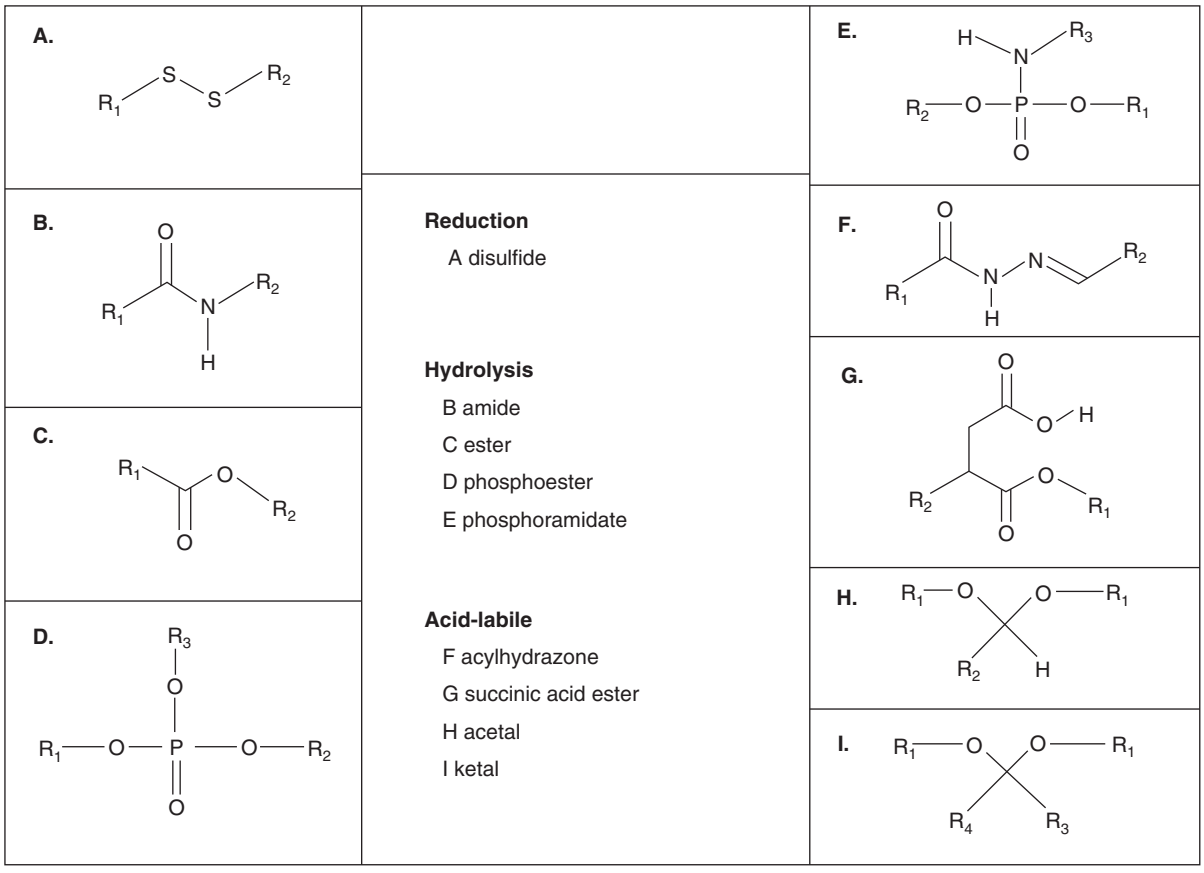


**Figure 1. Overview of basic biodegradable linkages.**

carboxylic acid group was found. However, it was reported by Bignotti *et al*. that mechanism for the role of carboxylic acid group is not clearly understood yet. However, they found that PAAs obtained from amino acids such as glycine or β-alanine apparently degrade faster than the similar kind of PAA with no carboxyl substituent. But interestingly, it was found that PAAs derived from bisacrylamido acetic acid (BAC) degrade at slower rate [20]. It was also reported that mechanism of PAA degradation seems to be purely hydrolytic, as no vinyl groups, such as those that would have derived from a β-elimination reaction, could be determined by nuclear magnetic resonance (NMR) spectroscopy.

As a result of PAAs degradability, the use of water as a reaction medium increases the average molecular weight of the product with reaction time until it reaches maximum and later steadily decreases. It was found that polyaddition reaction in aqueous media competes with the hydrolytic cleavage of amide bonds. The maximum attainable molecular weight in water depends on the monomer structure and concentration, as well as on the reaction temperature, being higher for higher concentrations and lower temperatures. At relatively high temperatures, for instance 40 – 60°C, the maximum molecular weight is attained more quickly but is lower [21].

### 2.2 Solubility and molecular mass

Molecular masses of PAAs such as number average and weight average described so far were in a range of 5000 – 40,000 (number average) and 10,000 – 70,000 (weight average) with poly dispersity indices of ~ 2 which generally depends on the isolation method [5].

Solubility of PAAs is a major governing factor for their success and most PAAs are soluble in water and in organic solvents such as chloroform, dimethyl sulfoxide (DMSO), lower alcohols and polar solvents. However, amphoteric PAAs dissolve only in water. PAAs show the intrinsic viscosities in organic solvents or in aqueous media generally range from 0.15 to 1 dl/g. Generally, PAAs show relatively large hydrodynamic volumes in solution if compared with vinyl polymers with same range of molecular weights, showing a tendency to assume an extended chain conformation in solution [20].

### 2.3 Crystallinity

Many PAAs in the solid state are generally crystalline owing to their regular structure. Sometimes crystallization can be induced by solvent treatment. It has been practically found that crystalline PAAs have melting point in the range of 80 – 120°C. However, melting point increases as high as



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270°C (with decomposition) when cyclic structures are present [22].

### 2.4 Acid–base properties of PAAs

Almost all PAAs contain tertiary amine groups in their main chain so they are generally classified as polyelectrolytes. Normally, the values of protonation constants (logK) of polyelectrolytes depend on the degree of protonation of the whole macromolecule. In case of PAAs, with additional tertiary amino group in the side chains, thermodynamic values are similar to those of non-macromolecular models. In these polymers, however, viscometric titrations did not show any jump either after first or second protonation step. This is probably due to the fact that anion in PAAs can interact with one or another of the two neighboring carbonyl groups. This leads to greater conformational freedom and hence the viscometric titrations do not show any jump even after second protonation step.

## 3. Classification of degradable PAAs

The PAAs are a family of peptidomimetic polymers prepared by Michael addition reaction of amines to macromonomers and by grafting of LMW PEI with polypeptide.

### 3.1 Degradable linear PAAs

Hydrolytic stability of PAAs have been increased compared with poly(amino ester)s as the amide group is less sensitive to hydrolysis than the ester group. These PAAs represent a versatile group of polymers and their linear form with alternating amide and tertiary amine functions can be easily prepared by reaction of difunctional primary amines or bis(secondary amine)s to bisacrylamide derivatives. The tertiary amine groups in the main chain can be protonated, giving the polymer an overall basic and polycationic character and generally a good solubility in water. **Figure 2A** represents the general overview of the synthesis scheme for PAAs. Lin *et al*. have synthesized and studied the properties of a number of these polymers and have pointed out its high potential for biomedical applications due to their low toxicity and biodegradability [23]. Jones *et al*. synthesized linear PAAs by Michael addition reaction of amine compounds to bisacrylamides with various charge densities and chain stiffness in terms of their DNA binding, polyplex stability and transfection efficiency [24]. The results indicated that polyplexes of meythylene-*N,N*′-bis(acrylamide) (MBA)-methyamine (MMA) and 1,4-bis(bisacryloyl)piperazine (BAP)-2-methylpiperazine (2MP) showed low transfection efficiency due to their poor DNA binding ability and colloid stability, whereas polyplexes of MBA-*N,N*′-dimethylethylenediamine (DMEDA) showed higher transfection efficiency than that of MBA-2MP and was comparable with Lipofectamine, suggesting that charge density of the polymers and the structural flexibility influence DNA binding capability of polymers and colloid stability of polyplexes and, consequently the transfection efficiency [25].

Also, they developed a series of amphoteric PAAs having a carboxylic acid group in the bisacrylamide unit to have a pH-dependent conformational change on protonation of the carboxylate and amino groups in these polymers [26]. The results indicated that polymers prepared from 2,2-bis(acrylamido) acetic acid (BAC) and 2MP showed higher hemolysis at pH 6.5 and a comparable transfection efficiency compared with that of PEI 25K due to the endosomolytic activity.

Lin *et al*. also demonstrated that linear PAAs containing secondary and tertiary amines were readily synthesized by Michael addition polymerization of 1-(2-aminoethyl)piperazine (AEP) and equimolar bisacrylamide. These PAAs effectively condense DNA into nanosized polyplexes (< 150 nm) with cationic surface charge. All PAAs have a favorable high buffer capacity that may favor the endosomolytic pathway of polyplexes of these polymers. The polyplexes formed from PAA polymers with reducible disulfide linkages, like p(cystamine bisacrylamide (CBA)-AEP) and its CBA-derived copolymers, showed remarkably superior transfection and lower cytotoxicity compared with those of the reference polymers PEI and poly(2-(dimethylamino)ethyl methacrylate) (pDMAEMA), thus showing that the PAAs with disulfide linkage are very promising carriers for safe and efficient gene delivery [23].

### 3.2 Reducible PAAs

The incorporation of disulfide bond as reducible linker in the linear PAAs has received much attention in gene delivery because the disulfide can be cleaved inside the cells by reducing enzymes such as glutathione reductase and glutathione [25]. The disulfide is relatively stable in the extracellular environment whereas it is rapidly degradable inside the cells [26]. The basic reaction scheme for bioreducible PAAs is shown in **Figure 2B**.

Lin *et al*. developed a series of reducible PAAs containing secondary and tertiary amine groups in their main chain and different structures in the bisacrylamide segments [23]. The results indicated that polyplexes from PAAs with disulfide linkages gave significant higher transfection efficiency than those from PAAs without the disulfide linkages with low toxicity and the contents of disulfide linkage in PAAs affected gene expression. They also reported that polyplexes of branched disulfide-containing PAAs mediated much higher transfection efficiencies than PEI 25K with low toxicity in different cell types [27]. Furthermore, they studied the effects of different pendant groups [28] with variable charge density and buffer capacity of the pendant groups [29], and amino type and amino spacer length in the pendant groups [30] of reducible PAAs on the transfection efficiency. The results showed that increase in the buffer capacity of the polymers and the presence of pendant chains in the polymer enhanced the transfection efficiency due to the increased polyplex–cell membrane interaction [28]. Also, the copolymer of CBA-histamine (HIS)/dimethyl aminopropylamine (DMPA) having a HIS/DMPA mole ratio of 70/30 mediated higher transfection efficiency



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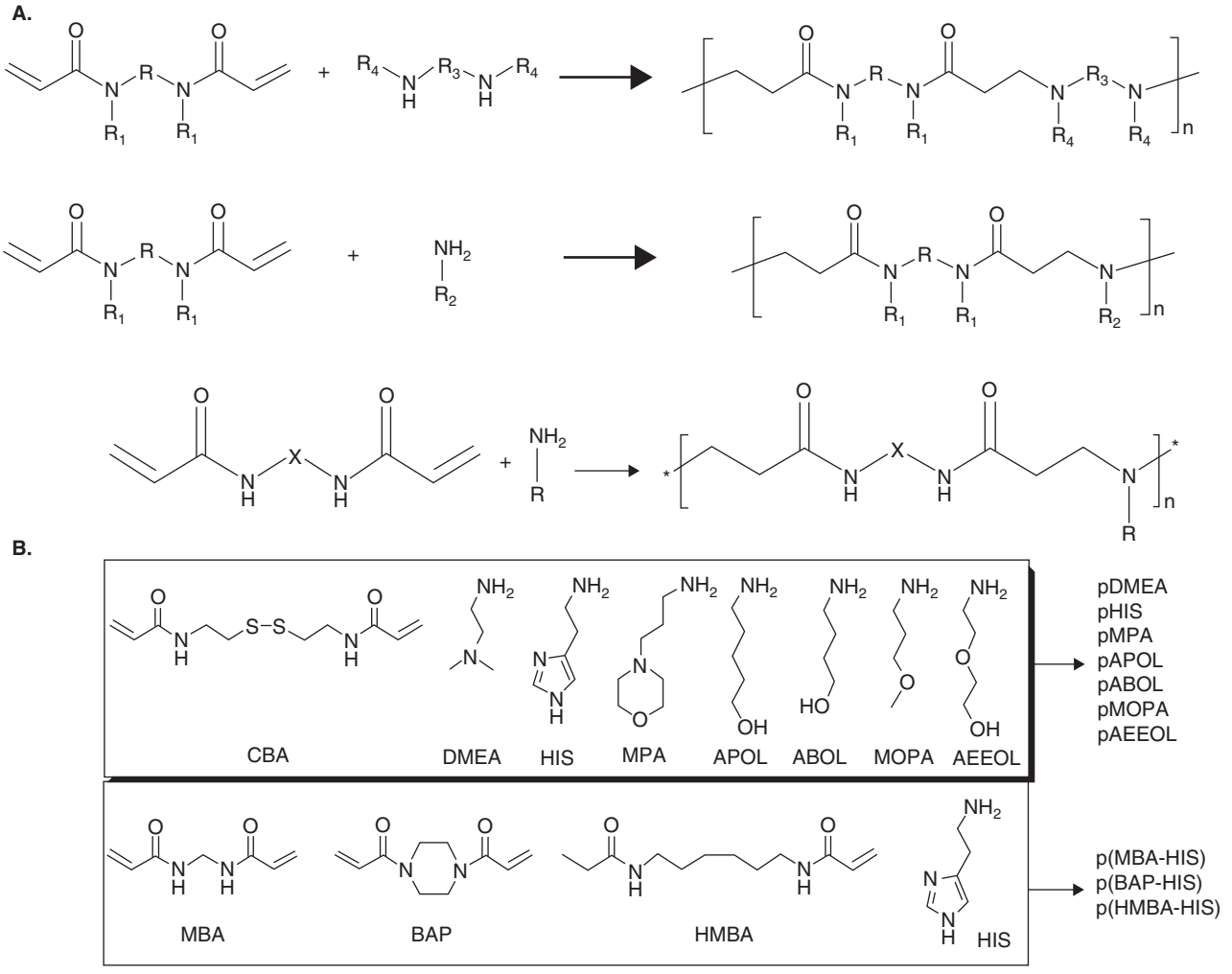


**Figure 2. (A) General synthetic scheme for poly(amido amine)s (PAAs). (B)** Synthesis scheme for bioreducible PAAs and non-reducible (PAAs)[a] by Lin *et al.*

[a]*SS*-PAAs were coded in terms of the used primary amine monomer.

than their analog p(CBA-HIS) and p(CBA-DMPA) homopolymers both in the absence and presence of serum due to the high buffer capacity at the mole ratio of 70/30 [29]. Furthermore, reducible PAAs containing secondary amine functions in the side chain transfected COS-7 cells *in vitro* with transfection efficiencies similar or even higher than that of PEI 25K with low cytotoxicity although increase of the amino spacer length from ethylene to propylene between the amino units in the side chains showed lower transfection efficiency with increased cytotoxicity, suggesting that type of amino group and the amino spacer length affects transfection efficiency and cytotoxicity of the polymer [30].

Lin *et al.* also designed and evaluated linear poly(amido amine) homo- and copolymers (PAAs) containing secondary and tertiary amino groups and different amounts of disulfide linkages in the main chain. This study indicated that the polyplexes from the PAAs containing disulfide linkages in the main chain (*SS*-PAAs) induce higher transfection efficiency against COS-7 cells *in vitro* and meanwhile have essentially lower cytotoxicity than those analogs lacking the disulfides. These results lead them to explore further effects of functional side groups in the *SS*-PAAs. Therefore, bioreducible PAAs with a variety of functional side groups (*SS*-PAAs) are synthesized and studied as non-viral gene delivery vectors. Polyplexes of the *SS*-PAAs with hydroxylalkyl side groups were able to transfect COS-7 cells *in vitro* with transfection efficiencies significantly higher than those of branched PEI in the absence of serum. Moreover, in the presence of 5% serum, a high level of gene expression could be obtained by optimization of the transfection time. Importantly, the *SS*-PAAs and their polyplexes revealed essentially absence or only very low cytotoxicity at concentrations where the highest transfection activity was observed [28].



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### 3.3 PEGylated PAAs

Conjugation of poly(ethylene glycol) (PEG) to cationic polymers certainly has been proven as a useful method to overcome various extracellular barriers. Polyplexes formed from PEGylated polymers and DNA exhibit significant biophysical properties, such as improved colloidal stability in systemic circulation, reduced interaction with blood components and cell surfaces, thus resulting in prolonged circulation and lower toxicities. However, compared with positively charged polyplexes, PEGylated polyplexes exhibited significantly decreased transfection capabilities because their neutral surface impairs efficient cellular association and thereby internalization. Among several approaches, one approach is to overcome this limitation by attachment of a specific ligand to the end of the PEG chain of PEGylated polyplexes, which may exhibit enhanced cellular uptake via receptor-mediated endocytosis and, additionally, targeted gene delivery for specific cells and tissues [31]. Wood *et al*. reported on galactosylated PEGylated PAA dendrimers for targeted gene delivery *in vitro* and showed that the hybrid polymer systems induce highly efficient transfection against HepG2 human hepatocytes [32].

Recently, Khouri *et al*. successfully synthesized and evaluated PEGylated PAMAM dendrimer G4.0 conjugates with bisaryl hydrazone linkages (i.e., G4.0-BAH-PEG) as a new vector. They incorporated BAH linkages into the vector which significantly enhanced the buffering capacity of the vector, even with a high degree of PEGylation (42 PEG chains per dendrimer). These conjugates formed tight complex with plasmid DNA at low weight ratios and exhibited improved cytocompatibility. Gene transfection studies in 293T and HN12 cells showed that this PEGylated carrier is capable of both transfecting more cells and inducing higher green fluorescent protein (GFP) expression than unPEGylated carrier. It showed that use of a BAH linkage for coupling of PEG to the dendrimer helps to increase the buffering capacity of the functionalized dendrimer and results in enhanced gene transfection [33].

### 3.4 Poly(aspartate-graft-PEI)

Poly(aspartic acid) as a polypeptide is biocompatible, biodegradable and has a low cytotoxicity. However, as a gene carrier, a positive charge should be introduced. Therefore, Tang *et al*. conjugated LMW PEI (MW: 600 Da) to the poly[(aspartic acid)-co-lysine] (PSL) prepared by thermal polycondensation of aspartic acid and lysine, and compared the transfection efficiency *in vitro* [34]. The results showed that the PEI-grafted PSL/DNA complexes mediated significant transfection efficiency in NT2 and COS-7 cells higher than PEI 600 Da in the absence and presence of serum. Xiong *et al*. conjugated LMW PEI (MW: 800 Da) to the polyaspartate prepared by ring-opening polymerization of β-benzyl-L-aspartate-*N*-carboxyanhydride and evaluated for its biocompatibility *in vivo* [35]. The results indicated that the polymers caused the inflammation and apoptosis/necrosis in the liver and spleen of 24 rodents post-injection, however, the optimized polymer and PEI 800 did not show tissue damage and apoptosis by day 5 whereas PEI 25K exhibited apoptosis and necrosis in the kidneys and spleen although they did not perform transfection efficiency. Recently, our group simply synthesized α,β-poly(L-aspartate-graft-PEI) (PAE) by ring opening reaction of poly(L-succinamide) (PSI) with LMW PEI (Mn: 423) and evaluated transfection efficiency *in vitro* and *in vivo* [36].

The results indicated that the PAE/DNA complexes mediated higher transfection efficiency in different three cell lines than PEI 25K as shown in **Figure 3** with low cytotoxicity due to the proton sponge effect of PEI and the complexes also showed good gene expression *in vivo* and were dominantly distributed in kidneys, liver, spleen and lung after intravenous administration. Also, PAE was synthesized by the ring-opening reaction of PSI with branched PEI (MW: 600 and 1200 Da) as shown in **Figure 4** and the transfection efficiency *in vitro* was evaluated [37]. The results indicated that the most efficient gene transfection of the polymer/DNA complexes was similar to that of PEI 25K in 293T, HeLa and HepG2 cells due to the proton sponge effect although the transfection efficiency of the branched PEI-grafted polymer is lower than that of linear PEI-grafted polymer due to higher cytotoxicity of branched PEI-grafted polymer.

## 4. Small interfering RNA delivery by PAAs

RNA interference (RNAi)-mediated gene silencing discovery has recently increased our understanding of the molecular mechanisms of 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 [38,39]. However, efficient delivery of small interfering RNA (siRNA) is still a major bottleneck in their success, besides their non-uniform 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 ever report of siRNA delivery using PAA was using reducible PAA. The reducible cationic copolymer, synthesized via Michael-type polyaddition of 1,6-diaminohexane and CBA (poly(DAH/CBA)), tightly condensed the PGE(2)-siRNA conjugate to form nanosize polyplexes having a diameter of 100 – 150 nm. The PGE(2)-siRNA/poly(DAH/CBA) polyplexes decomplexed to release PGE(2)-siRNA in a cytosolic reducing environment due to the degradation of the reducible poly(DAH/CBA). The cellular uptake of the PGE(2)-siRNA/poly(DAH/CBA) polyplex was increased in rat cardiomyocytes (H9C2 cells) due to PGE(2) receptor-mediated endocytosis. When H9C2 cells were transfected with siRNA against Fas, a key regulator of ischemia-induced apoptosis, the PGE(2)-Fas siRNA/poly(DAH/CBA) polyplex delivery system led to a significant increase in Fas gene silencing, resulting



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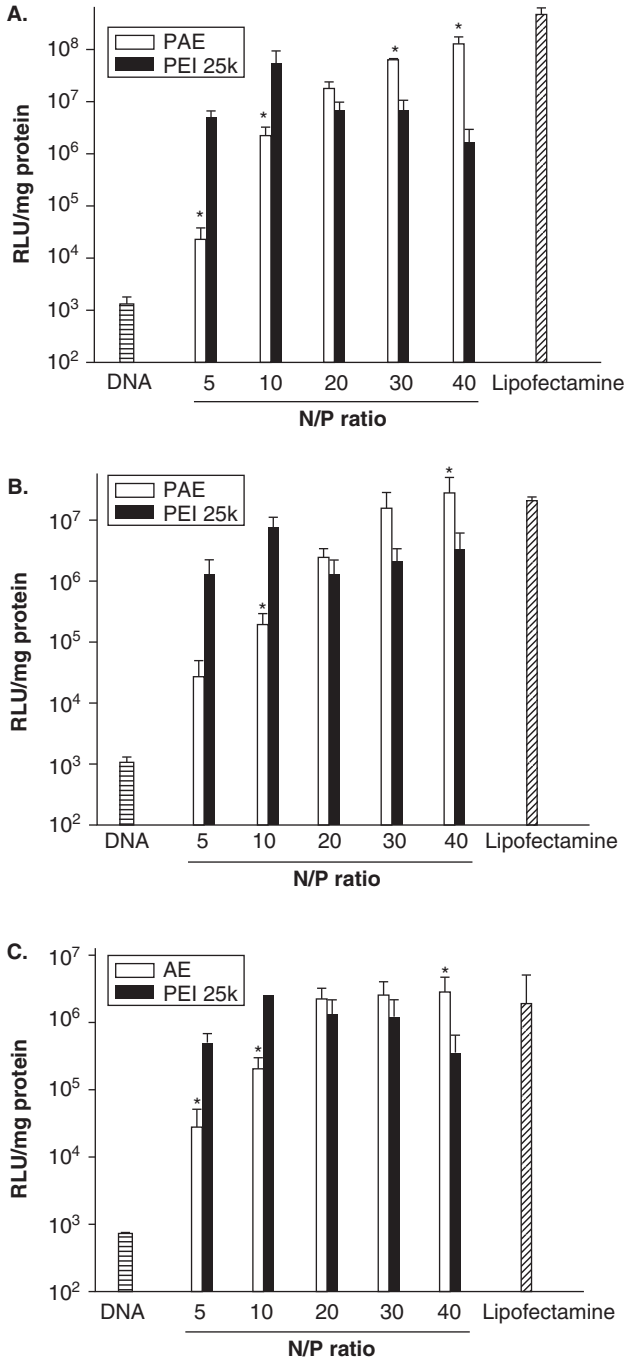


**Figure 3. Transfection efficiency of copolymer/pGL3 control at various N/P ratios and in different cells lines: (a) 293T, (b) HeLa and (c) HepG2 cells.**

in inhibition of cardiomyocyte apoptosis. The PGE(2)-Fas siRNA/poly(DAH/CBA) polyplex did not induce interferon-alpha in peripheral blood mononuclear cells. These results suggest that the PGE(2)-Fas siRNA/poly(DAH/CBA) polyplex formulation may be clinically applicable as a cardiomyocyte-targeted Fas siRNA delivery system to inhibit apoptosis in cardiovascular disease [40].

Recently, Vader *et al*. also showed that incorporation of ethylene diamine (EDA) in the polymer resulted in increased siRNA condensation. Efficient condensation of siRNA was shown to be important for uptake by cell; however, at the same time, excess of polymer decreased siRNA uptake for polymers with high amounts of EDA. Silencing efficiency did not correlate with uptake, since silencing increased with increasing w/w ratio for all polymers. It was found that more than 80% knockdown was found for polyplexes formed with polymers containing 25% or 50% EDA, which was combined with low cytotoxicity [41].

The same group also reported the synthesis of PAA with tunable charge densities by Michael addition polymerization of CBA with variable ratios of 4-amino-1-butanol (ABOL) and EDA or triethylenetetramine (TETA). It was observed that at least 20 – 30% EDA or TETA amino units in the copolymers is necessary to encapsulate siRNA into small and stable polyplexes (< 200 nm). Interestingly, it was also found that polyplex formation and its stability was not further improved by incorporation of higher amounts of EDA or TETA in the copolymers, but the increased hemolytic activity as well as cytotoxicity was observed due to cationic charge in these copolymers. Copolymers with 20% EDA showed excellent gene silencing properties *in vitro* (70% luciferase knockdown in H1299 cells) with negligible cytotoxicity [42].

## 5. Conclusions

Degradable polymers among non-viral vectors for gene delivery have been increasingly studied because the HMW PEI limits its use as gene carrier *in vitro* and *in vivo* owing to the cytotoxicity. In this review, degradable PAAs as the peptidomimetic polymers comprise a class of degradable cationic polymers with many desirable properties for the gene delivery. These polymers can be simply generated with commercially available chemicals. Degradable PAAs can be classified mainly as linear, reducible and grafted PAAs. However, the PAAs are more stable than PEAs due to the presence of amide linkages. These degradable PAAs have been proven efficient in transfection in both *in vitro* and *in vivo* with low cytotoxicity although the structure–activity relationship of polymer should be studied in more detail.

## 6. Expert opinion

Degradable polymers for gene delivery have been increasingly investigated over the past 5 – 10 years because the HMW 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 unpacking the polymer/DNA or polymer/siRNA complexes and release of DNA (or siRNA).



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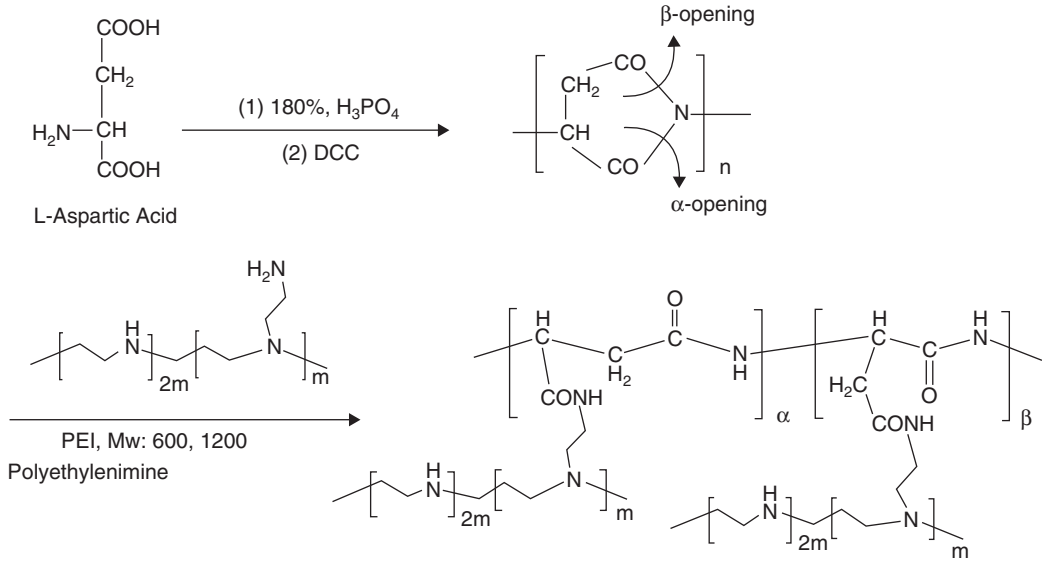


**Figure 4. The synthesis scheme of α,β-poly(L-aspartate-graft-PEI).**

PAAs-based polymers such as linear, branched and grafted with degradable or reducible linkers are very promising because these polymers mediate higher transfection or higher gene silencing than PEI 25K. Moreover, they are safe and biocompatible with potential properties such as prolonged circulation half-life, bio-responsiveness and target-specific degradability. PAAs also opt for spatial and temporal delivery of nucleic acids as they are proved superior to PEI 25K. 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 pharmacodynamics aspects including non-specific 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

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