Expert Opinion

Cationic nanoparticles for cancer therapy

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Importance of the field: The lack of selective delivery of therapeutic molecules to cancer cells remains a problem in cancer therapy. As a result of this non-selectivity, cytotoxic agents are delivered to both healthy and cancerous cells, resulting in severe side effects for the patient, eventually causing termination of therapy or ineffective therapy resulting in progression or recurrence of the disease. In this context, cationic polymers with net positive surface charge emerge as a promising option owing to their very strong cellular interaction properties and good cellular uptake.

Areas covered in this review: In this review, the structure, characteristics and preparation techniques for cationic nanoparticulate drug delivery systems are discussed in the light of cytotoxicity associated with cationic polymers and strong complement activation properties of cationic carrier systems on injection. *In vivo* behavior and biodistribution of cationic nanoparticles are also reviewed for a better understanding of biological interaction of cationic nanoparticles.

What the reader will gain: This review will give an insight to the properties of cationic polymers, including their advantages and drawbacks and drug/gene delivery systems based on cationic polymers intended for cancer therapy.

Take home message: Cationic polymer-based nanoparticles emerge as a promising group of nanosize carrier systems to the tumor cell level with a wide range of modification and application possibilities.

Keywords: cationic polymer, chitosan, complement activation, cyclodextrin polymers, cytotoxicity, dendrimers, nanoparticles, pharmacokinetics, polyethylene imine, poly-**L**-lysine, polymethacrylates

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1. Introduction

With > 10 million new cases every year, cancer remains a major fatal disease worldwide [1]. In many countries, > 25% of deaths are due to cancer [2]. Cancer rates are expected to increase 50% by 2020, according to a current report from the World Health Organization, the International Agency for Research on Cancer (IARC) [1]. The high death rates of cancer suggest that challenges regarding cancer therapy should be considered and worked on by researchers in drug delivery. Most of the current chemotherapeutics on the market are low-molecular-mass agents with high pharmacokinetic volume of distribution, both of which contribute to their cytotoxicity. Moreover, the low molecular mass of these chemicals makes them easily excreted, hence a higher concentration is ultimately required, and consequently a higher toxicity is unavoidable. Also, these drugs when administrated alone lack specificity and cause significant damage to healthy tissues. This results in serious, unwanted side effects such as bone marrow suppression and hair loss [3,4].

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Article highlights.

- Positive surface charge of cationic polymers helps mask the negative DNA charge and thereby facilitates entrance into cells and transfection of most cell types.
- A polynucleotide/drug-loaded particle should have a diameter < 100 nm to be suitable for systemic gene therapy or intracellular drug delivery.
- Tumor growth and angiogenesis were suppressed with intratumoral injection of chitosan with interleukin-12 plasmid.
- Although the surface charge of cationic nanoparticles seems to be an advantage for cellular uptake, some major drawbacks of such systems include aggregation, instability, toxicity and rapid clearance by the mononuclear phagocyte system.
- It is believed that cationic nanoparticles may find their optimum application for cancer therapy in local and mucosal application.

This box summarizes key points contained in the article.

The tumor has its physiological barriers, such as vascular endothelial pores, heterogeneous blood supply and heterogeneous architecture [5,6]. For a treatment to be successful, it is very important to overcome these barriers [7]. Successful cancer treatment is very much dependent on the method of delivery and the selectivity of the chemotherapeutic agent to tumor cells as well as prolonging the retention of the drug in tumor mass. Colloidal drug delivery systems of submicrometer size emerge as promising delivery systems. This was emphasized recently with the FDA approval in 2005 of Abraxane® (Abraxis Bioscience LLC, USA), the first nanotechnology product of the pharmaceutical industry consisting of paclitaxel bound to albumin nanoparticles, for the treatment of breast cancer.

Targeted nanocarriers are able to deliver their load (drug, peptide, oligonucleotide, DNA) selectively to tumor tissues by means of the enhanced permeation and retention (EPR) effect, which is a direct result of leaky vasculature characteristic of tumor blood vessels and retention at the tumor site owing to the absence of a functioning lymphatic drainage system at the tumor site. Nanoparticles have attracted the attention of scientists because of their multifunctional character. The treatment of cancer using targeted drug delivery nanoparticles is the latest achievement in the biomedical field [2,8]. Nanocarriers with uniform and well-defined particle size and shape are of growing interest in biomedical applications because of their ability to cross cell membranes and to reduce the risk of premature clearance from the body [9].

Nanoparticles can be prepared with a wide variety of polymers and copolymers with alternative molecular mass, hydrophilic/lipophilic properties and surface charge, all of which affect the final properties of resulting nanoparticles. Cationic polymers have drawn attention, particularly in the last decade, as a result of their favorable cellular interaction and uptake properties. Cationic polymers have the advantage of forming aggregates with proteins with a reduced size, which is an important difference from cationic lipids, which also form similar complexes or aggregates with DNA but rather larger in size.

Positive surface charge of cationic polymers helps mask the negative DNA charge and thereby facilitates entrance into cells and transfection of most cell types [10]. Cationic polymers have been extensively reported to form nanosize complexes with DNA [11-13] and small interfering RNA (siRNA) [14-16], and the delivery of encapsulation of anticancer drugs alone or with DNA simultaneously for enhanced gene therapy has been reported in the literature [17-19]. Most cationic polymers possess amine groups that are protonable at acidic pH. When these polymers are inside the endosome, they accept protons and consequently resist the drop in pH, which can destabilize the encapsulated DNA or oligonucleotide, negatively affecting gene transfection [20].

It is believed that cationic polymers are able to form effective nanosize delivery systems for active molecules such as anticancer agents, DNA, oligonucleotides and peptides owing to their favorable physicochemical properties, and therefore provide advantages over anionic or neutral polymers and systems derived from them. Unique properties of cationic polymers that enable them to deliver genes or active molecules to the tumor site at the cellular level are their ability to form condensates or complexes with macromolecules of negative charge such as DNA or proteins, strong interaction of cationic polymers with biological membranes and therefore tumor cells, and facilitated entrance into tumor cells for effective transfection or cytotoxic effect. On the other hand, these unique properties also cause some unwanted effects, particularly due to the strong positive surface charge, which is discussed further in Section 4 of this review.

The focus of this review is on nanosize systems prepared using cationic polymers, emphasizing the properties of major cationic polymers used in this field. Nanoparticles including dendrimers are discussed in terms of their preparation, characteristics, efficacy in cancer therapy, safety and challenges.

2. Major cationic polymers used in the preparation of nanoparticles for cancer therapy

The main difference between cationic polymers and cationic lipids is that cationic polymers do not contain a hydrophobic moiety and are completely soluble in water [2]. Compared with cationic liposomes, cationic polyplexes have the advantage of compressing DNA molecules to a relatively smaller size [21,22]. This can be crucial for gene transfer and intracellular drug delivery, as small particle size may be helpful for improving transfection efficiency and cellular internalization. Modifications to these polymers such as molecular mass, geometry (linear versus branched) and ligand attachment have been reported extensively in the literature [23,24].

The most widely studied polymers in the literature for gene therapy or delivery of anticancer agents to the tumor cell level are represented schematically in **Figure 1** and briefly defined for their physicochemical and biological properties in the following subsections [25,26].

2.1 Chitosan

A natural polymer of cationic nature is chitosan, which has been demonstrated to show absorption enhancer properties through mucosa, controlled drug release and bioadhesion. Chitosans are modified through degree of deacetylation and grafting of side chains for targeting purposes [27,28].

Chitosan plays an important role as a cationic polymer for cancer therapy because it has been reported to show anticancer effects *per se*. Chitosan has been demonstrated to cause apoptosis and death of bladder tumor cells through caspase-3 activation [29]. A similar phenomenon has been reported for a metastatic breast cancer model with a chitosan gel [30]. Chitosan also stimulates macrophages to mature into cytotoxic macrophages and suppresses tumor growth in mice [31], and directly inhibits tumor cell proliferation by inducing apoptosis [32].

Recently, chitosan nanoparticles have been shown to cause necrotic death of liver cancer cells by means of neutralization of cell surface charge, decrease in mitochondrial membrane potential and induction of lipid peroxidation [33]. Chitosan shows an anticancer effect on oral administration, which can be advantageous for patient compliance. The extent of such activity may differ according to tumor type. A similar study, using a chemically induced tumor model, demonstrated that chitosan given in the diet is able to suppress the development of tumor lesions in mice colon [34]. Therefore, the anticancer activity of cationic polymer chitosan should always be taken into consideration when evaluating the anticancer effectiveness of chitosan-based drug delivery systems loaded with active molecule [35-38].

Chitosan is also able to form positively charged polyplexes and/or encapsulate active molecules in nanoparticle matrix [39,40], however the transfection efficiency has been found to be slower than that of polyethylene imine (PEI) owing to slower endosomal escape of chitosan polyplexes [20,41].

Chitosan has been largely favored as a potential nanoparticle carrier owing to some of its favorable properties. It is a mucoadhesive polymer that has the ability to enhance drug absorption by rearranging the tight junction proteins [42,43]. Chitosan nanoparticles are taken up by the endosomes, allowing the drug to overcome the permeability barrier of the epithelia [43]. Chitosan nanoparticles provide protection against enzymatic degradation and have been shown to be able to control the release of genes or drugs in a controlled, sustained manner.

As a raw material chitosan is abundant, and it is easier and cheaper to manipulate compared with other drug delivery systems such as cationic liposomes or formulations made from other materials.

2.2 Polyethylene imine

Polyethylene imine, a commercially available cationic polyamine, is one of the most successful and widely studied cationic polymers [44-48]. It is widely regarded as a gold standard for the comparison of transfection efficiencies among non-viral vectors. Transfection efficiency of PEI has been found to be dependent on parameters including molecular mass, degree of branching, N/P ratio and complex size [49]. PEI has a high density of protonable amino groups as every third atom is amino nitrogen, which results in high buffering ability at practically any pH. Once inside the endolysosomal compartment, PEI can efficiently destabilize the endosome, releasing the polynucleotide in the cytoplasm, as seen in **Figure 2**. PEI nanoparticles in comparison with PLGA/PLA polymers have been reported to deliver DNA adsorbed and/or complexed to the nanoparticle surface, which can be considered as an alternative to polyplexes of PEI and DNA [50].

PEI, being an efficient cationic polymer, is associated with toxicity issues, which emerge as a drawback for the acute or chronic use of delivery systems based on this polymer [51,52]. In this context, researchers reported two types of PEI-induced cytotoxicity [53]. First is an immediate toxicity associated with free PEI and second is a delayed toxicity caused by cellular processing of PEI/DNA polyplexes [54]. On injection, free PEI was found to interact with negatively charged serum proteins and erythrocytes, precipitated in huge clusters and adhered to cell surface [55], destabilizing plasma membrane and inducing immediate toxicity. Conjugation of hydrophilic polymer poly(ethylene glycol) (PEG) was proposed as a solution to this problem in several studies. PEG can also shield the positive charge of the polyplexes, reducing the interaction with blood components while in circulation and thus prolonging circulation time and delaying opsonization [56-58].

The linear structure was another factor causing PEI-induced toxicity. Lethal side effects were observed in mice when linear PEI polyplex was administered intravenously. Active targeting of PEG-PEI polyplexes was possible through grafting of transferrin to PEG, which resulted in a fivefold increase in transfection efficiency and lower toxicity [59].

2.3 Poly-L-lysine

Poly-L-lysine (PLL) is obtained through polymerization of *N*-carboxy-anhydride of lysine. This biodegradable polymer forms nanosize complexes (< 100 nm) with polynucleotides owing to the presence of protonable amine groups on the lysine moiety. Polyplexes of PLL are reported to have good cellular uptake but lower transfection efficiency when compared with PEI [60-64]. Researchers believe that this may be due to reduced endosomal escape resulting from the lack of protonable amine groups at acidic pH, which eventually leads to the release of DNA [65,66].

The lack of buffering capacity of endosomal acidic pH leads to the enzymatic degradation of polyplexes. PEG coating/shielding has been studied to overcome this problem

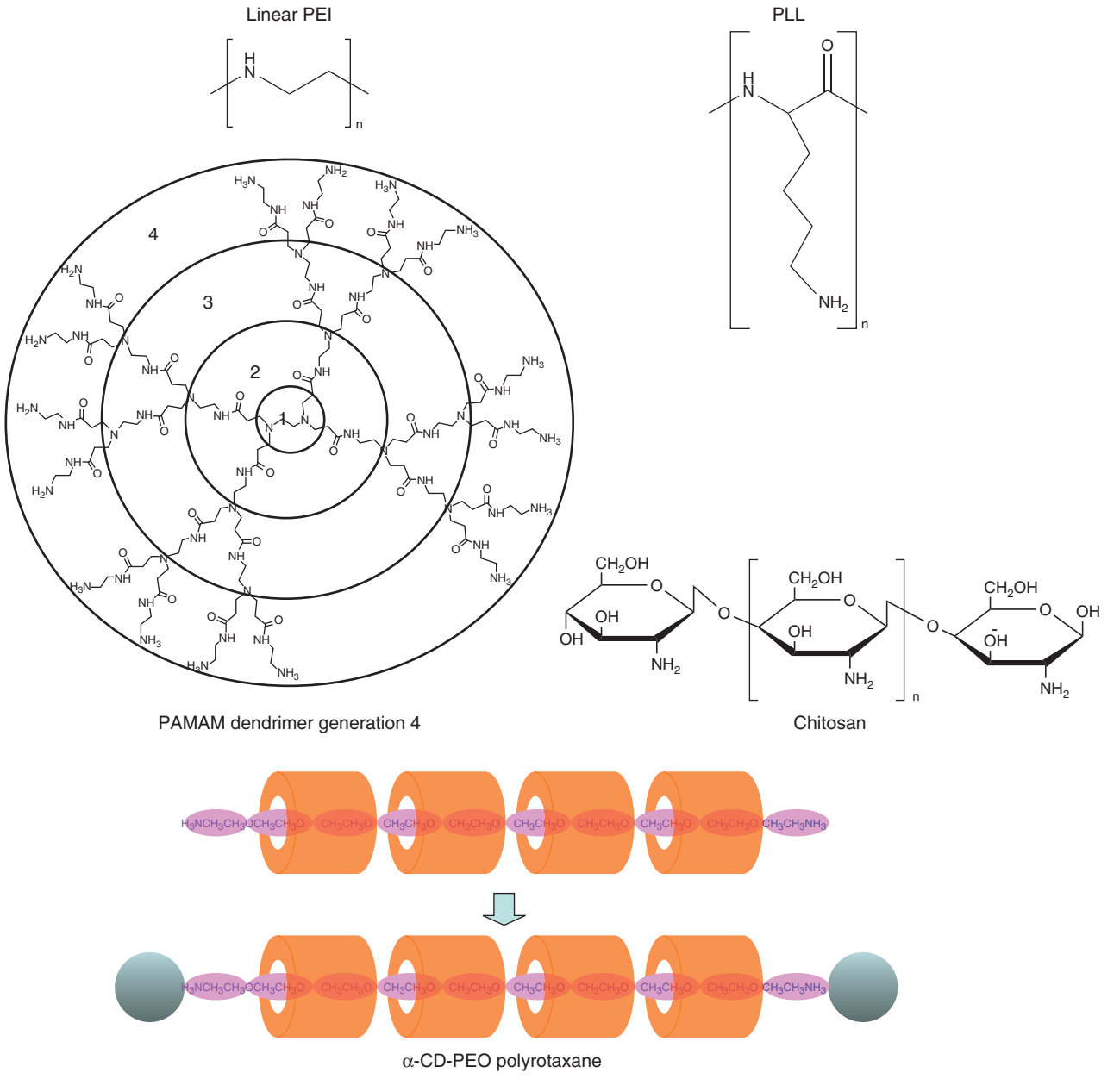

Figure 1. Chemical structures of some of the most frequently used cationic polymers for gene or drug delivery to tumor site [74,143].

by masking positive charge, improving the stability of the polyplex, prolonging circulation time and protecting the polynucleotide from enzymatic degradation. An alternative to PEGylation was conjugation with membrane-disruptive peptides and fusogenic peptides [61,67].

Transfection efficiency of PLL was increased by a degradable PLL analogue, poly(a-[4-amino-butyl]-L-glycolic acid) (PAGA) without any cytotoxic effects [68]. PLL is also used as a coating polymer to obtain anticancer drug delivery systems with positive surface charge.

2.4 Polymethacrylates

Polymethacrylates are cationic polymers with vinyl-base and they also possess the ability to form polyplexes on condensation of polynucleotide in the nanometer range. These polymers have a wide range of molecular masses and chemical structures. However, polymethacrylates with only tertiary amine groups are reported to have a similar transfection efficiency potential to PEI, which is accepted as the gold standard in terms of transfection with the advantage of a more favorable biocompatibility profile [69,70].

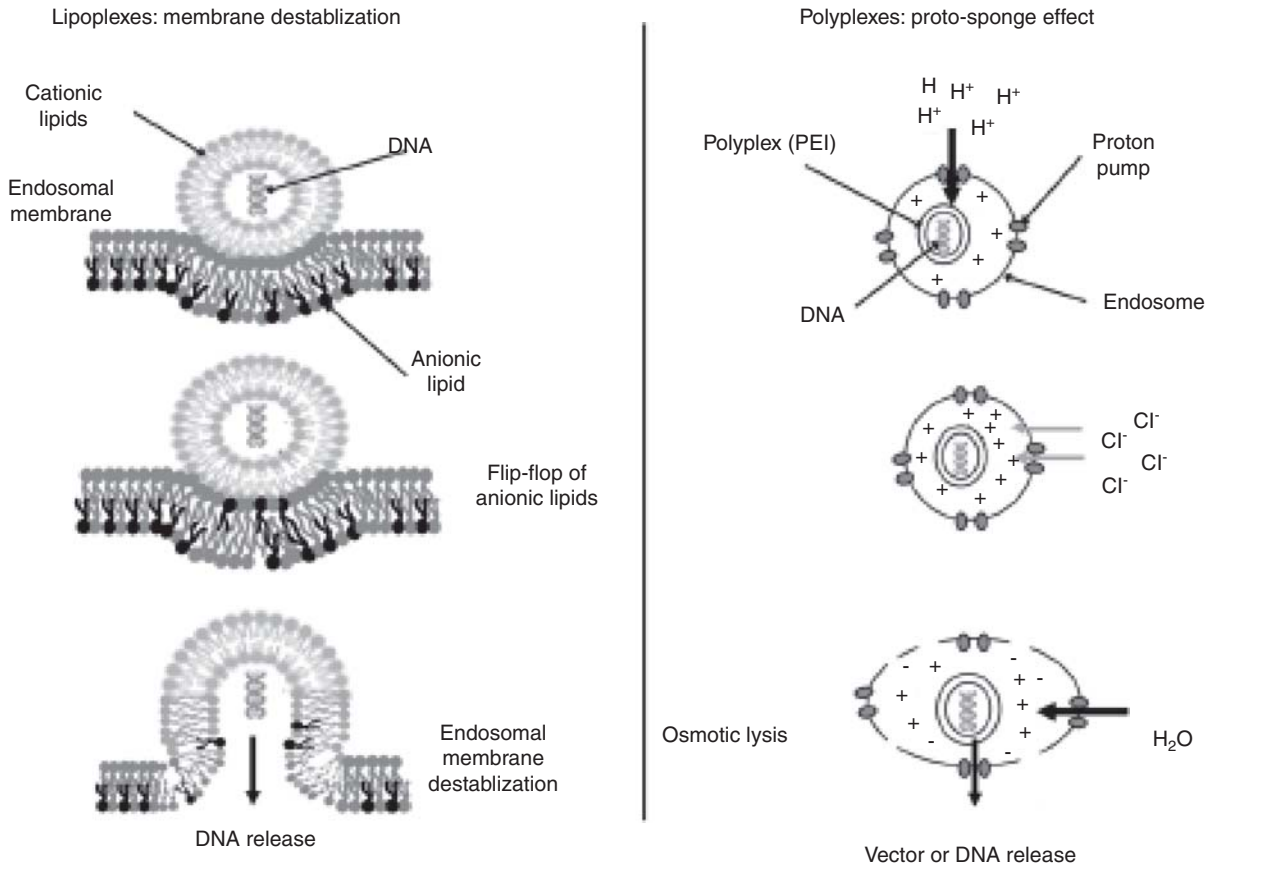

Figure 2. Schematic representation of endosomal escape for lipoplexes and polyplexes.
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Graft copolymerization of methacrylate core to PEI shell resulted in nanoparticles capable of transfection and DNA delivery [71,72]. Commercially available polymethacrylate Eudragit®E100 (Rohm Pharma, Germany) was combined with FDA-approved polymers PLGA/PLA to give nanoparticles of a cationic nature using cationic surfactant cetyltrimethylammonium bromide (CTAB) with significantly high transfection efficiency [20,73].

2.5 Imidazole-containing polymers

Cationic polymers possessing an imidazole heterocycle have been demonstrated to be favorable for transfection efficiency because the imidazole heterocycle displays a pK_a ~ 6 with a buffering capacity for the endosomal pH range and facilitating vesicular escape by a proton sponge mechanism as depicted in **Figure 2** because endosome acidification is required for efficient transfection [74].

To enhance the efficacy of gene delivery, some researchers have worked on the balance between the free ε-amino groups of the lysine moieties that make possible complex formation with DNA with the number of imidazole heterocycles that are responsible for endosomal escape [75,76].

2.6 Cyclodextrin-containing cationic polymers

Supramolecular hydrogels based on self-assembly of the inclusion complexes between cyclodextrins (CDs) with biodegradable block copolymers could be used as promising injectable drug delivery systems for sustained controlled release of macromolecular drugs. More importantly, the polyplexes of CD-containing cationic polymers with DNA could be PEGylated through a supramolecular process using inclusion complexation between the CD moieties and a modified polyethylene oxide (PEO). New cationic polyrotaxanes composed of multiple oligoethylenimine-grafted CDs threaded and end-capped on a block copolymer chain were designed and synthesized as a new class of polymeric gene delivery vectors, where the chain interlocked cationic cyclic units formed an integrated supramolecular entity to function as a macromolecular gene vector [77-79]. Cyclodextrins were used to modify and functionalize cationic polymers, or serve as a core or template for synthesis of new cationic polymers with star architectures [80-82].

Recently, by taking advantage of the supramolecular structures of CD-based polyrotaxanes, a new class of cationic supramolecules was developed for gene delivery [83].

PAMAM (polyamidoamine) dendrimer (G3) conjugate with α-cyclodextrin (α-CDE) showed potent effects with negligible cytotoxicity compared with several transfection reagents in various cells [84].

Self-assembled CD-DNA nanoparticles called CDplexes were prepared from polycationic thiolated amphiphilic cyclodextrins, as seen in **Figure 3** [85-88]. Researchers reported an economic, diversity-orientated strategy to obtain polycationic amphiphilic CDs that can be controlled in terms of density of amino groups, flexibility and presence of extra H-bonding functional groups achieving higher transfection efficiency than that of PEI and lower toxicity. The authors also suggested that the presence of apolar CD cavity may contribute to the delivery of other functional molecules intended for cellular targeting, nuclear localization and visualization [85-88].

2.7 Poly(β-amino ester)s

Poly(β-amino ester) (PBAE) is a new class of cationic polymers of biodegradable and non-toxic nature. PBAE polyplexes have been studied in terms of molecular mass, polymer end groups, complex size and N/P ratio [89]. Polyplexes showed transfection efficiencies similar to PEI and low cytotoxicity *in vitro* [89]. PBAE in combination with PLGA was used for DNA vaccination. PBAE/PLGA microparticles succeeded in significant reduction of tumor size in mice [90]. PBAE/PLGA particles were also able to delay the release of plasmid DNA for several days [91].

Recently, polyethylene oxide-modified PBAE nanoparticles were designed as a pH-sensitive system for tumor targeting of water-insoluble anticancer drugs [92]. Biodistribution studies of tritium-labeled [H^3]-paclitaxel encapsulated into PEO-PBAE nanoparticles revealed long systemic circulation resulting from surface modification by PEO and effective delivery of drug to tumor mass [92]. A similar formulation of PEO-PBAE nanoparticles was used for the delivery of siRNA and paclitaxel in comparison to PEO-PCL nanoparticles to overcome multi-drug resistance. The authors suggested that PEO-PBAE could provide a more advantageous platform for the delivery of polyanions such as siRNA [93].

In another study, a blend nanoparticle consisting of PBAE-encapsulated paclitaxel and PLGA-encapsulated C6-ceramide, an apoptotic signaling molecule, was administered to nude mice bearing human breast carcinoma cell line MCF7 and multi-drug resistant cell line $MCF7_{TR}$. It was believed that paclitaxel would be rapidly released from the pH-sensitive cationic polymer PBAE and ceramide would be slowly released from PLGA. As a result, PLGA/PBAE blend nanoparticles succeeded in a higher plasma concentration for encapsulated drug paclitaxel and longer residence time in tumor mass [94]. Safety and therapeutic efficacy of PEO-PBAE nanoparticles were evaluated in tumor-bearing mice. Tumor growth inhibition and lower toxicity profile regarding body weight and blood parameters were reported [95].

3. Cationic nanoparticulate delivery systems

3.1 Preparation techniques of cationic nanoparticles

3.1.1 *Polyplexes*

A polynucleotide/drug-loaded particle should have a diameter < 100 nm to be suitable for systemic gene therapy or intracellular drug delivery. For tumor drug delivery, nanoparticles should be < 400 nm to benefit from the EPR effect. Nanoparticles should be stable and resist nonspecific uptake in the circulation, but be quickly destabilized to release DNA once they are taken up by target cells. For commercialization, nanoparticles must be produced on a large scale, from simple components using robust methods, to be cost-effective. In the literature, two methods are used often to prepare cationic polymer-DNA complexes.

(1) *Direct mixing* is used to formulate traditional polyplexes. The procedure consists of rapidly mixing aqueous suspensions of cationic polymers with plasmid DNA. This results in condensed DNA surrounded by a cationic bilayer and minimizes particle diameter [96].
(2) The *detergent dialysis* method [97,98] was initially used for preparing relatively stable cationic lipid-DNA particles by dissolving DNA and cationic lipid mixture in a detergent solution, which is followed by a subsequent dialysis process to remove the detergent. Particles are stable for a longer period and are more active in the presence of serum-containing medium [99,100]. *In vivo* performance is not affected by this method [101].

Apart from polyplexes that are a spontaneous outcome of polynucleotide condensation by cationic polymers, nanoparticles can be obtained by directly using cationic polymers such as chitosan, or by coating of nanoparticles that are not cationic by grafting/copolymerization, or by simple electrostatic interactions with cationic polymers, lipids or surfactants. A third class of cationic nanoparticulate delivery systems is dendrimers. These three types of cationic drug delivery system are discussed further in the following sections.

3.1.2 *Nanoparticles prepared directly from cationic polymers*

Nanoparticles of cationic polymers such as chitosan, PEI and PLL were formulated to deliver mostly nucleic acid and some anticancer agents to tumor cells effectively. Owing to their favorable cellular interaction and internalization properties, they have been considered as promising delivery systems for cancer therapy through both systemic and local administration.

These nanoparticles have been studied extensively for SiRNA delivery with polymers including PEI, chitosan, PLL and PEG-based polymers. They are polycations or polycation-containing block copolymers, and can form specialized interpolyelectrolyte complexes or block ionomer complexes with siRNA, respectively. Strong electrostatic

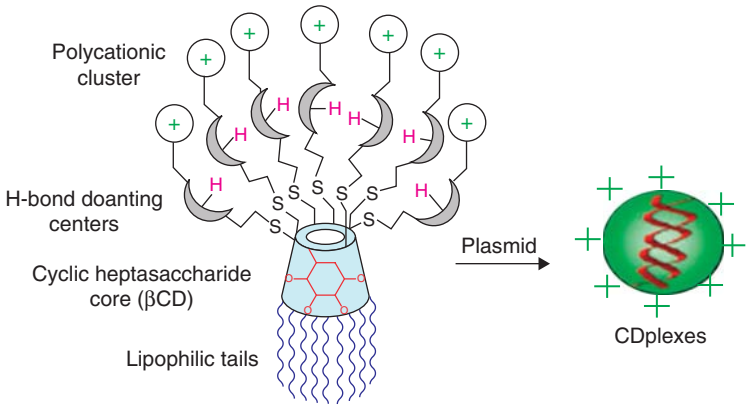

Figure 3. Polycationic amphiphilic cyclodextrins and formation CDplexes with plasmid DNA.
Reproduced with permission from [88].

interactions between oppositely charged polyelectrolytes allow for 'self-assembly', which can substantially hinder or prevent enzymatic degradation of the incorporated polynucleotide in the bloodstream [102,103]. PEO [104] and poly [(*N*-2-hydroxypropyl) methacrylamide] (PHPMA) [105-107] are often chosen as the hydrophilic, non-immunogenic, neutral block and synthetic quaternary amine polymers or bioconjugates as the cationic segments for nucleic acid binding [108,109].

Another different application of cationic nanoparticles is gadolinium neutron-capture therapy, which is a cancer therapy using gadolinium neutron-capture reaction *in vivo* by thermal neutron irradiation. The principal problem with gadolinium is that it remains in insufficient quantity in the tumor tissue after intratumoral administration. Gadopentetic-chitosan nanoparticles were injected by the intratumoral route to mice having developed B16F10 melanoma tumors. The therapy consisted of a thermal-neutron irradiation, 8 h after administration of gadopentetic-chitosan nanoparticle. Gadopentetic-chitosan nanoparticles allowed significant suppression of the tumor growth, with a mean tumor volume variation < 15% after 14 days, despite the radioresistance of the melanoma model [110].

3.1.3 *Cationic polymer/lipid/surfactant-coated nanoparticles*

Another approach for the design of cationic nanoparticles is coating of polymeric nanoparticles with positively charged polymers, surfactants or lipids to provide better cellular uptake for cancer therapy. By providing the positive surface charge through coating, it was believed that encapsulation of hydrophilic molecules such as peptides, DNA or drugs could be improved as well as protection of polynucleotides from enzymatic degradation.

Nanosize carriers possessing cationic coating are the basic approach in the design of magnetoliposomes for tumor delivery [111]. These carriers consist of magnetic nanoparticles wrapped in a phospholipid bilayer. Furthermore, their surface can be modified to achieve active targeting to tumor tissue, and localization of vesicles inside tumor cells can be enhanced by rendering positive surface charge [112]. Cationic magnetoliposomes proved to be effective for hyperthermia treatment in a variety of tumor types [113-116].

Chitosan coating through electrostatic charge interactions has been drawing increased attention for tumor drug delivery in the past few years. To enhance the intracellular delivery potential of plasmid DNA, polybutyl cyanoacrylate (PBCA) and chitosan were used to prepare PBCA nanoparticles by emulsion polymerization and complexes prepared through the complex coacervation of nanoparticles with DNA. Electrophoretic analysis suggested that nanoparticles with positive charges could protect the DNA from nuclease degradation and cell viability assay showed that nanoparticles have a low cytotoxicity to human hepatocellular carcinoma (HepG2) cells [117].

Chitosan-coated polyisohexylcyanoacrylate (PIHCA) nanoparticles have also been developed for intravenous delivery of siRNA and have been shown to possess efficacy against the *RhoA* cancer target gene [118]. Intravenous administration was performed in this study and no evidence of toxicity was observed after 30 days [35].

Positively charged nanoparticles are gaining increasing importance for drug delivery through intravenous, oral or ocular administration [119]. Such particles have generally been reported to be obtained by emulsion polymerization of alkylcyanoacrylates in the presence of the cationic polysaccharide, chitosan [110,119]. The use of a low-molecular-mass biocompatible chitosan instead of the usual cationic surfactant, such as CTAB, resulted in reduced toxicity and increased the adsorption of nucleic acids [110].

The author's group has recently worked on cellular uptake and anticancer efficacy of chitosan nanoparticles compared with poly-ε-caprolactone nanoparticles coated with cationic polymers chitosan or PLL. The study revealed that intravesical

chemotherapeutic agent Mitomycin C could be successfully encapsulated, providing longer residence time, higher local drug concentration and prevention of drug loss during bladder discharge. As far as cellular interaction was concerned, CS-PCL was the most efficient formulation for uptake of fluorescent markers Nile Red and Rhodamine 123. In particular, CS-PCL nanoparticles loaded with Rhodamine 123 sharing hydrophilic properties with mitomycin C were selectively incorporated by bladder cancer cell line, but not by normal bladder epithelial cells, as seen in **Figure 4**. CS-PCL nanoparticles seem to be promising anticancer delivery systems, suggested by their efficacy against mouse bladder cancer cell line MB49 [120].

Nanoparticles can also be coated with cationic surfactants by incorporation of the cationic surfactant during preparation, by incubation of nanoparticles in the cationic surfactant during preparation, or by incubation of nanoparticles in the surfactant solution. Cationic surfactant coating is believed to render positive charge to nanoparticles, therefore improving their interaction with cells and tissues. Another reason for cationic coating with surfactants would be significant reduction in size, leading to 'stealth' properties and prolonged circulation time. In the literature, surface coating with the following surfactants has been reported: DMAB (didodecyldimethyl ammonium bromide), DTAB (dodecyltrimethyl ammonium bromide), CTAB, DODAB (dimethyl dicotyldodecyl ammonium bromide), benzalkonium chloride and cetrimide [121].

PLGA nanoparticles stabilized with cationic surfactant DMAB were studied for oral delivery of paclitaxel and resulted in equivalent anticancer efficacy to intravenously administered paclitaxel in cremophor, and this equivalent effect was obtained with 50% lower dose of paclitaxel encapsulated in nanoparticles [122].

A similar nanoparticle formulation consisting of PLGA nanoparticles coated and stabilized with a variety of cationic material including DODAB, DC-chol (3β-[*N*-(dimetyhylaminoethane carbamoyl)] cholesterol), cetrimide, chitosan and protamine was evaluated for pulmonary epithelial delivery of plasmids. A prolonged and high level of gene expression was observed for positively charged coated PLGA nanoparticles. These coated nanoparticles were also found to be less toxic than PEI nanoparticles. Highest cellular uptake was observed with chitosan and DC-chol coating [123].

Targeted cationic nanoparticles capable of active targeting to tumor were formulated by blending PLGA and cationic lipid DOTAP (*N*-[1-(2,3-dioleoyloxy) propyl]-*N,N,N*-trimethyl ammonium methyl sulfate) [124] to encapsulate interleukin-12. Asialofetuin was incorporated as targeting ligand using a modified solvent evaporation process. This system increased significantly levels of luciferase gene expression in the liver on intravenous administration [125].

3.1.4 *Cationic dendrimers*

Dendrimers are synthetic molecules at average protein size possessing a highly branched shape. This shape renders a very large surface area, which enables the encapsulation or adsorption of therapeutic agents or other biologically active molecules. The unique properties [126] of dendrimers are their high degree of branching, multivalency, globular architecture and well-defined molecular mass.

Recent progress has been made in the application of biocompatible dendrimers to cancer treatment, including their use as delivery systems for potent anticancer drugs such as cisplatin and doxorubicin, as well as agents for both boron neutron-capture therapy and photodynamics therapy. Recent research has shown that dendrimers possess many unique properties that may be promising for increasing bioavailability and selectivity of antineoplastic drugs, as seen in **Table 1** for cisplatin complexed to dendrimer [2].

Many commercial small molecule drugs with anticancer activity have been successfully associated with dendrimers such as PAMAM, poly(propylene imine) (PPI or DAB) and poly(etherhydroxylamine) (PEHAM) dendrimers, by means of either physical interactions or chemical bonding. Targeted delivery is possible via targeting moieties conjugated to dendrimer surface or owing to the EPR effect.

Cationic dendrimers show cytotoxicity; however, derivatization with fatty acid or PEG chains, reducing the overall charge density and minimizing contact between cell surfaces and dendrimers, can reduce toxic effects [9] similarly to other cationic polyplexes. Covalently coupled methotrexate dendrimer conjugates are stable under identical conditions in water and buffered saline.

Cytotoxicity tests have shown that methotrexate as the dendrimer inclusion complex has an activity identical to the free drug *in vitro*. By contrast, folic acid-targeted dendrimer with covalently conjugated methotrexate specifically kills receptor-expressing cells by intracellular delivery of the drug through receptor-mediated endocytosis [127].

4. Challenges associated with cationic nanoparticles

Systemic therapy of different cancer types emerges as a major challenge for safe and effective drug delivery in the sense that drug-loaded nanoparticles are expected to survive in the bloodstream without being degraded or taken up by macrophages [128-132]. When they reach the tumor site, they need to extravasate into the tumor tissue and bind specifically to tumor cells. Cellular internalization of drug delivery systems and intracellular barriers such as endosomal escape, cytoplasm trafficking and nucleus entry act as major hurdles for effective treatment of tumors [133,134].

Although the surface charge of cationic nanoparticles seems to be an advantage for cellular uptake, some major drawbacks of such systems include aggregation, instability, toxicity and rapid clearance by the mononuclear phagocyte system (MPS). The main reason for the accumulation of positively charged nanoparticles in MPS organs is believed to be the necessity of high concentrations of drug-loaded particles to

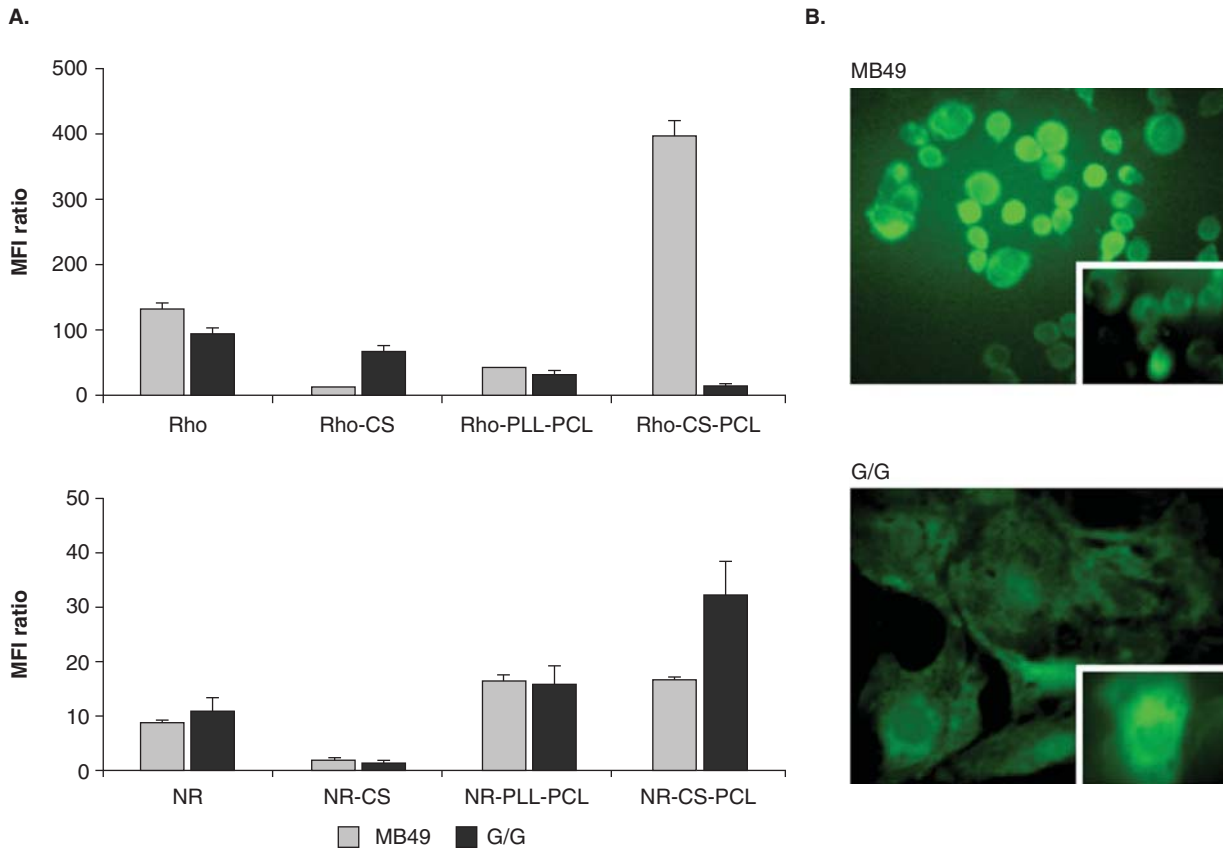

Figure 4. Cellular uptake of cationic nanoparticle into healthy and cancerous cells. A. MFI ratio was calculated with MFI value of corresponding formulation versus autofluorescence of untreated cells. **B.** Fluorescence photomicrographs of MB49 mouse bladder carcinoma cell line and G/G mouse bladder epithelial cell line treated with Rhodamine-loaded cationic nanoparticles prepared from poly-ε-caprolactone and coated with cationic polymer chitosan.

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MFI: Mean fluorescence intensity.

Table 1. AUC value (μg Pt/ml blood or μg cisplatin/ organ) for 48 h (n = 5 mice/data point).

Organ	Cisplatin	Cisplatin–dendrimer complex
Tumor	5.3	25.4
Blood	9.4	10.7
Liver	51.6	17.0
Kidney	57.6	138.1

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provide therapeutic dose, which results in the aggregation of cationic nanoparticles in clusters. It is true that cationic nanoparticles are not toxic themselves, however they can cause embolism in the lungs on aggregation.

It is basic knowledge that in colloidal drug delivery systems, particles of similar charge having an electrostatic repulsion greater than van der Waals forces tend to be physically stable and do not aggregate. As far as cationic polyplexes are concerned, a different scenario emerges. This scenario concerns more complex mechanisms such as bridging the gap between particles by means of electrostatic surfaces of opposite charge on the particles. The charge of the polyplex is dependent on the nature of the condensing agent and the ratio of the condensing agent (cationic polymer)/DNA. A major concern is the stability of polymer/DNA complexes under physiological buffer conditions [135].

The advantage of good interaction between positively charged cationic particles with negatively charged biological membranes may turn into a drawback because the same strong interaction exists between the particle and the erythrocyte membrane [136]. A similar phenomenon is the rapid opsonization and macrophage uptake of positively charged particles in the bloodstream [137-139]. To prolong the circulation, particles should be as small and as neutral as possible. PEG grafting can be a solution to mask the positive charge that leads to rapid clearance of the particles. On interaction

of cationic nanoparticles with plasma proteins, surface charge is altered from positive to negative, leading to destabilization of the complex and premature release of DNA [69,140-142].

5. Expert opinion

Cationic nanoparticles have recently emerged as promising carrier systems for the effective delivery of nucleic acid or anticancer drugs to tumor cells. The principal advantages of these delivery systems seem to be their strong cellular interaction and cell membrane disruption properties, which result from their net positive surface charge. The wide variety of polymers that can be used for the preparation or coating of nanoparticles with positive charge is another important advantage for the development of this research field. The main polymers preferred for drug or nucleic acid delivery to tumor cells are chitosan, polyethyleneimine, poly-L-lysine and polymethacrylates forming dendrimers. The active molecule to be encapsulated in the nanoparticulate carrier system plays an important role in the design of cationic delivery systems.

In the case of DNA or gene delivery, small polymers such as PEI and PLL give very promising results because they can form complexes with nucleic acid or its fragments and can fold these otherwise labile molecules to maintain their physical and chemical stability following administration to the body. Nucleic acid can be protected from physiological pH, enzymes and enzymatic degradation and protein binding in circulation when encapsulated into cationic nanoparticles or in the form of polyplexes.

Cationic polymers have stronger interaction and cellular uptake when compared with polymers with neutral or negative charge and therefore are considered to be promising carriers for mucosal application of anticancer drugs, especially for local chemotherapy such as intravesical chemotherapy for the treatment of bladder tumors.

On the other hand, favorable properties of cationic polymers induce undesired effects *in vivo* as a result of their complement activation property, which causes these systems to be rapidly cleared from the circulation on opsonization with plasma proteins, which hinders the delivery of the active molecule, be it drug or nucleic acid, to tumor cells. Electrostatic interaction of cationic vectors with negatively charged erythrocyte membranes can result in accumulation of the cationic carriers in the lungs, liver and spleen. These generally ineffective delivery systems become toxic owing to embolization of particles in the lung. The stability of DNA-cationic polymer complexes in physiological buffer conditions is another question to be answered and the stability of DNA polyplexes depends on the nature of the condensing agent used. These toxicity issues can be tackled by shielding surface charge of the polyplexes or nanoparticles by coating and grafting with PEG.

It has to be understood, however, that steric stabilization is not desirable for all steps of anticancer drug delivery with cationic vectors. Prolonged circulation time and lower toxicity may be achieved by steric stabilization, but the vector needs to liberate its content, the active molecule, once it is inside the tumor tissue. Polymer coating may hinder the release of the drug as well as target cell interaction and can be a barrier to obtaining therapeutic response.

Cost-effectiveness and large-scale production are also important in the production and registration of a therapeutic system, and chitosan seems to be the most promising cationic polymer among its analogues because it is considered non-toxic and produced on a large scale. When cationic polymers are compared among themselves for general nanoparticle properties, particle size seems to be between 10 and 200 nm, with surface charges between + 5 and + 50 mV depending on the formulation, polymer composition and preparation technique.

Cationic polymer vectors can be applied through different routes, including oral, parenteral and mucosal routes for local or systemic therapeutic effect, therefore further research and development studies are required to elucidate fully the cellular interaction, biological activity and potential toxicity of these promising delivery systems for effective cancer therapy.

It is believed that cationic nanoparticles may find their optimum application for cancer therapy in local and mucosal application.

Declaration of interest

The author states no conflict of interest and has received no payment in preparation of this manuscript.

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