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CRITICAL REVIEW

Cationic polymers and their therapeutic potential

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The last decade has witnessed enormous research focused on cationic polymers. Cationic polymers are the subject of intense research as non-viral gene delivery systems, due to their flexible properties, facile synthesis, robustness and proven gene delivery efficiency. Here, we review the most recent scientific advances in cationic polymers and their derivatives not only for gene delivery purposes but also for various alternative therapeutic applications. An overview of the synthesis and preparation of cationic polymers is provided along with their inherent bioactive and intrinsic therapeutic potential. In addition, cationic polymer based biomedical materials are covered. Major progress in the fields of drug and gene delivery as well as tissue engineering applications is summarized in the present review.

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1. Introduction

Polymeric systems bearing positive charges or synthesized in the presence of novel cationic entities, incorporated on their backbone and/or as side chains, are considered as cationic polymers. These systems exhibit unique physico-chemical properties and their ability that allows further modification renders them appealing for biological applications. The 21st century has marked substantial research in the field of cationic polymers and their derivatives through various developments even resulting in clinical trials.[1–4] Cationic and anionic polymers possess significant potential in their own arena and are

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Sangram Keshari Samal received his PhD degree in Biomaterials from the School of Biomolecular Science, University of Pisa, Italy. During his PhD, he was a visiting fellow at BWH, HST-MIT and Tufts University, USA. He undertook his first post-doctoral research on magnetic biomaterials for bone regeneration at Consiglio Nazionale delle Ricerche, Bologna, Italy. At present he is a post-doctoral fellow at the Polymer Chemistry & Biomaterials Group, Department of Organic Chemistry, University of Ghent, Belgium. His research interest is mainly focused on functionalization of polymers and evaluation of their potential for various therapeutic applications.

Mamoni Dash

Mamoni Dash received her PhD (2010) degree in Biomaterials from the University of Pisa, Italy. She undertook post-doctoral research firstly (2010–2011) in the research group of Professor Emo Chiellini, BIOLab, University of Pisa, Italy, and subsequently (2011–Present) at the Department of Organic Chemistry, University of Ghent, Belgium, in the research group of Professor Peter Dubruel. Her research interest lies in the interdisciplinary area of polymers, and currently focuses on the development of polymers as biomaterials for biomedical applications.

both extensively investigated for various therapeutic applications. Anionic polymers have the ability to form ionic complexes with cationic biomolecules including cationic drugs, basic peptides and blood proteins leading to several therapeutic applications.[5–8] In comparison to anionic polymers, cationic polymers are extensively explored and form electrostatic complexes with anionic biomolecules, nucleic acids and proteins. In addition their inherent bioactive properties such as stimuli responsiveness, antimicrobial, antioxidant, antitumor and anti-inflammatory make cationic polymers more promising for further enhanced therapeutic potential.

Interest in cationic polymers results from their potential to form polyelectrolyte complexes with nucleic acids (DNA, RNA and PNA). The cationic polymers mediate transfection *via* the condensation of nucleic acids, provide protection from enzymatic degradation and facilitate cellular uptake and endolysosomal escape, thus becoming excellent candidates for gene delivery. However, their development has also expanded to other applications including drug conjugation and delivery, tissue engineering and therapeutic applications. Widely studied cationic polymers include poly(ethyleneimine) (PEI), poly-L-(lysine) (PLL), poly[2-(*N*,*N*-dimethylamino)ethyl methacrylate] (PDMAEMA) and chitosan. While these polymers bear inherent cationic charges, others have also been developed by introduction of cationic moieties such as cationic cyclodextrin and dextrans. Most cationic polymers bear amine functions that can be protonated. The relative number of the protonable amines differs for each cationic polymer. In addition, different polymer architectures exist including linear, branched, hyperbranched and dendrimer-like. Some of these,

Sandra Van Vlierberghe

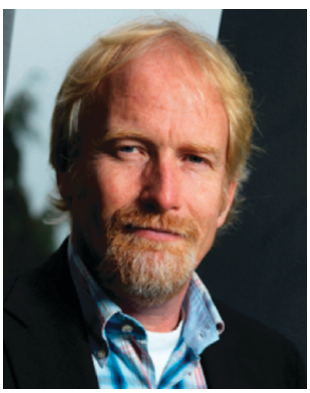
Sandra Van Vlierberghe finalized her PhD entitled 'Cell-Interactive Biopolymer-based Hydrogels designed for Tissue Engineering' in 2008 in the Polymer Chemistry & Biomaterials Group at Ghent University. At present, she is working as a senior post-doctoral fellow at the same institution, supported by the Research Foundation Flanders (FWO-Flanders). She has published more than 50 papers and is a promoter of 5 PhD students. In addition, Dr Van Vlierberghe is Editorial Board Member of BIOMAT.net, responsible for the book section and Executive Board Member of the Young Scientist Forum, which is affiliated to the European Society for Biomaterials. Her research interests are related to the development and modification of polymers for biomedical applications.

David L. Kaplan

David Kaplan is the Stern Family Professor of Engineering at Tufts University. He is Professor and Chair of the Department of Biomedical Engineering and also holds faculty appointments in the School of Medicine, Department of Chemistry and the Department of Chemical and Biological Engineering. His research focus is on biopolymer engineering to understand structure–function relationships, with emphasis on studies related to self-assembly, biomaterials engineering and functional tissue engineering. He has published over 500 papers and edited eight books.

Emo Chiellini

Emo Chiellini is a former Professor of Fundamentals of Chemical Technology, Faculty of Engineering, University of Pisa, Italy. Presently he is acting as free-of-charge Director of the BIOlab of the Department of Chemistry & Industrial Chemistry of the University of Pisa. He has been involved in numerous projects funded by EC and industries in the field of Polymer Science and Technology. During his almost 50 years of activity he has collected more than 480 publications, edited and co-edited 20 books, issued 30 patents and delivered 260 speeches in national, international conferences, scientific institutions and industries.



Clemens van Blitterswijk

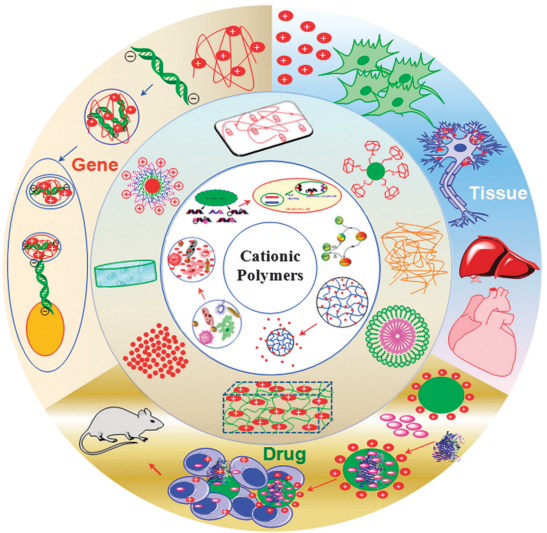
Clemens A. van Blitterswijk received his PhD in 1985 at Leiden University on artificial ceramic middle ear implants, for which he was awarded the Jean Leray award, the Marie Parijs award and the Klein award in the following years. He continued his research on bone and cartilage replacement, with extensions to muscle and skin substitutions. Today most of his research deals with regenerative medicine. Prof. van Blitterswijk has authored and co-authored more than 230 scientific papers and frequently acts as an invited speaker or chairman at international conferences in the field. For his more recent work, he received the George Winter award of the European society for Biomaterials and was appointed Fellow of Biomaterials Science and Engineering and member of the Royal Dutch Academy of Science.

such as PLL, are linear polymers, while other polymers like PEI exist both as linear and branched structures. Furthermore, some cationic polymers carry positive charges on their backbone as in PEI while others like PLL possess positively charged side groups. Block copolymers including polyethylene glycol–PLL (PEG–PLL) as well as comb-type copolymers like PLL-*g*-dextran with a polycationic backbone and grafted hydrophilic side chains have been developed. These strategies clearly highlight the platform for improvement through conjugation and inclusion of biodegradable or bioreducible properties resulting in the therapeutic success of cationic polymers.

There are several excellent reviews in the literature that highlight cationic polymers for gene delivery applications.[1,9] In the present review, we aim to broaden the focus from gene delivery systems to include alternative therapeutic applications. The first section of the review describes the development and modification of cationic polymers. In the subsequent part, recent progress encompassing various structural forms, including hydrogels, scaffolds, membranes, micelles, nanoparticles and dendrimers, will be discussed along with their responsiveness to external stimuli and bioactivity. The last section of the review highlights recent advanced applications, providing an overview of the success and limitations of these cationic polymeric systems for drug delivery, tissue engineering and gene therapy applications. The pictorial representation of inherent bioactive properties (2nd inner circle), architectures (3rd inner circle) and therapeutic applications (outermost circle) of cationic polymers is shown in Scheme 1. Concerns arising from the current state-of-the-art of cationic polymers will be summarized as well as avenues for future research evolving around the use of cationic polymers and the modification of their structural properties for various therapeutic applications.

2. Cationic polymers

Cationic polymers have potential as biomaterials for treatment of various human diseases. Cationic polymer properties are highly dependent on polymeric chain flexibility, H-bond formation, hydrophobic interactions, electrostatic forces or charge transfer potential, amine group and its neighboring functionalities, pK_a, and nucleophilic character. A wide range of cationic polymers have been investigated for various therapeutic applications and are generally divided into two categories, according to their origin: natural or synthetic.



Scheme 1 Overview of the inherent bioactive properties, architectures and therapeutic applications of cationic polymers.

2.1 Naturally derived cationic polymers

Natural cationic polymers are attractive candidates for therapeutic applications as they generally are non-toxic, derived from renewable resources, biocompatible, biodegradable and possess low immunogenicity. Most natural cationic polymers contain reactive sites, which can be easily modified to improve physicochemical properties. This section focuses on the synthesis of naturally derived cationic polymers. A summary of

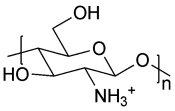
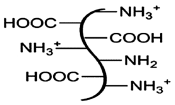
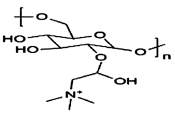
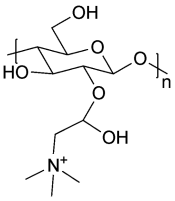
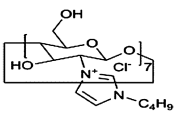
Lorenzo Moroni

Lorenzo Moroni received his PhD in 2006 at University of Twente on 3D scaffold technologies for tissue engineering. After working at Johns Hopkins University on hydrogels and stem cells, in 2008 he was appointed the R&D director of the Musculoskeletal Tissue Bank of Rizzoli Orthopaedic Institute. Here, he investigated stem cells from alternative sources for cell banking and developed novel bioactive scaffolds for musculoskeletal regeneration. He joined again the Tissue Regeneration Department at University of Twente in 2009 as an assistant professor. Currently, his research interests aim at generating new libraries of bioactive scaffolds to recruit and deliver stem cells in situ, and control their fate.

Peter Dubruel

Peter Dubruel is currently heading a group of over 30 people and has published over 70 A1 papers. Since the start of 2006, he has been involved in several EU projects (3 FP6 and 4 FP7, 1 as a coordinator). Since end 2006, he has delivered over 20 invited lectures. He has been the spokesperson of the Young Scientist Forum (YSF) from the European Society for Biomaterials (ESB) for more than 5 years. He is part of the editorial team of BIOMAT.net and the journal Biomaterials. In 2010 and 2012, he was awarded, respectively, the YSF Excellence Award from the Romanian Society for Biomaterials and the Jean Leray Award from the ESB.

Table 1 Overview of the most widely used natural cationic polymers in therapeutic applications

Cationic polymer	Nature	Structure	Application	Ref.
Cationic chitosan	Polysaccharide *N*-acetyl glucosamine and D-glucosamine		Drug delivery Tissue engineering Gene delivery	27, 67, 68 69–72 73–75
Cationic gelatin	Protein 18 Non-uniformly distributed amino acids		Drug delivery Tissue engineering Gene delivery	33, 37, 76 77–79 80–85
Cationic dextran	Polysaccharide Glucose units linked by α-1,6-linkages		Drug delivery Tissue engineering Gene delivery	43, 86–88 89, 90 46, 91–95
Cationic cellulose	Polysaccharide Linear glucose units linked by β-1,4-D-linkage		Drug delivery Tissue engineering Gene delivery	47, 96–98 99, 100 48, 51, 101
Cationic cyclodextrin	Polysaccharide Cyclic glucose units linked by α-1,4-linkage		Drug delivery Tissue engineering Gene delivery	102–106 107 108, 109

their chemical structures and their relevant applications is listed in Table 1.

2.1.1 Chitosan. Chitosan is a natural cationic copolymer composed of randomly distributed *N*-acetyl glucosamine and D-glucosamine, varying in composition, sequence and molecular chain length. The biocompatibility, non-toxicity, biodegradability, antibacterial activity, antioxidant activity and mucoadhesive properties impart versatility.[10–12] Chitosan is a weak polybase with a pK_a around 6.5, implying that its charge density varies in the pH range of 6–6.5. This imparts pH-responsiveness, which is beneficial for various therapeutic applications. Since the pK_a is near neutral, the soluble–insoluble transition occurs at pH between 6 and 6.5, which is a convenient range for biological applications (Fig. 1).[13]

The high charge density of chitosan at pH levels below the pK_a aids in polyelectrolyte formation, whereas a low charge density at neutral pH contributes to its low cytotoxicity and facilitates the intracellular release of biomolecules. The low charge density, however, leads to low solubility, aggregation and the poor stability of chitosan-based formulations, depending on the type of chitosan applied. The degree of deacetylation (DD) and molecular weight (Mw)[14] alter the cationic properties

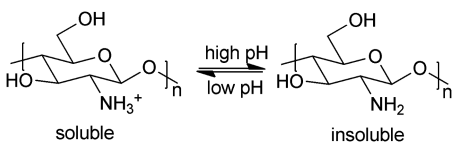


Fig. 1 Chitosan's inherent cationic nature below its pK_a.

of chitosan by varying the positive charge density and affect its cell-dependent transfection efficiency.[15,16] The cationic nature of chitosan enables the formation of polyelectrolyte complexes with negatively charged biomolecules, the interaction with cell membranes and more efficient transfection. The limitations of chitosan in therapeutic applications result from low solubility at physiological pH and from a high degree of swelling in aqueous environments leading to rapid drug release[9,17] in drug delivery devices based on chitosan as the continuous matrix. In order to overcome these difficulties, chemically modified chitosan derivatives have been synthesized. Chitosan exhibits three attractive reactive sites enabling modification, including one primary amine and two primary or secondary hydroxyl groups per glucosidic unit. The modification of chitosan using cationic moieties has been performed by quaternization of the amino group or by grafting small molecules or polymer chains onto the chitosan backbone. However, modifications do not imply a change in the fundamental properties of chitosan but introduce new properties. Modified chitosan provides a derivative with specific functional features to match applications, for example, improving transfection requires the preservation of primary amines.

The quaternization of chitosan has been investigated by several research groups.[18,19] This strategy provides good control over the cationic character without affecting its pH independency, which is desirable to improve the stability of ionic complexes. In addition, the solubility of chitosan in water was increased rendering it soluble over a wide pH range. The reaction of chitosan with methyl iodide under basic conditions is the most straightforward route for quaternizing chitosan. Among all quaternized chitosans described, *N*,*N*,*N*-trimethyl

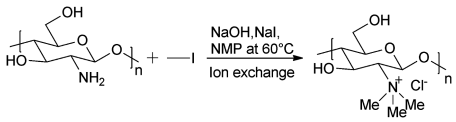


Fig. 2 Synthesis of *N*,*N*,*N*-trimethyl chitosan chloride.

chitosan chloride (TMC) is the most widely applied.[20,21] Quaternization improves the mucoadhesive properties of chitosan depending on the degree of quaternization, which makes this chitosan derivative a good candidate for gene delivery. Typically, TMC is synthesized by a two-step reaction. In the first step chitosan is reacted with methyl iodide in the presence of sodium hydroxide in *N*-methyl-2-pyrrolidinone (NMP) at 60 °C. In the second step, the iodide ion is substituted by chloride using an ion exchange process (Fig. 2).[20,22]

Jia *et al.* reported the synthesis and the antibacterial activity of quaternary ammonium salts of chitosan including *N*,*N*,*N*-trimethyl chitosan, *N*-propyl-*N*,*N*-dimethyl chitosan, *N*-furfuryl-*N*,*N*-dimethyl chitosan and *N*-diethylmethylamino chitosan.[23] In addition, quaternized chito-oligomers also possessed antibacterial activity.[24] By exploiting the cationic nature of chitosan, several drug conjugate approaches have been developed toward therapeutic applications. Yang *et al.* synthesized folic-acid conjugated chitosan as a carrier for 5-aminolevulinic acid and determined its targeting and uptake efficiency in different human colorectal cancer cell lines (HT29 and Caco-2) by folate receptor-mediated endocytosis.[11] Folic acid–chitosan conjugates were synthesized by chemically linking folic acid to chitosan using 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC).[25] These conjugates were prepared at different molar ratios ranging from 1 : 0.02 to 1 : 0.2 for the amine groups of chitosan to folic acid, respectively.

In a similar way, folic acid and transactivating transcriptional activator peptide (TATp) were conjugated to a novel chitosan derivative octadecyl-quaternized lysine-modified chitosan (OQLCS). Paclitaxel, which demonstrates poor solubility in water, was successfully encapsulated into the OQLCS liposomes.[26] OQLCS was also used for preparing polymeric liposomes coated with folate-PEG.[27] *N*-((2-Hydroxy-3-trimethylammonium)propyl)chitosan chloride (HTCC) was also reported to show high encapsulation of paclitaxel.[28] This water-soluble cationic derivative of chitosan was synthesized *via* reaction with glycidyl-trimethyl-ammonium chloride (Fig. 3).[29] HTCC demonstrated good solubility and permeation-enhancing effects in neutral environments. Enhancement of permeation involves electrostatic interactions between positively charged HTCC and negatively charged sites in tight cell junctions resulting in drug transport *via* transiently opened tight junctions. Moreover, HTCC interacts with negatively

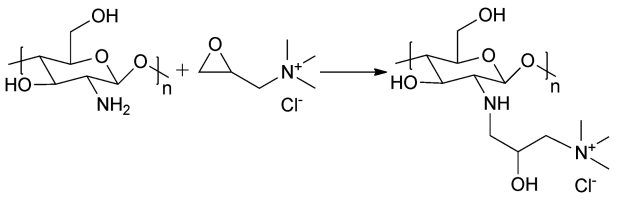


Fig. 3 Synthesis of *N*-((2-hydroxy-3-trimethylammonium)propyl) chitosan chloride (HTCC).

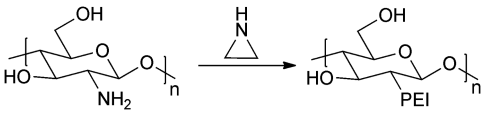


Fig. 4 Synthesis of PEI-*g*-chitosan derivatives possessing improved buffering capacity.

charged mucin glycoproteins, endowing them with increased mucoadhesive properties compared to chitosan.

An increase in positive charge density has also been achieved by grafting PLL to chitosan. The chitosan-*g*-PLL polymer exhibited improved DNA-binding ability, reduced cytotoxicity, and an increased transfection efficiency compared to both PLL and 25 kDa PEI.[30] The conjugation of PEI with chitosan increased the buffering capacity of chitosan-based polyplexes (Fig. 4). The transfection efficiency of the chitosan–PEI derivative was comparable to that of 25 kDa PEI while the cytotoxicity was significantly reduced for the chitosan–PEI derivative.[31]

2.1.2 Gelatin. Gelatin is a natural polymer derived from collagen, commonly applied for pharmaceutical and medical purposes because of its biodegradability and biocompatibility in physiological environments.[32–36] Gelatin is composed of 18 non-uniformly distributed amino acids with both positive and negative charges. The inherent cationic property of gelatin is basically due to lysine and arginine residues. The denaturation process through which gelatin can be obtained from collagen is performed by acidic or basic treatment, resulting in gelatin A with an isoelectric point (IEP)[4] of 6–9 and gelatin B with an IEP of 4.7–5.4 respectively. The alkaline process targets the amide groups of asparagine and glutamine and hydrolyses them into carboxyl groups, yielding gelatin with a higher density of carboxyl groups, making it negatively charged and lowering its IEP. In contrast, an acidic pre-treatment does not significantly affect the amide groups. This results in two oppositely charged gelatin types. Gelatin shows cationic behavior at pH values below its IEP *via* protonation of amino groups (Fig. 5). The cationic density is higher for acidic gelatin and lower for basic gelatin. The US Food and Drug Administration (FDA) classified gelatin as a safe excipient, which is currently used as a constituent of various biomaterials.[37]

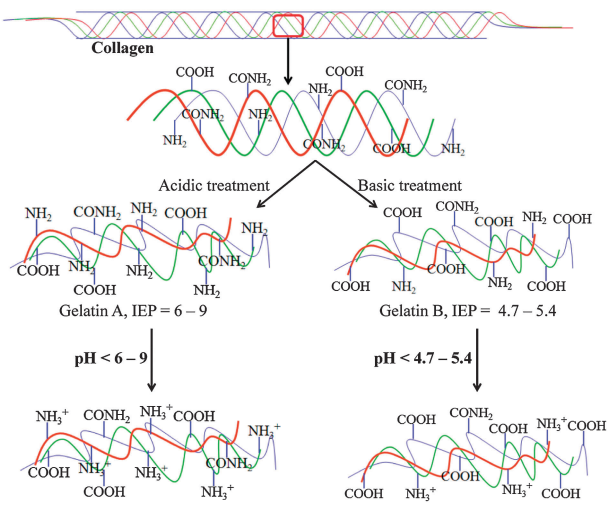


Fig. 5 Gelatin's inherent cationic nature at pH values below its IEP.

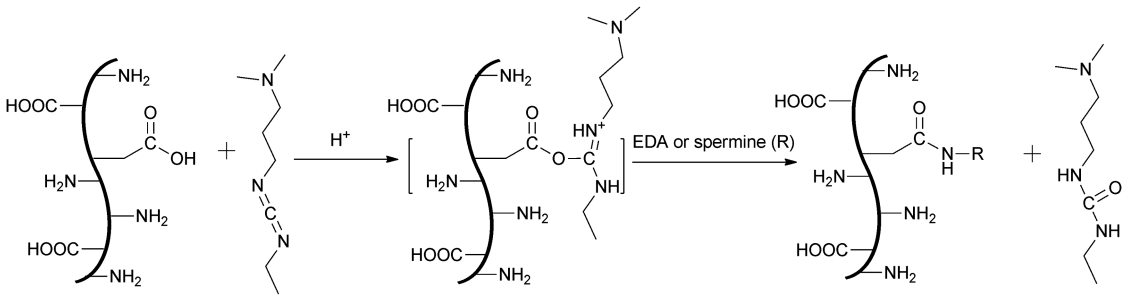


Fig. 6 Cationization of gelatin using EDA or spermine and EDC.

Gelatin can be cationized by protonation of the amine group, which occurs below its pK_a or by introducing amino groups onto the gelatin backbone, usually realized *via* carbodiimide chemistry. Morimoto *et al*. have synthesized cationic gelatins by coupling ethylenediamine (EDA) or spermine through an EDC-mediated reaction. It should be noted that this reaction, which establishes amide bonds between carboxylic groups of gelatin and amino groups of EDA or spermine, promotes crosslinking (Fig. 6).[38]

Gelatin is generally cationically derivatized to enable interactions with biomolecules of anionic nature without having a pH dependency. For example, Xu *et al*. applied cationic gelatin nanoparticles for the non-viral delivery of plasmid DNA encoding insulin-like growth factor (IGF)-1 to adult canine articular chondrocytes *in vitro*.[39] The results of the study revealed that chondrocytes transfected with IGF-1 using cationic gelatin nanoparticles were able to maintain steady IGF-1 overexpression when subsequently grown in cationic gelatin scaffolds for up to 2 weeks in three-dimensional (3D) culture. Cationic gelatin and its aminated derivative were evaluated for the controlled release of three acidic peptide/protein drugs with different M_Ws[14] and IEPs.[38]

Transfection of cationized gelatin and plasmid DNA complexes into monocyte-derived immature dendritic cells was demonstrated by Inada *et al*.[40] Cationization of gelatin was again achieved by introducing amine residues onto the carboxylic groups of gelatin which not only induced complex formation between vector compounds and negatively charged plasmid DNA through electrostatic interaction, but also enhanced the physical adhesion of plasmid DNA to a negatively charged cell surface. High transfection efficiency was obtained upon transfection of enhanced green fluorescent protein (EGFP). In a recent study by Fujii *et al*., a cationic gelatin conjugate composed of Hemagglutinating Virus of Japan Envelope (HVJ-E) and sodium borocaptate was developed. The safety, bio-distribution and effectiveness of this conjugate for boron neutron capture therapy were evaluated using a multiple liver tumor model.[41]

2.1.3 Cationic dextran. Dextran is an FDA-approved highly water-soluble branched polysaccharide composed of glucose units mainly linked by α-1,6-linkages. This homopolysaccharide is suitable as a polymeric carrier due to its biodegradability, wide availability, ease of modification and solubility in water irrespective of the pH. As a result, the potential application of cationic dextran has also been exploited. A series of dextran-based cationic polymers including diethylaminoethyl–dextran and dextran–spermine have been

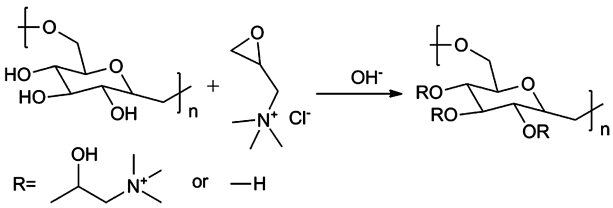


Fig. 7 Reaction pathway for developing a cationic dextran derivative.

prepared for the efficient delivery of nucleic acids.[42] As an alternative to protamine in anticoagulant therapy, Kaminski *et al*. prepared cationic derivatives of dextran by the substitution of hydroxyl groups with glycidyltrimethylammonium chloride (GTMAC) (Fig. 7). The degree of substitution of the polymers ranged from 0.50 to 0.65 GTMAC groups per glucose unit. These cationic derivatives of dextran formed complexes with unfractionated heparin and the binding efficiency correlated with the degree of cationic modification.[43]

Dextran–spermine-based conjugates (Fig. 8) have been prepared by reductive amination between oxidized dextran and spermine.[44] Spermine, a naturally occurring linear polyamine, is involved in cellular metabolism and is a polycation at physiological pH. Dextran was first oxidized with potassium periodate and the obtained dialdehyde derivative was then reacted under basic conditions with spermine. Dextran–spermine displayed especially high transfection efficiency, which was attributed to the unique complexation properties between DNA and the grafted spermine moieties. Dextran–spermine and their derivatives have shown high transfection of plasmid DNA both *in vitro* and *in vivo*.[45]

Utilizing this pioneering work, Cohen *et al*. combined the unique characteristics of acetyl dextran (Ac-DEX) and spermine (Fig. 9) for siRNA delivery. Ac-DEX possesses several characteristics suitable for the delivery of bioactive agents such as

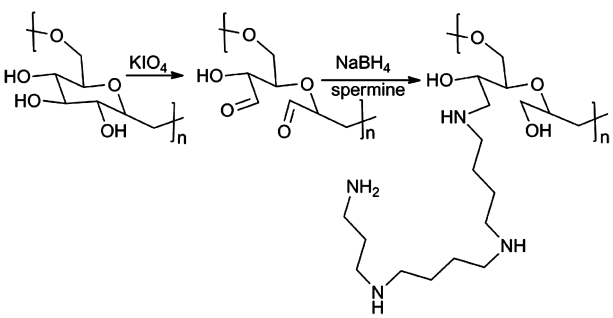


Fig. 8 Reaction pathway for developing dextran–spermine conjugates.

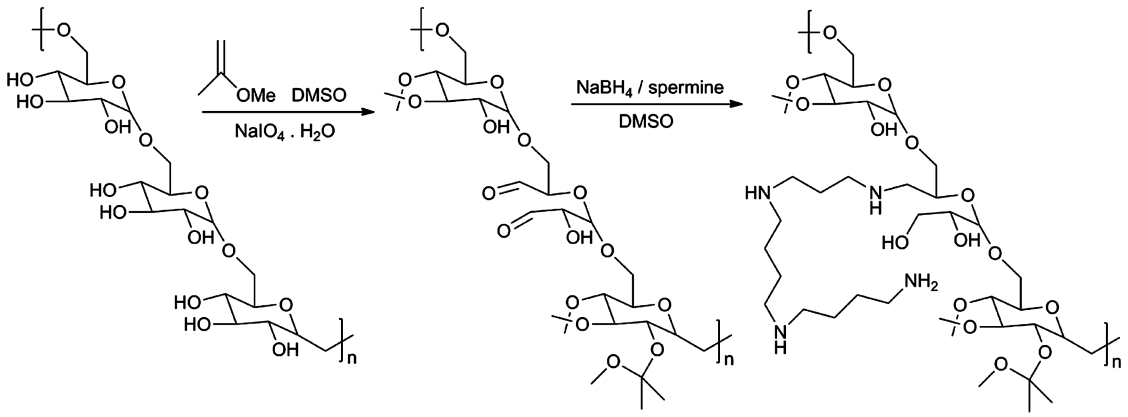


Fig. 9 Reaction pathway for developing spermine–modified Ac-DEX.

proteins. The novel system combined easy synthesis steps and biocompatibility with the advantage of controlled release that is sensitive to physiologically relevant acidic conditions. Acid-catalyzed hydrolysis of spermine–Ac-DEX generated spermine-modified dextran, which could further be metabolized *in vivo* by enzymes.[46]

2.1.4 Cationic cellulose. Cellulose, a linear β-1,4-D-glucan, is the most abundant polymer offering itself as the most common organic compound spread out all over the world. Cationic cellulose derivatives, which possess many useful characteristics including hydrophilicity, biodegradability and antibacterial properties, have numerous therapeutic applications.[47–49] An important cationic cellulose derivative has been prepared by the etherification of cellulose using glycidyl ammonium salts or alkylene epoxides in the presence of a suitable alkaline catalyst, usually NaOH.[50] Song *et al.* reported for the first time the homogeneous quaternization of cellulose in an aqueous solution.[51] Cellulose was dissolved in a NaOH–urea aqueous solution followed by addition of 3-chloro-2-hydroxypropyl trimethyl ammonium chloride (CHPTA) under alkaline conditions as an etherifying agent. Under these conditions, an epoxide was produced *in situ* and quaternized cellulose was subsequently formed through reaction between cellulose sodium alkoxide and the epoxide formed or the CHPTA added. This reaction results in the formation of diols as a side product. The quaternized cellulose derivatives obtained in the aqueous system have proven to be promising agents to be applied as gene carriers. Despite the successful preparation of cationic cellulose, cationic cellulose derivatives prepared directly from cellulose *via* a homogeneous process have been scarcely reported because of the insolubility of cellulose in water and in most organic solvents due to their strong inter- and intra-molecular hydrogen bonding. The group of Song *et al.* extended the work on quaternized cellulose by preparing a novel amphiphilic quaternized cellulose derivative, hydrophobically modified quaternized cellulose (HMQC). HMQC derivatives were obtained by a two-step synthesis as illustrated in Fig. 10. In the first step, water-soluble quaternized cellulose with a nitrogen content of 3.85% and degree of substitution of 0.76 was homogeneously synthesized from cellulose directly in a NaOH–urea aqueous solution. Thereafter, hexadecyl bromide was reacted with the residual hydroxyl groups of cellulose to increase the number of alkyl chains and thus hydrophobicity to the polymer. HMQC micelles were evaluated as a delivery carrier for poorly water-soluble drugs.[47]

Among the other cellulose derivatives, hydroxypropyl cellulose (HPC) and hydroxyethyl cellulose (HEC) are the most widely applied.[52] HPC-based materials have been approved by FDA and are widely used in food and drug formulations. Xu *et al.* prepared well-defined comb-shaped copolymers composed of HPC backbones and cationic PDMAEMA side chains to be studied as novel non-viral gene vectors. The short PDMAEMA chains were grafted onto the long HPC backbone (HPD) *via* atom transfer radical polymerization (ATRP) using bromo-iso-butyryl terminated HPC (HPC-Br) as the macroinitiator. The PDMAEMA chains of HPD were further partially quaternized to produce quaternary ammonium HPD (quaternized HPD), as indicated in Fig. 11.[48]

The transfection properties of cationic HEC/plasmid DNA (pDNA) nanoparticles for gene delivery applications were investigated by Fayazpour *et al.*[49] They evaluated the DNA complexation properties of two types of cationic HEC including polyquaternium-4-cellulose (PC-4) and polyquaternium-10-cellulose

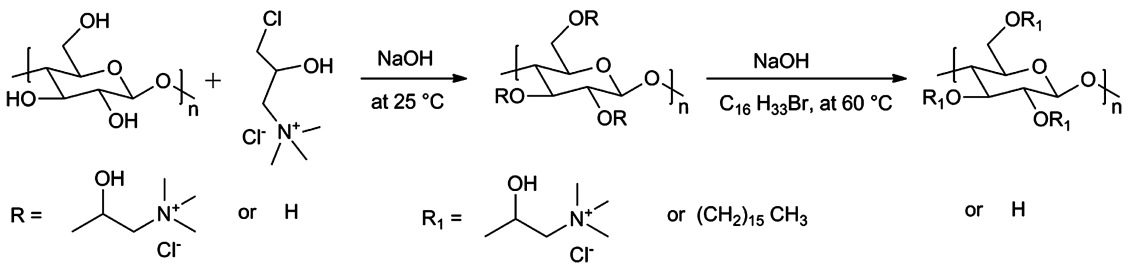


Fig. 10 Reaction pathway for the production of HMQC.

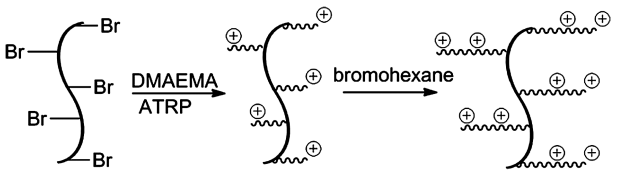


Fig. 11 Cationic comb-shaped copolymers composed of a HPC backbone and short, partially quaternized PDMAEMA side chains.

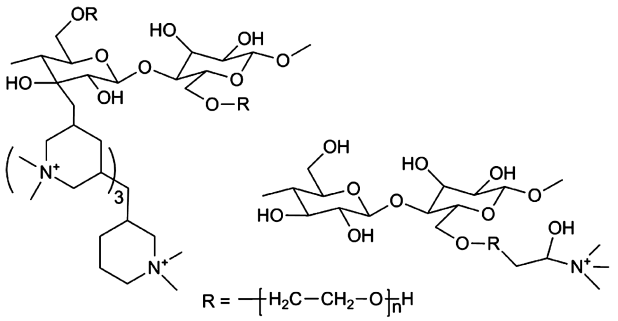


Fig. 12 Molecular structures of PC-4 and PC-10.

(PC-10) (Fig. 12). In both PC-4 and PC-10, the sugar monomers were substituted with PEG. However, in PC-4, the quaternary ammonium groups were directly linked onto the cellulose backbone, while, in PC-10, the quaternary ammonium groups were present at the end of the PEG chains.

2.1.5 Cationic cyclodextrin. Cyclodextrins (CDs) are sugar derivatives produced by bacteria grown on starch. CDs are torus-shaped, cyclic oligomers of glucose containing six to eight glucose units linked by α-1,4-bonds. The interior part of the macrocyclic structure consists of hydrocarbon and oxygen linking the glucose units, resulting in a hydrophobic cavity surrounded by a water-compatible exterior part. The most common CDs are composed of six (alpha, α), seven (beta, β) or eight (gamma, γ) glucopyranose units, but the term 'cyclodextrin' most commonly refers to β-cyclodextrin, which consists of seven glucose units. The main advantages of CDs include their monodisperse saccharide structure, their relative ease of specific chemical modification and their favorable toxicology. Interestingly, oligosaccharides possess low immunogenicity and have multiple sites available to introduce cationic or cell-targeting moieties. Cationic CD derivatives have shown a great ability to bind nucleotides and to enable enhanced delivery by viral vectors. In addition, CDs have already been incorporated into polycationic polymers and dendritic vectors. These unique properties of cationic CDs have been utilized for several therapeutic applications.[53–56] Yang *et al.* synthesized a series of novel, cationic star polymers with oligoethylenimine (OEI) arms of different lengths being linked to the α-CD core. The hydroxyl groups of the six glucose units of α-CD were activated and grafted with multiple OEI arms to form a cationic star polymer. The activation was performed using 1,1′-carbonyldiimidazole (CDI), followed by reaction with a large excess of OEI resulting in α-CD-OEI star polymers (Fig. 13).

In order to ensure that there was no intra- or intermolecular crosslinking, the molar ratio of CDI or OEI to α-CD was maintained at above 100.[57] The group of Qian *et al.* prepared a range of novel cationic β-CD polymers and evaluated them as potential drug carriers. They observed that cationic β-CD of high M_W and low cationic charge density exhibited good drug inclusion and dissolution abilities. The synthesis of cationic β-CD was performed *via* one-step condensation using epichlorohydrin choline chloride.[58] The incorporation of drug was also performed *via* β-CD–PEI conjugates. Lu *et al.* reported a β-CD–PEI conjugate comprising 5-fluoro-2′-deoxyuridine and studied its efficiency as a gene delivery system for glioma cancer therapy. CDI was again used in this study for the activation of 5-fluoro-2′-deoxyuridine to be conjugated to β-CD–PEI.[59]

The application of CD-containing polymers for gene delivery purposes was pioneered by Davis *et al.* They have evaluated the potential to incorporate cyclodextrin into cationic polymers.[53,60,61] One of their strategies was based on the polycondensation of cationic bifunctionalized co-monomers and bifunctionalized CD monomers. Cationic polymers capable of binding to DNA were obtained by the reaction of the bis(hydrogen carbonate) salt of 6*A*,6*D*-dideoxy-6*A*,6*D*-di(2-amino ethane thio)-β-CD hexahydrate with dimethylsuberimidate (DMS) (Fig. 14). Electrostatic complexation of the resulting cationic CD polymers and negatively charged pDNA (~5 kbp) resulted in 100–150 nm sized polyplexes with *in vitro* cell transfection efficiency comparable to that obtained with PEI and Lipofectamine™ while preserving a reduced toxicity.[52]

A straightforward design of polycationic CD-based facial amphiphiles as monodisperse molecular systems for efficient gene delivery and a diversity-oriented synthetic strategy was developed by Di-Moscoso *et al.* The overall architecture of these polycationic CDs enabled fine-tuning in terms of the density of cationic groups, their flexibility and the presence of additional hydrogen-bonding functionalities while maintaining a C7-symmetric disposition.[62] Recently, they also developed a modular strategy for the preparation of well-defined polycationic amphiphilic β-CDs capable of complexing and compacting DNA into homogeneous nanoparticles. The polyaminothiouriedo β-CDs developed possess a cluster of

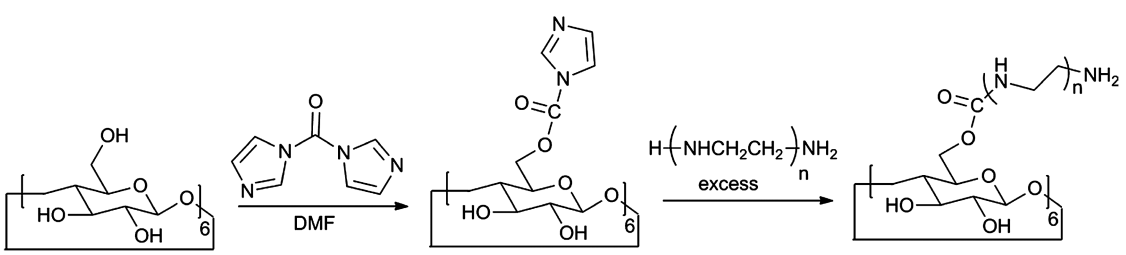


Fig. 13 Synthesis and structures of α-CD-OEI star polymers.

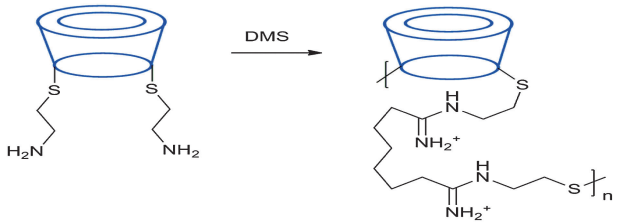


Fig. 14 Reaction pathway for the development of CD-containing cationic polymers.

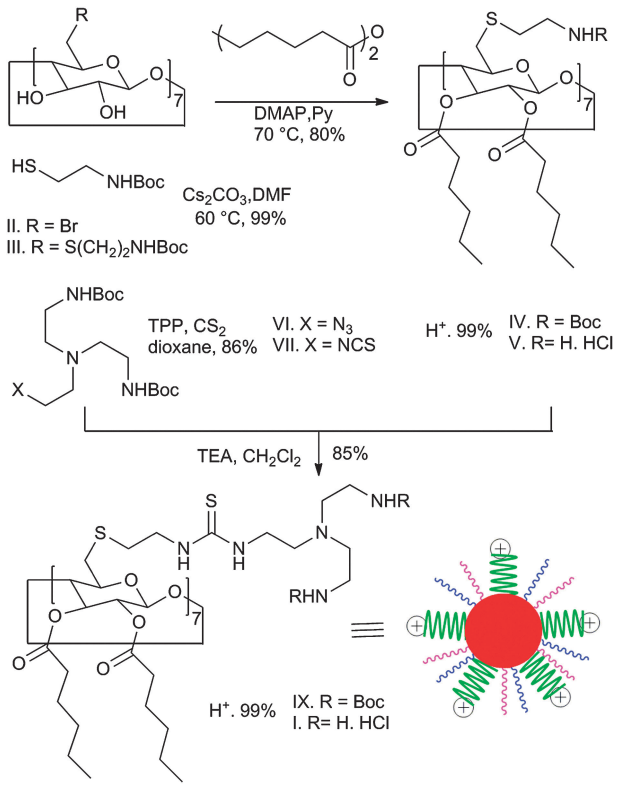
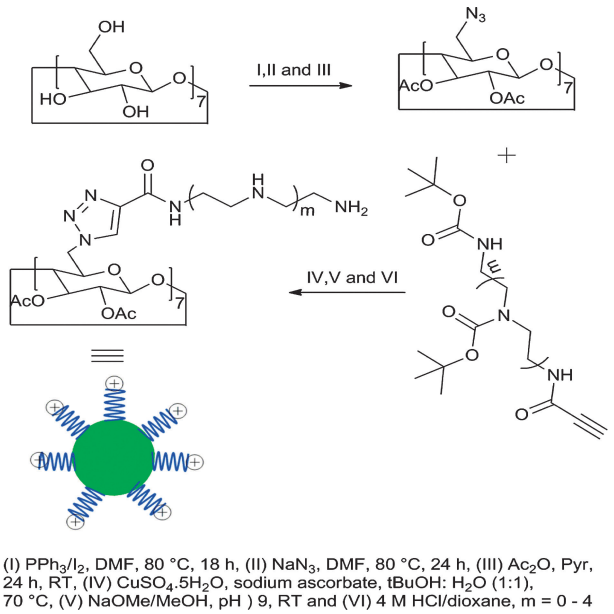


Fig. 15 Reaction pathway for the synthesis and chemical structure of polyaminothioureido amphiphilic β-CD.

fourteen primary amino groups that can act cooperatively with a belt of thiourea segments for the reversible complexation of the plasmid polyphosphate skeleton through both electrostatic interactions and hydrogen bonds. They also contain an additional circle of tertiary amino groups. The preparation of the β-CD precursor was completed in four steps starting from commercially available β-CD. The reaction sequence involved primary face-selective bromination with *N*-bromosuccinimide, followed by the nucleophilic displacement of the halogen groups by *N*-Boc-protected cysteamine and subsequent homogeneous acylation of the secondary hydroxyls with hexanoic anhydride and *N*,*N*-dimethylaminopyridine (DMAP) in pyridine. The final step involved the acid-promoted hydrolysis of the carbamate moieties (Fig. 15).[63]

A series of multivalent polycationic β-cyclodextrin "click clusters" with discrete M_W have been synthesized and examined as therapeutic pDNA carriers by combining a perazido β-CD core moiety with oligoethyleneamine dendrons *via* click coupling. An acetylated-per-azido β-CD was reacted with a



(I) PPh_3/I_2, DMF, 80 °C, 18 h, (II) NaN_3, DMF, 80 °C, 24 h, (III) Ac_2O, Pyr, 24 h, RT, (IV) $CuSO_4.5H_2O$, sodium ascorbate, tBuOH: H_2O (1:1), 70 °C, (V) NaOMe/MeOH, pH) 9, RT and (VI) 4 M HCl/dioxane, m = 0 - 4

Fig. 16 Synthesis of cationic β-CD polymers.

series of alkyne dendrons using Cu(I)-catalyzed azide alkyne 1,3-dipolar cycloaddition. The formed click cluster possessed the 1,2,3-triazole linker with the series of dendrons containing internal secondary amines and terminal primary amines (Fig. 16).[64]

The host–guest concept was exploited by Amiel *et al.* taking advantage of the CD inclusion capacity to induce polyCDplex formation. They utilized a neutral epichlorohydrin cross-linked β-CD polymer with cationic compounds possessing a high affinity for β-CD (*e.g.* positively charged adamantane or cholesterol derivatives).[65] The authors anticipated that the charge density of the supramolecular polymer can be fine-tuned by varying the proportion of the cationic guest thereby offering the possibility to modulate the DNA complexing abilities. In another study a methodology was described for the preparation of cationic, single-isomer CD applicable for chiral separation of amino acids and anionic pharmaceuticals by capillary electrophoresis.[66] The cationic water soluble cyclodextrin, mono-6^A-(1-butyl-3-imidazolium)-6^A-deoxy-β-cyclodextrin chloride (BIMCD), involved five reaction steps. The key reaction steps included the preparation of CD tosylate followed by a treatment with a chloride ion-exchange resin (Fig. 17).

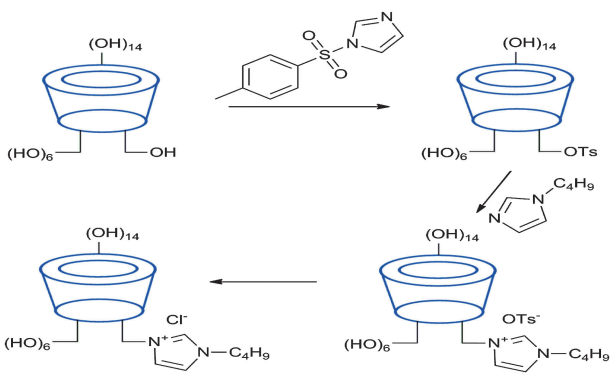
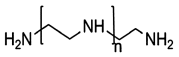
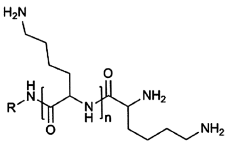
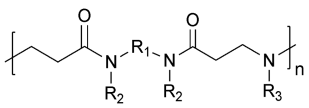
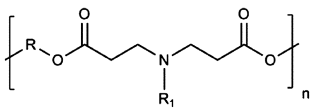
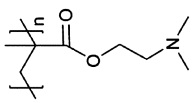


Fig. 17 Reaction pathway of BIMCD.

Table 2 Overview of the most widely used synthetic cationic polymers in therapeutic applications

Cationic polymer	Nature	Structure	Application	Ref.
PEI	LPEIs contain all secondary amines BPEIs contain primary, secondary and tertiary amines		Drug delivery Tissue engineering Gene delivery	170–172 173–176 119, 177–183
PLL	Homopolymer of the amino acid L-lysine.		Drug delivery Tissue engineering Gene delivery	184–186 187, 188 189–193
PAA	Synthetic cationic polymer containing tertiary amino groups.		Drug delivery Tissue engineering Gene delivery	194–197 198, 199 200–202
PAE	Amine containing polyesters		Drug delivery Tissue engineering Gene delivery	155, 203, 204 152, 205, 206 4, 153, 207–209
PDMAEMA	Synthetic cationic polymer containing tertiary amino groups.		Drug delivery Tissue engineering Gene delivery	210, 211 212, 213 164, 166, 214, 215

2.2 Synthetically derived cationic polymers

Both natural as well as synthetic cationic polymers have been evaluated for therapeutic purposes. The main difficulties with batch to batch variation of natural polymers can be overcome by synthetic polymers. Synthetic polymers enable improved control over properties and modifications. The bioactive moieties and functional groups can be readily incorporated into the synthetic polymeric system to result in specific M_Ws and block structures with degradable linkages if required. These properties determine both the therapeutic potential and its degradation properties for the polymer. In the upcoming paragraphs, an overview is provided of the most commonly applied synthetic cationic polymers. A summary of their chemical structures and applications is given in Table 2.

2.2.1 Polyethyleneimine. PEI is the most prominent and extensively used cationic polymer containing primary, secondary and tertiary amino functions. It is synthesized in both linear and branched forms and exists in different M_Ws. At room temperature, linear PEI (LPEI) is a solid while branched PEI (BPEI) is a highly viscous liquid. Synthesis of BPEI is realized *via* acid-catalyzed polymerization of aziridine

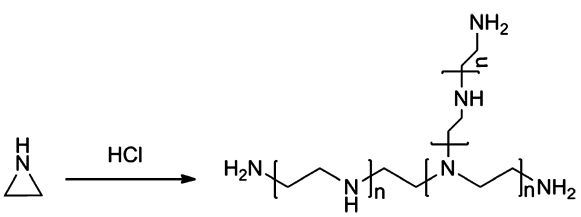


Fig. 18 Synthesis of BPEI by acid-mediated of aziridine.

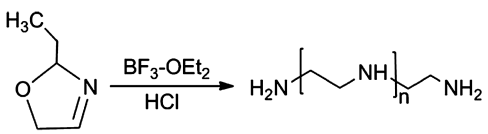


Fig. 19 Synthesis of LPEI by ring opening polymerization.

(Fig. 18), whereas LPEI is obtained *via* ring opening polymerization (ROP) of 2-ethyl-2-oxazoline followed by hydrolysis (Fig. 19).[110,111] PEI is composed of chemically reactive amino groups and can be applied for a wide range of chemical modifications which offers PEI desirable physicochemical properties. The protonable amino group of PEI can electrostatically interact with negatively charged biomolecules including drugs and nucleic acids forming polyelectrolyte complexes.[112,113] PEIs with a high molar mass and a high degree of branching lead to the formation of small and enzymatically stable polyplexes showing high transfection efficiency. However, their nondegradability, cytotoxicity and low hemocompatibility have limited their therapeutic applications.[114] It has been shown that LPEI is less effective in condensing DNA compared to the branched form for similar M_W.

In addition, the polyplex stability is higher for complexes with more primary amines, making BPEI a more suitable transfection vector.[115] BPEI contains primary, secondary and tertiary amines in a ratio of 1 : 2 : 1. At physiological pH, the polymer has a high amount of amine functions and is protonated to only approximately 25%. As BPEI has the ability to capture a large amount of protons in an acidic environment, it can be considered as an ideal buffer for variations in pH. The latter is a useful property in the endosomal escape route. ExGen500 and jet PEI are some of the commercially available LPEIs that have already

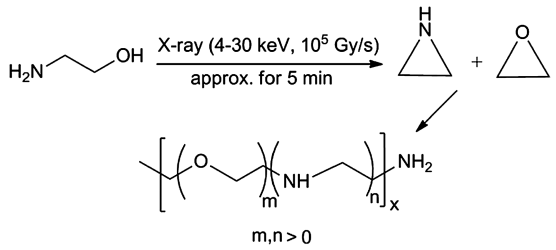


Fig. 20 Preparation of LPEI-*co*-PEG using X-ray irradiation.

reached clinical trials for the local treatment of bladder carcinoma and HIV disease.[116]

The first detailed characterization of LPEI was published by Saegusa *et al*. starting from the living cationic ROP of 2-oxazoline and the subsequent hydrolysis of poly(2-oxazoline) under alkaline conditions.[117] Since then, poly(2-oxazoline)s have often been used as precursors for LPEI upon hydrolysis under alkaline or acidic conditions. Recently, Tauhardt *et al*. studied the synthesis of LPEI by acidic hydrolysis of poly-(2-ethyl-2-oxazoline) and optimized the highest hydrolysis degree within the shortest time range using a microwave synthesizer as shown in Fig. 19.[118]

In another recent study, LPEI-*co*-PEG was synthesized by synchrotron X-ray (4–30 keV, 10^5 Gy s^{-1}) irradiation. X-ray irradiation is a strong radiation source with potential to generate free radicals without additional catalysts and/or initiators to be added (Fig. 20). In this process, ethanolamine was introduced as a monomer into an aqueous solution and the mixture was irradiated using synchrotron X-rays for approximately 5 min, resulting in a copolymer with a mono-dispersive M_W.[119]

Acid-degradable amino ketal branches have been incorporated into secondary amines of LPEI to facilitate endosomal escape.[120] Acrylamide groups have been conjugated to secondary amines of LPEI *via* a Michael addition reaction leading to acid degradable ketalized LPEI and the conjugation was done using an acrylated ketal monomer *N*-trifluoroacetamido ethoxy propan-2-yloxy ethyl acrylamide (TEPEA), in the presence of triethylamine (TEA) which served as a deprotonating agent and a preventer of the hydrolysis of ketals (Fig. 21). This approach resulted in a broader buffering capacity of primary, secondary and tertiary amines and in the subsequent release of siRNA into the cytoplasm, thus resulting in efficient RNA interference (RNAi). Drug conjugation to LPEI was also achieved, by conjugating it with dexamethasone, which enhanced the transfection efficiency of LPEI.[121]

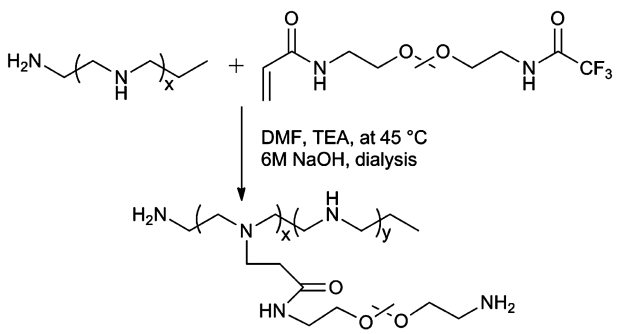


Fig. 21 Synthesis of ketalized LPEI.

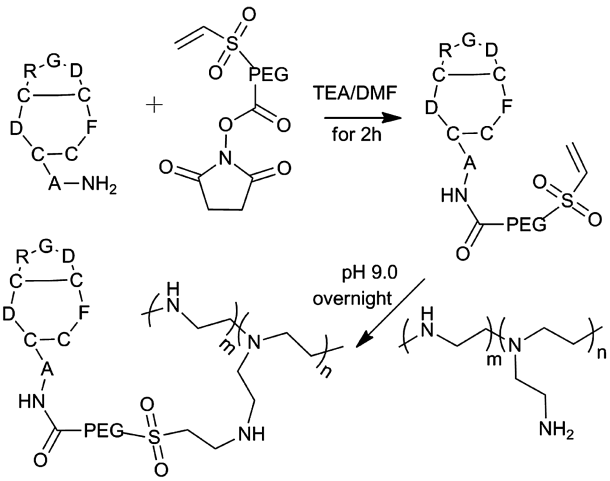


Fig. 22 Synthesis of PEI–PEG–RGD.

Kim *et al*. developed an angiogenic endothelial cell-targeting polymeric gene delivery system, PEI-*g*-PEG-RGD.[122,123] This delivery system was developed by incorporating the $\alpha_v\beta_3/\alpha_v\beta_5$ integrin-binding RGD peptide (ACDCRGDCFC) into PEI using a hydrophilic PEG spacer (Fig. 22). In the two step synthesis, the first step involved the reaction of the *N*-hydroxy-succinimide (NHS) group of heterobifunctional PEG-NHS (*N*-hydroxysuccinimide–vinyl sulfone polyethylene glycol) with the primary amine of the peptide that was preactivated using an excess of TEA. In a subsequent step, the conjugated RGD-PEG was incubated with PEI in buffer (pH 9).

Appelhans *et al*. proposed a rapid synthesis method for the development of hyper-branched PEI decorated with different oligosaccharide architectures as carrier systems for drugs and bioactive molecules to perform *in vitro* and *in vivo* experiments. For the development of various oligosaccharide substitution degrees, they established an easy, one-pot approach on the PEI surface (Fig. 23).[124] Reductive amination of hyper-branched PEI with readily available oligosaccharides resulted in sugar-functionalized PEI cores with oligosaccharide shells of different densities.

Non-biodegradability is one of the major shortcomings of PEI to find wide therapeutic applications.[125,126] An example to overcome this drawback was represented by the introduction of short PEI chains into a longer chain using biodegradable linkers in order to develop biodegradable PEI derivatives possessing high transfection efficiency and low toxicity.[127] In another approach, the synthesis of biodegradable PEI involved either the incorporation of reducible disulfide linkages or esters. Park *et al*. designed and synthesized a reducible LPEI derivative, linear poly(ethylenimine sulfide) (LPEIS), with M_W ranging from about 10 to 20 kDa by oxidative polycondensation of bismercapto-ethyleneimine oligomers.[128] The transfection efficiency increased with increasing amine density of LPEIS and even approached that of 25 kDa PEI (Fig. 24).

In addition to disulfide linkages, PEI derivatives with acid labile ester linkages have been explored by several research groups to create biodegradable PEI derivatives.[129]

2.2.2 Poly-L-lysine. PLL is a cationic homopolymer of the amino acid L-lysine and is composed of a large number of

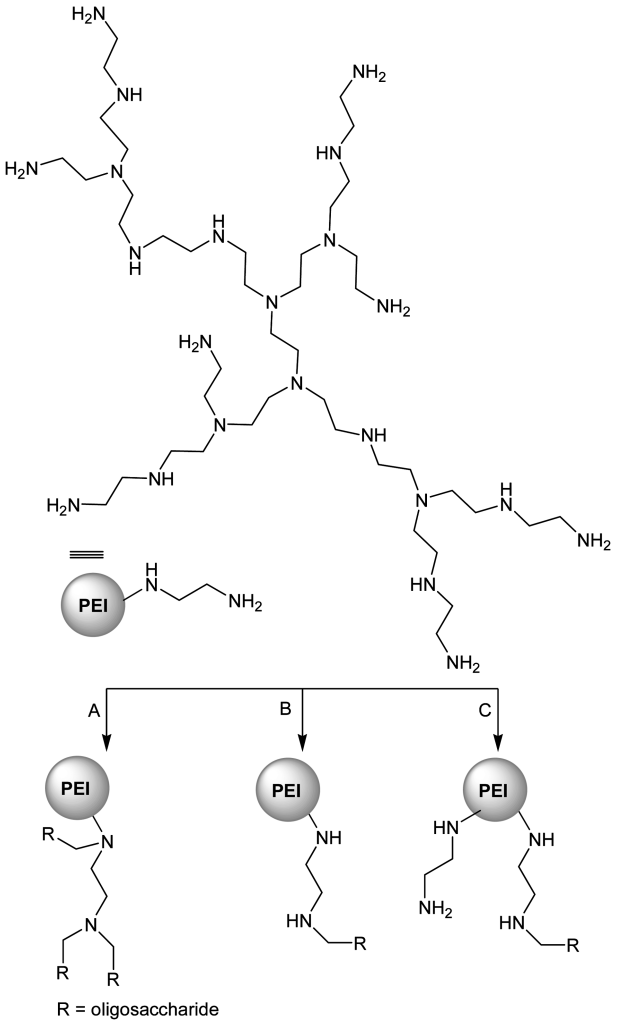


Fig. 23 Hyper-branched PEI conjugated with different oligosaccharide architectures.

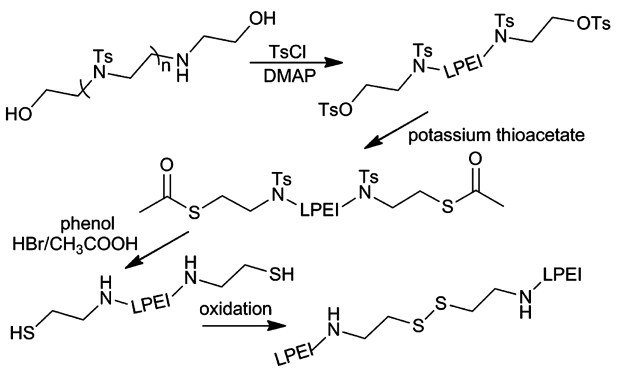


Fig. 24 Synthesis of LPEI derivatives with disulfide linkers.

primary amines which can interact with negatively charged biomolecules through electrostatic interaction when protonated. Synthesis of PLL proceeds by conversion of a primary amine protected L-lysine monomer into the cyclic *N*-carboxy-(*N*-benzyloxycarbonyl)-L-lysine anhydride (Fig. 25).[130,365,366] The *N*-carboxy-anhydride undergoes ring-opening polymerization using a primary amine initiator. Control over the M_W can be achieved through the use of specific monomer to initiator feed ratios.

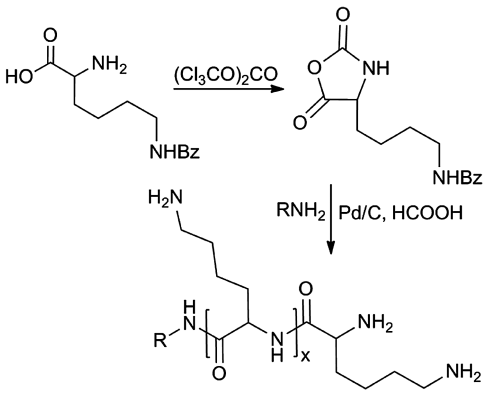


Fig. 25 Reaction pathway for the development of PLL.

PLL is one of the first polycations used for polyplex formation. The primary amine groups of lysine in PLL, which are partly protonated in a physiological environment, electrostatically interact with negatively charged phosphate groups of DNA to form nanoparticulate polyelectrolyte complexes. Due to the neighbouring group effects in the polymer chains, only part of the primary amine groups of PLL is protonated at physiological pH. Therefore, PLL shows a rather low buffering capacity (pH 5.7–7.7) and consequently low transfection efficiency. In addition, it has been indicated that high M_W PLL is cytotoxic and shows a tendency to aggregate and precipitate depending on the ionic strength[131] and protects DNA from degradation only to a minor extent. Accordingly, PLL which shows limited transfection potential is commonly applied as a control in studies demonstrating the advantages and disadvantages of other polyplexes. Research further revealed that less stable complexes were formed with PLL having M_W of less than 3000 Da, indicating that the number of primary amines from PLL is important for the complex formation. In an attempt to overcome these limitations, several approaches have been elaborated including the incorporation of dendritic PLL derivatives. In an effort to reduce cytotoxicity and to improve DNA release through endocytosis, various biodegradable PLL conjugates have been prepared. Researchers have attempted to avoid polyplex precipitation by preparing PLL–PEG block copolymers.[132] These copolymers also exhibited improved buffering potential. Moreover the artery wall binding peptide (AWBP, Cys-Gly-Arg-Ala-Leu-Val-Asp-Thr-Leu-Lys-Phe-Val-Thr-Gln-Ala-Glu-Gly-Ala-Lys) was covalently attached to a PLL-*g*-PEG copolymer.[133] AWBP–PEG–PLL was synthesized by the reaction between the vinyl sulfone group of PEG-*g*-PLL and the thiol group of cysteine in AWBP. Transfection efficiencies of AWBP–PEG–PLL–pDNA complexes were 150–180 times higher than that of the used controls of PEG-*g*-PLL–pDNA and PLL–pDNA. Hepatic cell/tissue targeting was induced by conjugating either PLL or PLL–PEG derivatives to lactose.[134] Pegylated PLL dendrimers have also been developed for drug conjugation.[135,136] Kaminskas *et al.* studied pegylated dendrimers for the pH-dependent release of doxorubicin (Dox).[137] Leukemia specific JL1 antigen was conjugated to PLL to target leukemia T-cells.[138]

The main drawback of linear PLL-based gene transfer, the relatively high cytotoxicity, was overcome by the preparation of PLL-based dendrons. The first synthesis of PLL dendrons

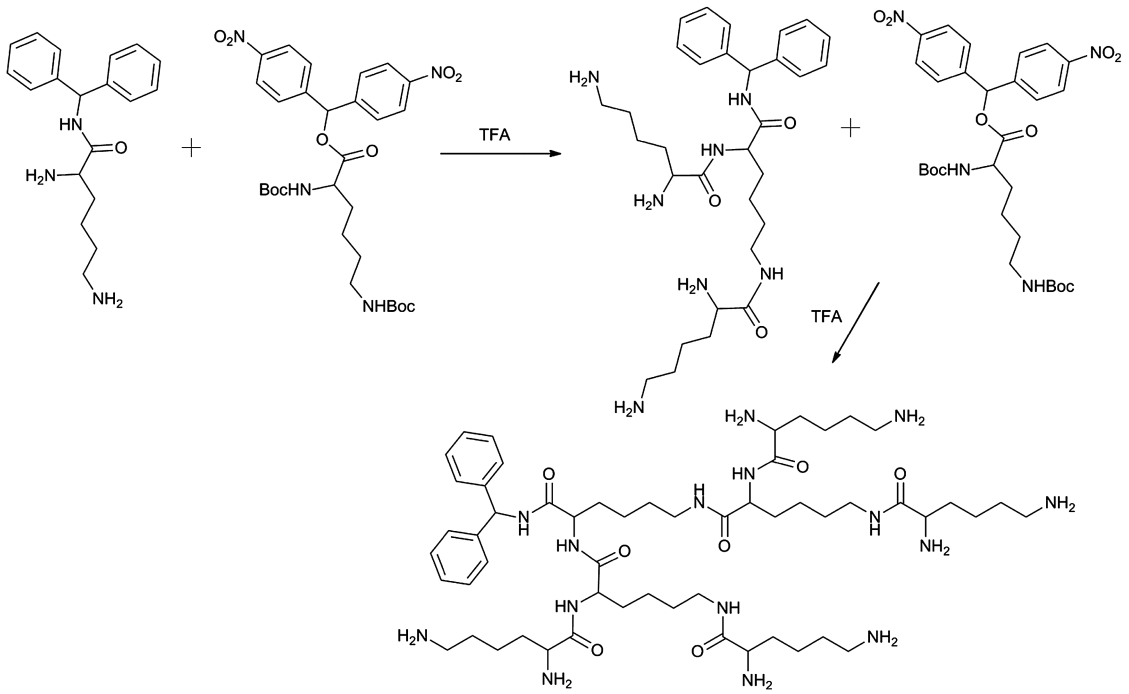


Fig. 26 Synthesis of PLL based dendrimers.

was suggested by Denkewalter *et al.* In his study a divergent route was described starting from a two-directional asymmetric core derived from L-lysine and benzhydrylamine.[367] The coupling between the core and a Boc-protected lysine derivative activated by a *p*-nitrophenylester was followed by acid deprotection (Fig. 26). This has resulted in the first generation dendron structures which could undergo additional coupling steps to afford higher generation structures. Thereafter, various PLL dendrimers have been prepared using several approaches.[139]

Lu *et al.* synthesized PLL dendrimers with a cubic octa-(3-aminopropyl)silsesquioxane core. These compounds exhibited significantly higher transfection efficiency.[140] Biodegradable PLL dendrimers were prepared by introducing lysine or succinimyldipropyldiamine (SPD) units.[137] PLL dendrimers Generation (G) 5 were constructed where one of the available amine groups in each lysine or SPD molecule in the outer surface layer was conjugated to the 1100 Da PEG chain and the other available amine was conjugated to Dox *via* a (hydrazinosulfonyl) benzoic acid linker. Arginine end groups were introduced onto second generation PLL dendrigraft by Sideratou *et al.* (Fig. 27). The primary amino groups of second generation PLL dendrigraft reacted with 1*H*-pyrazole-1-carboxamidine hydrochloride and *N*,*N*-diisopropylethylamine, thereby producing the guanidinylated derivatives.[139] These arginine end-functionalized derivatives of second generation PLL dendrigraft spontaneously interacted electrostatically with insulin at pH 7.4 and room temperature with an efficiency ranging from 94 to 97%. The complexes showed a slow release of insulin of about 70% during 6 hours.

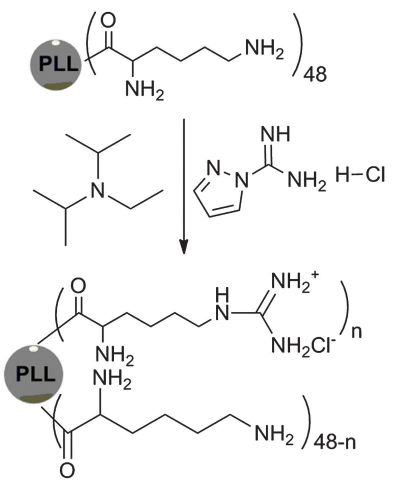


Fig. 27 PLL dendrigraft and derivatives.

2.2.3 Poly(amidoamine)s. Poly(amidoamine)s (PAAs) are a unique family of synthetic cationic polymers with many desirable properties including biodegradability, biocompatibility, water solubility and a lower toxicity compared to other cationic polymers. PAAs can be obtained by Michael-type polyaddition of primary amines or bis-secondary amines to bis-acrylamides. The developed polymer consists of *tert*-amino and amido groups regularly arranged along the main chain (Fig. 28).[141] This polymerization process occurs using a wide range of monomers leading to structural variations and enables efficient modification of their properties for predetermined applications. Moreover, the prepared PAAs are inherently highly functional polymers, with flexibility towards the easy incorporation of side chain substituents for special purposes. Linear PAA polymers are known to have good DNA binding characteristics and are efficient gene transfer agents.

Zintchenko *et al.* synthesized PAAs using "green", biocompatible catalysts including $CaCl_2$ showing superior activity over the salts of transition metals for Michael addition polymerization.[142]

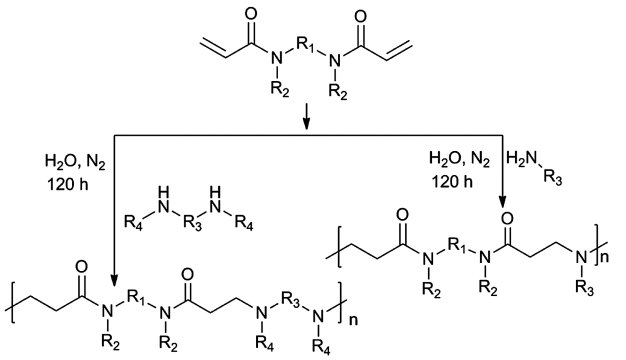


Fig. 28 Reaction pathway for the development of linear PAAs. R1, R2, R3 and R4 can be any alkyl residues in order to introduce carboxyl, amide, ester or ether groups.

Interestingly, a novel class of pH-sensitive PAAs with enhanced degradability were prepared by Jain *et al.* These PAAs were designed to remain stable at physiological pH but degrade more quickly into a series of small molecules in the pH range 5.0–6.0 which is the typical pH environment of lysosomes, endosomes or tumor tissue.

These developed polymers contain acetals or ketals along the main chain which can be degraded by acid catalyzed hydrolysis into low M_W compounds.[143] PAAs–Dox conjugates (*i.e.* ISA1Dox and ISA23-Dox) have been prepared from PAAs with amino pendant groups and Dox acylated with *cis*-aconitic anhydride as indicated in Fig. 29.[144] Employing Michael-type polyaddition between various oligoamine and disulfide-containing cystaminebisacrylamide (CBA), Lin *et al.* have developed a series of structurally well-defined linear bioreducible PAAs with multiple disulfide bonds in their main chain. These linear polymers exhibited a strong DNA condensation ability with high buffer capacities, a good transfection efficiency and a low cytotoxicity.[145] Frost *et al.* presented a series of cationic disulfide-PAAs (Fig. 30) which were synthesized *via* Michael polyaddition of the primary amine monomers 4-amino-1-butanol, α-amino-omega-carboxy-poly(ethylene glycol) hydrochloride (cPEG–NH_2) and CBA. cPEG–NH_2 side chains were included to induce a stealth effect.

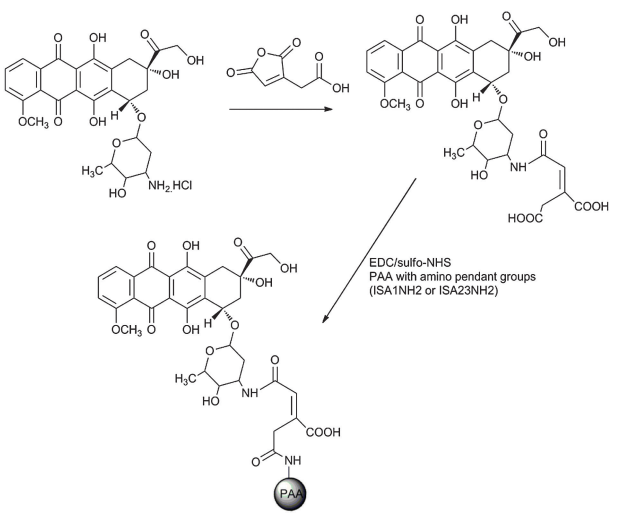


Fig. 29 Synthesis of ISA23Dox or ISA1Dox conjugates.

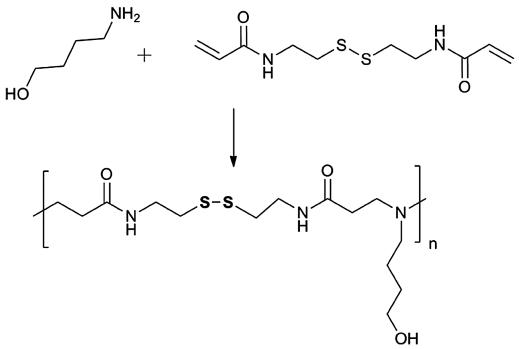


Fig. 30 Chemical structures of commonly applied SS-PAAs.

Due to the cleavage of multiple disulfide linkages in the main chain of the polymer, the stealth effect is disabled when the drug carrier reaches a reductive environment.[146]

2.2.4 Poly(amino-*co*-ester). Poly(amino-*co*-ester)s (PAEs) are a class of synthetic and hydrolytically degradable polyamines originally developed and investigated extensively as cationic polymers for DNA delivery. Langer *et al.* first synthesized PAE by the conjugation of a primary amine or a bis(secondary amine) monomer with a diacrylate ester monomer (Fig. 31). The M_W and end-chain functionality of the resulting polymer could be controlled by changing the monomer ratios, the reaction time and the temperature. The functional groups at the chain end containing amine-terminated polymers enhanced cellular uptake and DNA delivery.[147]

In general, PAE completely degrades into its monomeric constituent units within 5 hours and exhibits no negative effects on cell viability.[148] A library of these PAEs was developed and showed transfection efficiencies comparable to PEI, PLL and Lipofectamine 2000 in both COS-7 cell lines and HUVECs.[149] A series of high M_W PAEs have also been prepared *via* copolymerization of diesters with amino-substituted diols in the presence of *Candida antarctica lipase B* (CALB) as a catalyst.[150,151] Moreover, the PAE copolymers have also been enzymatically synthesized using various diesters and amino-substituted diols as comonomers in diphenyl ether solution in the presence of Novozym 435 as a catalyst (Fig. 32).

The authors further extended the study by including hydrophobic repeating units. They applied a novel method for the synthesis of terpolymers from lactone, diethyl sebacate and *N*-methyldiethanolamine using CALB as catalyst (Fig. 33).[4] Brey *et al.* introduced a triacrylate function during the synthesis

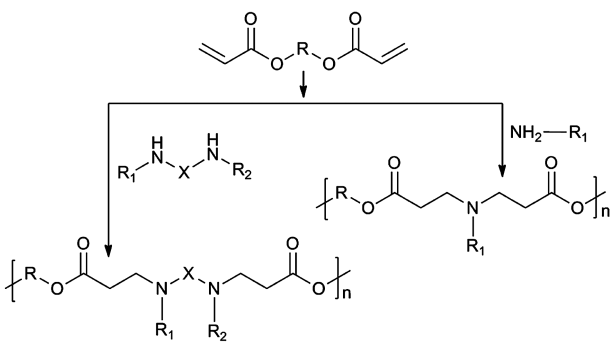


Fig. 31 Reaction pathway for the development of PAE.

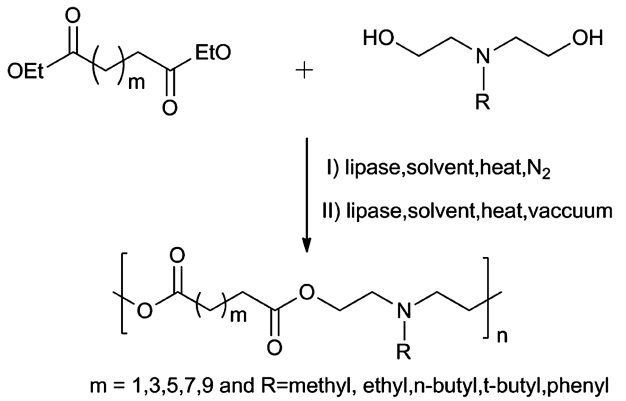


Fig. 32 Enzymatic copolymerization of diesters with amino-substituted diols.

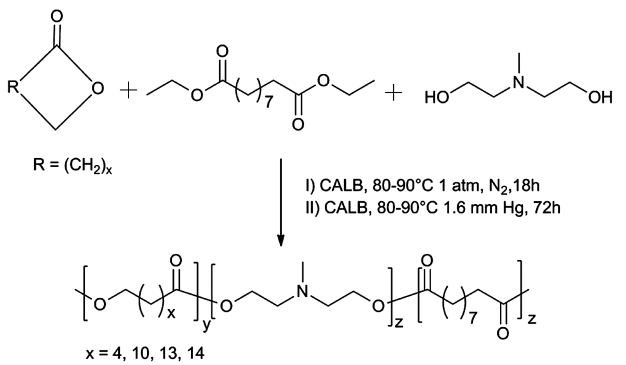


Fig. 33 Terpolymerization of lactone with diethyl sebacate and *N*-methyldiethanolamine.

of PAEs to evaluate the importance of macromer branching.[152] PAEs with different end groups were synthesized by conjugating different amine monomers to diacrylates in different molar ratios.[153]

In an effort to develop degradable polycations for gene delivery, the synthesis of novel PAEs has also been performed using the primary amine monomer 2-(pyridyldithio)-ethylamine (PDA).[154] The resulting polymers contained pyridyldithio moieties in the side chains displaying fast and selective reactivity towards thiols, without any alteration in the charge density of the polymer backbone. The synthesis of three PDA-based PAEs was performed by direct mixing of equimolar monomer concentrations of PDA and diacrylates. Another interesting approach to improve the biodegradability was the evaluation of copolymers of PAE–PEG block copolymers. In a recent study, Ko *et al.* prepared pH-responsive methyl ether PEG–PAE block copolymers to be applied as antitumor agents. The pH-responsive block copolymer was prepared by a Michael-type step polymerization of monoacrylated PEG, hexane-1, 6-diol, diacrylate (HDD) and 4,4′-trimethylene-dipiperidine (TDP). The monoacrylated PEG acted as a hydrophilic moiety while HDD/TDP functioned as the hydrophobic/pH-responsive moiety,[155] in order to decrease the cytotoxicity of low M_W PEI 25 kDa and the transfection efficiency of PAEs with DNA.[156]

2.2.5 Poly(2-*N*,*N*-dimethylaminoethylmethacrylate). One of the most important pH responsive polymers studied to date

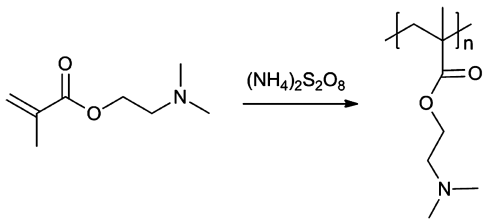


Fig. 34 Synthesis of PDMAEMA.

is PDMAEMA, which is a water-soluble cationic polymer. The tertiary amine groups of PDMAEMA are partially protonated in a physiological solution owing to the average pK_a of the amine groups at 7.5. Due to the inherent cationic charge, the polymer has potential as a gene transfer agent.[157] Synthesis of PDMAEMA occurs *via* free radical polymerization of 2-(*N*,*N*-dimethylamino)ethyl methacrylate (DMAEMA) initiated by ammonium peroxydisulphate (Fig. 34).[158] Evaluation of PDMAEMA based polyplexes indicated a combination of transfection efficiencies and acceptable cytotoxicity.[159] Since the polymer destabilizes endosomes and dissociates easily from the DNA once delivered into the cytosol, PDMAEMA polyplexes have been successful with respect to *in vitro* transfection efficiency.[160] Moreover, various modifications of the polymer structure have already been investigated in an attempt to further improve transfection efficiency.

Preliminary evaluations indicated the highest transfection efficiency with acceptable cytotoxicity for PDMAEMA/DNA ratios of 6/1 (w/w) for polymers possessing M_Ws higher than 300 kDa. The successful *in vitro* transfection efficiency of PDMAEMA polyplexes was attributed to the ability of the polymer both to destabilize endosomes as well as to dissociate easily from the plasmid once delivered into the cytosol.

A series of PDMAEMA chains with different M_Ws were synthesized using ATRP (Fig. 35). Starting from a rhodamine B-based ATRP initiator, PDMAEMA chains were end-capped with a fluorophore label to facilitate real-time cell imaging.[161] Plamper *et al.* described the synthesis of well-defined PDMAEMA star-shaped polymers with up to 24 arms using ATRP in the presence of multifunctional initiators. Oligofunctional initiators were used with 2-bromoisobutyryl initiating fragments, copper bromide (CuBr) as a catalyst and hexamethyltriethylenetetramine as a strong ligand in anisole as solvent.[162]

Oupický *et al.* recently reported an elegant approach to prepare reducible PDMAEMA polymers.[163] Reversible Addition–Fragmentation Chain Transfer (RAFT) polymerization of DMAEMA using a bifunctional chain transfer agent, 1,4-bis(2-(thiobenzoylthio)prop-2-yl)benzene (BTBP), yielded α,ω-dithioester functionalized PDMAEMA with a tailored M_W (3900–5500 Da) and a low polydispersity (1.06–1.09). The aminolysis of α,ω-dithioester PDMAEMA resulted in α,ω-dithiol-functionalized PDMAEMA, which, upon oxidative polycondensation, yielded a reducible PDMAEMA with a nearly 10-fold increase in M_W and with multiple disulfide bonds present in the polymer backbone (Fig. 36).

Various modifications to the PDMAEMA structure have also been investigated in an attempt to further improve transfection efficiency. Dubruel *et al.* varied the ratio of ammonium groups of PDMAEMA to pyridine, imidazole

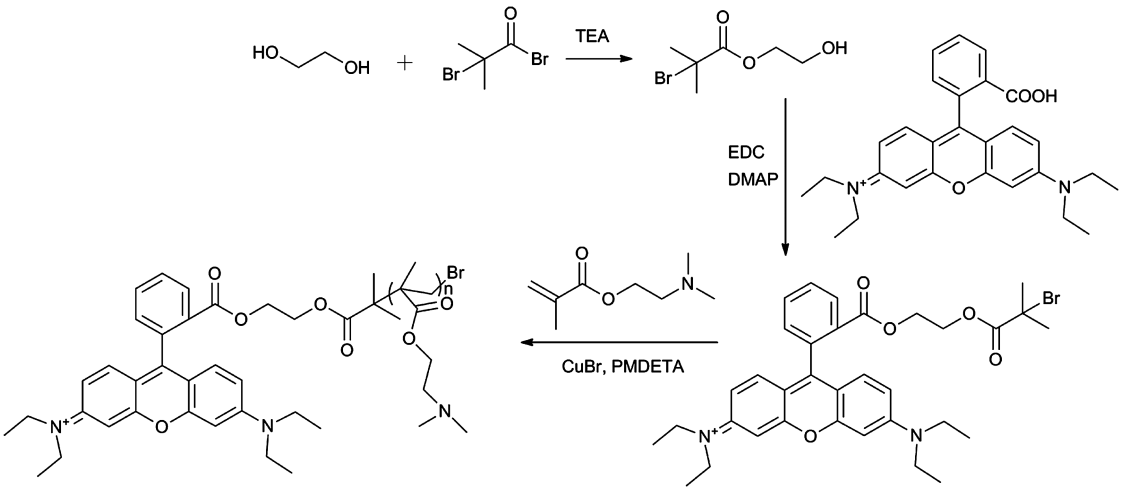


Fig. 35 Preparation of rhodamine B end-labeled PDMAEMA.

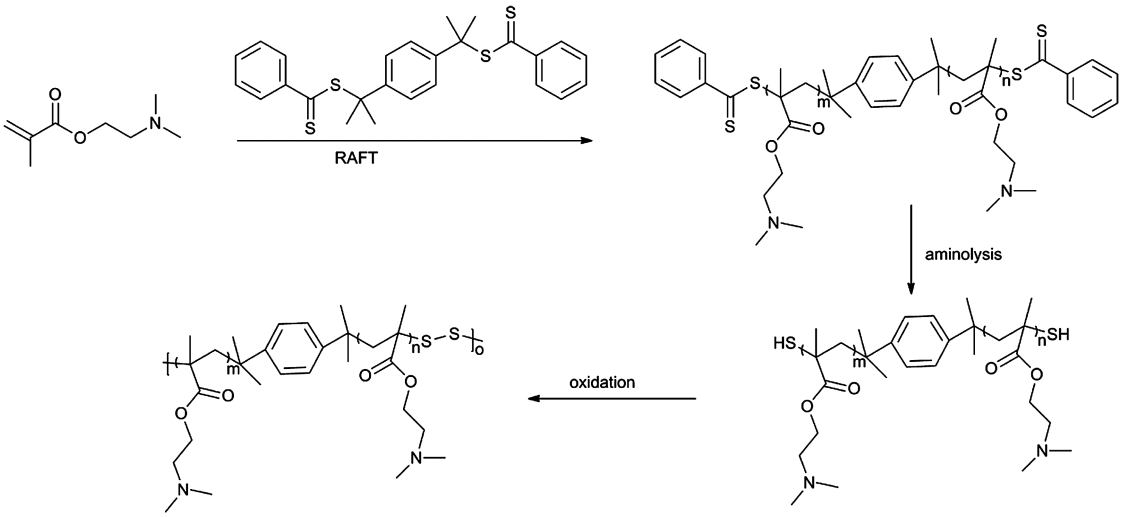


Fig. 36 Synthesis of reducible PDMAEMA.

and carboxylic acid functionalities with the final aim to improve endosomal escape. In contrast to what was anticipated, copolymerization of DMAEMA with pyridine containing monomers significantly reduced transfection efficiency, while imidazole and carboxylic acid derivatization completely eliminated transfection, further illustrating the imperfection of the "proton sponge" hypothesis.[164] The grafting of PEG chains onto PDMAEMA-based polymers inhibited the formation of aggregates.[165] Qiao *et al.* synthesized PEGylated PDMAEMA using ATRP. Interestingly, PEGylated PDMAEMA induced cytokine production by murine macrophages and showed its efficiency as a DNA vaccine vector enhancing adaptive immune responses by activating innate immunity.[166] Guo *et al.* synthesized methoxy-PEG-*block*-poly ε-caprolactone (PCL)-*g*-PDMAEMA by combining ROP and ATRP. Methoxy-PEG-*block*-(PCL-*g*-(bromo 2-methyl propionate-ε-caprolactone) is used as the macroinitiator which was prepared by ROP of caprolactone using methoxy PEG as the initiator and $Sn(Oct)_2$ as the catalyst (Fig. 37).[167]

pH and temperature responsive copolymers of PDMAEMA have also been prepared by grafting chitosan *via* homogeneous ATRP.[168] Chitosan was prepared as a macro-initiator by phthaloylation of the amino groups of chitosan and subsequent acylation of its hydroxyl groups with 2-bromoisobutyryl bromide (Fig. 38). The introduction of phthaloyl moieties reduced the inter and intra molecular hydrogen bond formation enabling dissolution of chitosan in organic solvents including dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF).

Recently, in an attempt to impart biodegradability to PDMAEMA, Zhang *et al.* introduced ester linkages in a PDMAEMA derivative. Free radical polymerization of the cyclic ketene acetal, 5,6-benzo-2-methylene-1,3-dioxepane and DMAEMA was performed using PEO as a macro-azo-initiator to increase the hydrophilicity of the polymers developed (Fig. 39). The resulting polymer showed a combination of low cytotoxicity and an efficient DNA transfection ability.[169]

3. Bioactive properties of cationic polymeric systems

Bioactive properties represent important parameters for the design and development of cationic polymeric systems and are

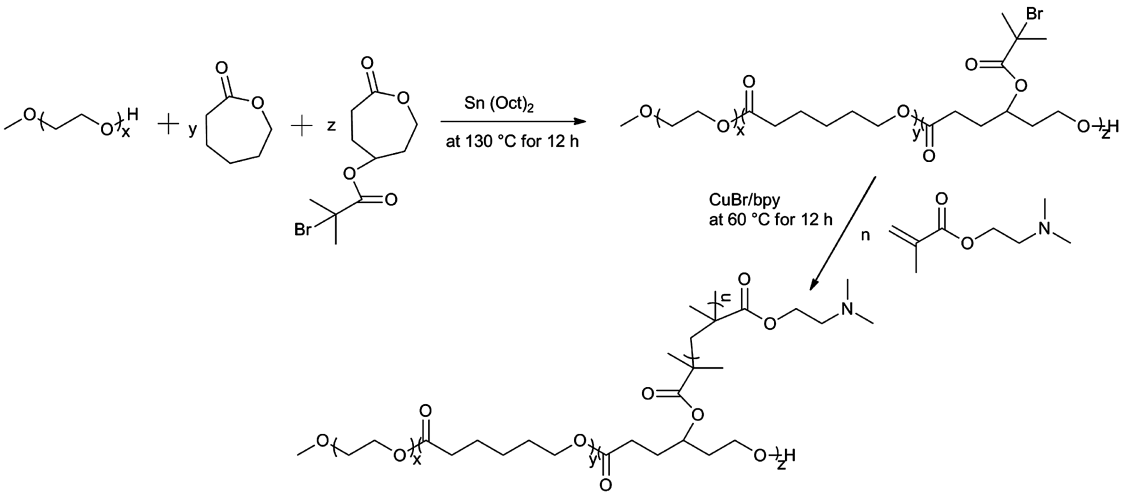


Fig. 37 Synthesis route of methoxy-PEG-*b*-PCL-*g*-PDMAEMA.

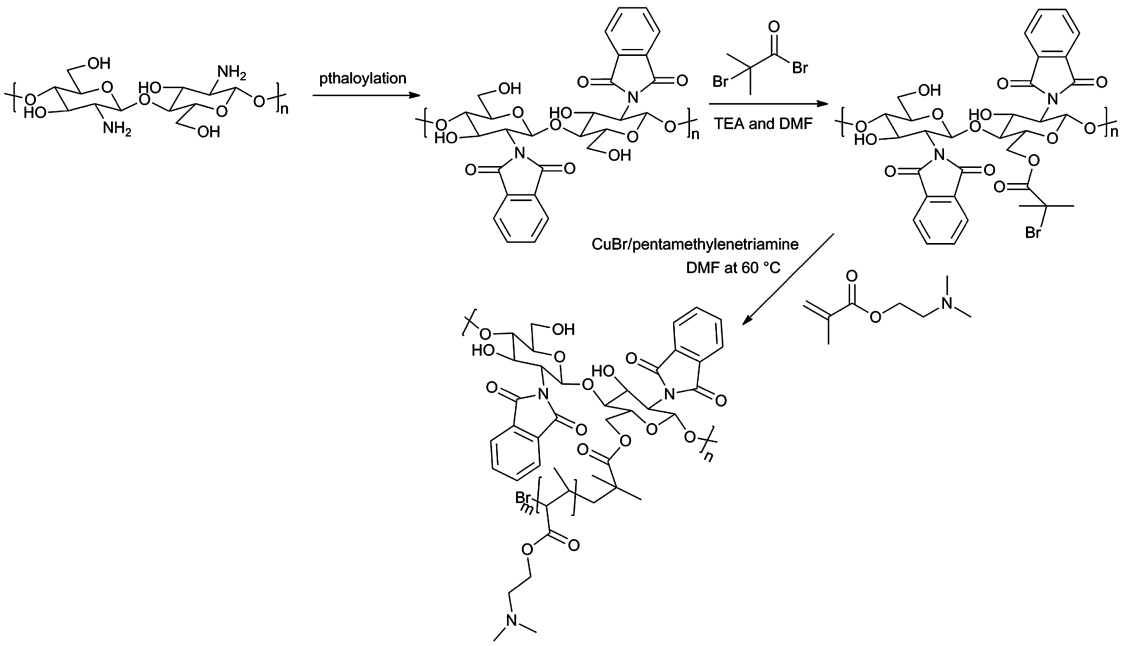


Fig. 38 Synthesis of chitosan-*g*-PDMAEMA copolymer *via* ATRP.

critical for their therapeutic applications. Some cationic polymers exhibit inherent bioactive properties while others have to be modified to achieve desired bioactive properties upon conjugation with bioactive molecules. To highlight the bioactive properties of cationic polymeric systems emphasis is placed on five important properties: (I) stimuli-responsive, (II) antimicrobial, (III) antioxidant, (IV) antitumor and (V) anti-inflammatory properties.

3.1 Stimuli-responsive cationic polymers

Advanced therapeutic research demands controlled intelligent systems for various therapeutic applications including controlled delivery, nucleic acid separation, controlled cell patterning and many others. Responsive cationic polymers are beneficial in this regard since the active agents can be delivered by a system that senses the signal caused by diseases, detects the magnitude of a signal and then releases the required amount of the therapeutic agent. Cationic polymeric systems with various chemical and structural responsive moieties exhibit the property of responsiveness to external stimuli such as temperature, pH, ionic concentration, light, magnetic field, electric field and chemicals. Polymers with multiple responsive properties have also been developed which elegantly combine two or more stimuli responsive mechanisms. In this section of the review the development of cationic polymers for several therapeutic applications will be discussed in detail.

3.1.1 Temperature responsive cationic polymers. Temperature responsive cationic polymers change their structural properties in response to temperature (Fig. 40) and hold potential for therapeutic applications. Temperature responsiveness can be achieved by incorporating or grafting temperature

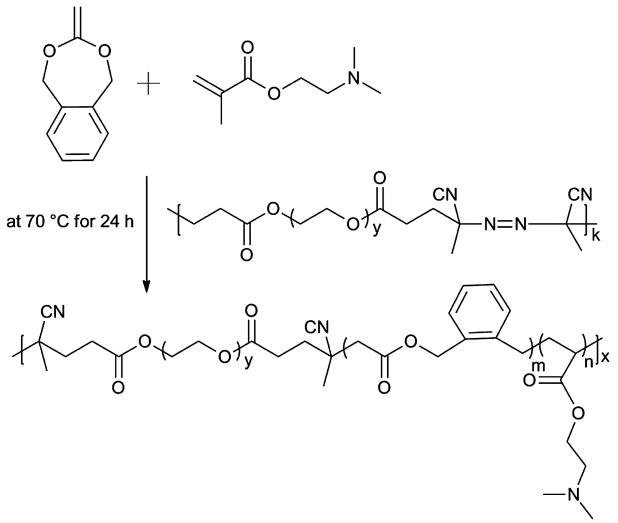


Fig. 39 Synthesis of poly(PEG-*co*-(BMDO-*co*-DMAEMA)) *via* free radical polymerization.

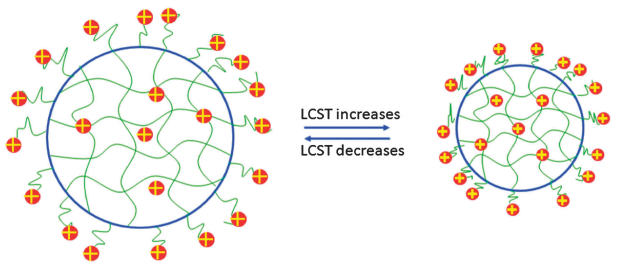


Fig. 40 Representation of a temperature responsive cationic polymer at different LCST.

responsive moieties such as pluronic F-127 or poly(*N*-isopropylacrylamide) (PNIPAM). Although thermo-sensitive cationic polymers are concomitant with several advantages there are still concerns regarding their toxicity, immunogenicity, and circulation time upon administration. In some cases the attachment of PEG can help to overcome these difficulties.

Lee *et al*. prepared temperature-sensitive PEI-pluronic nanocapsules by an interfacial crosslinking reaction between preactivated Pluronic F-127 and low M_W PEI using oil-in-water interface during a modified emulsification/solvent evaporation process. Green fluorescent protein (GFP) or vascular endothelial growth factor (VEGF) siRNA was conjugated to PEG *via* a disulfide linkage to form nanoscale complexes with PEI-pluronic nanocapsules. A brief cold shock to the transfected cells led to the rapid volume expansion of the nanocapsules which could burst out an endosome compartment, enabling the siRNA cargo to deliver into the cytosol region in a controlled manner, and subsequently silence a target mRNA.[216] The introduction of isobutyramide groups attached to the side chains of hyperbranched PEI has also shown to impart the cationic polymer with thermo-responsive properties. The synthesis of such hyperbranched polymers with isobutyric amide end groups was realized by an amidation reaction of PEI with isobutyryl chloride (Fig. 41).[217]

Zhao *et al*. synthesized long-circulating thermoresponsive cationic dendritic delivery systems by incorporating PNIPAM and biocompatible PEG onto PAA. This system improved drug-loading capability and also enabled prolonged drug release *via* manipulating the environment temperature above its low critical solution temperature (LCST). These thermosensitive PAA derivatives could control drug release simply by adjusting the temperature above or below the LCST (Fig. 42).[218]

3.1.2 pH responsive cationic polymers. The presence of ionizable functional groups on a cationic polymer dramatically alters its structural properties at, above and below a specific pH called its pK_a (Fig. 43). This rapid change in the net charge of pendant or backbone groups with respect to pH causes an alteration of the hydrodynamic volume or conformation of the polymer chains.

The pH responsive nature of cationic polymers can be used for biomolecule delivery in neutral or alkaline environments. At a pH above the pK_a, the pendant amine groups remain non-ionized leaving the polymeric chain in a collapsed state, while entrapping the biomolecule. As the pH decreases below

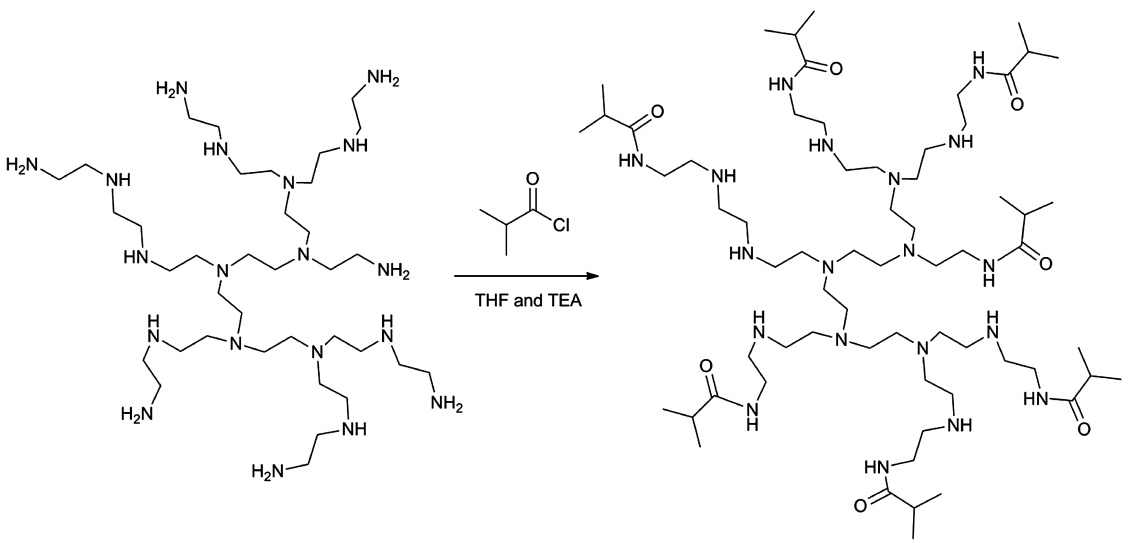


Fig. 41 Synthesis of hyper-branched PEI functionalized with isobutyric amide.

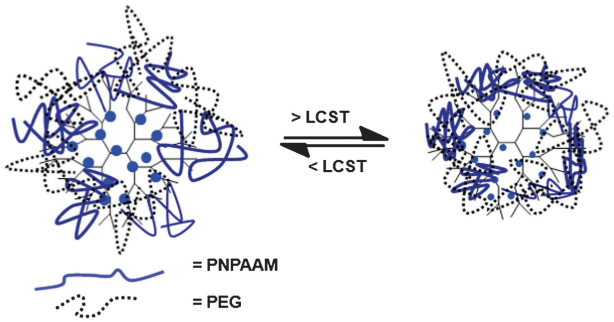


Fig. 42 Phase transition of PAA-*g*-PNIPAAm-*co*-PEG at LCST.

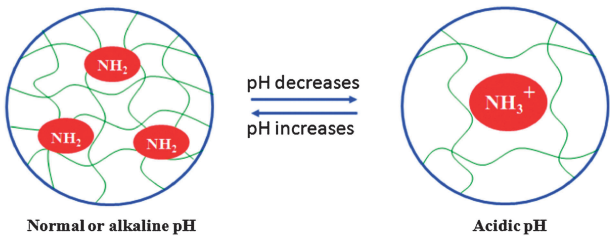
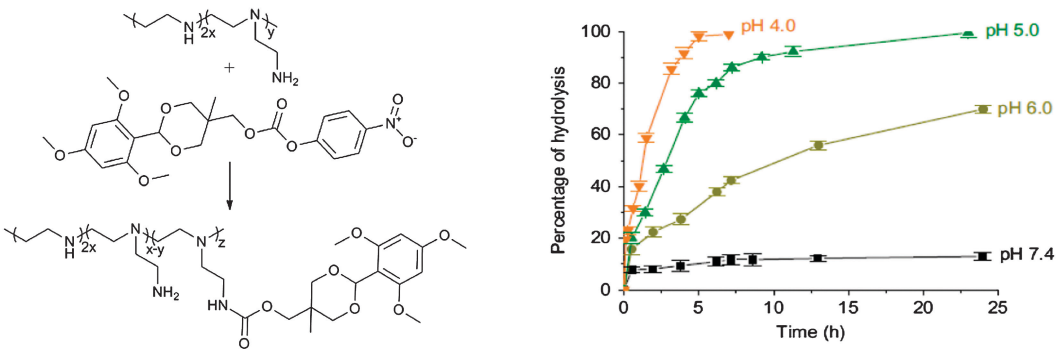


Fig. 43 Representation of a pH responsive cationic polymer.

the amine pK_a, the amine groups become protonated and the polymeric chain expands due to electrostatic repulsion, in this way releasing the entrapped biomolecules into the surrounding medium (Fig. 43). Cationic polymers like chitosan, PEI, PDMAEMA, PAA have basic functional groups such as primary, secondary, and tertiary amine groups that become ionized as the pH decreases. Jain *et al.* investigated the pH responsiveness of PAA after the incorporation of acetal or ketal linkages into the backbone. Acetal and ketal linkages degraded the polymer into low M_W hydrophilic compounds upon lowering of the pH. The polymers demonstrated a pH-dependent degradation profile with a significant increase in the hydrolysis rate as the pH was lowered from 7.4 to 5.0, which is the pH commonly found in lysosomes.[143] Park *et al.* prepared pH-stimuli-responsive near-infrared optical imaging nanoprobes composed of a biodegradable polymer poly(γ-glutamic acid) (γ-PGA)/(PAE).[219] The quenching property of indocyanine green at high concentrations, combined with a pH responsive PAE particle was evaluated. In an acidic environment the PAE particles disassembled and released the indocyanine green which showed enhanced fluorescence intensity that was nearly 4.5 times higher than that in a pH 7.4 buffer.

In a recent study pyranine-3 (HPTS, 8-hydroxypyrene-1,3,6-trisulfonic acid), a pH sensitive probe molecule, was ionically combined with a positively charged PLL to form confined aggregates that further assembled into silica nanoparticles to construct a pH sensitive microcapsule structure. HPTS is a highly water-soluble dye with low toxicity and has been extensively used as a multianalysis indicator for optical sensors for carbon dioxide, oxygen, ammonia and pH.[220] A gradual increase in deprotonation of the ammonium groups in PLL reduced the ionic interaction between the PLL and HPTS, which led to the release of HPTS molecules from the microcapsules. The amount of HPTS released was found to gradually increase with increasing pH of the medium from 8.5 to 10. PEI derivatives containing various degrees of substitution with 2,4,6-trimethoxybenzylidenetris (hydroxymethyl)ethane possessed pH sensitive behavior (Fig. 44A). The developed derivative showed successful DNA condensation and high transfection efficiency in HeLa, 293T, HepG2 and KB cells using plasmid pGL3 expressing luciferase as the reporter gene. The results also showed that the hydrolysis rate of the acetals of PEI-polyplexes was highly pH dependent (Fig. 44B).[221] In another study, the impact of pH variation on DNA–PEI complex formation, in aqueous solution and at constant ionic strength, was studied.[182] This study showed that DNA changes the overall conformation at pH 4 and indicates that complexes prepared at these lower values of pH are polydispersed and strongly bound whereas the complexes prepared at pH 8 show weaker binding between DNA and PEI with smaller aggregates, and possibly a more uniform population.

A microfluidics technique was used by Wei *et al.* to prepare cationic pH responsive microcapsules of PDMAEMA.[222] The cationic microcapsules obtained exhibited pH-sensitivity and the preparation conditions significantly affected the pH-responsive swelling of these microcapsules. Creusat *et al.* conjugated PEI to tyrosine which showed pH sensitivity along with excellent transfection efficiency for siRNA. The tyrosine–PEI conjugate allowed both endosomal rupture and siRNA liberation *via* pH-dependent dissolution of the PEI–tyrosine self-aggregates. The PEI based polyplexes were sensitive at



(A) (B)

Fig. 44 (A) Structure of PEI-*g*-trimethoxybenzylidenetris(hydroxymethyl)ethane [PEI-*g*-(TMB-THME)]and (B) pH-dependent hydrolysis of acetals in PEI-*g*-(TMB-THME)$_9$–DNA complexes (N/P ratio = 10/1). Adapted with permission from ref. 221. © 2011 Elsevier Limited.

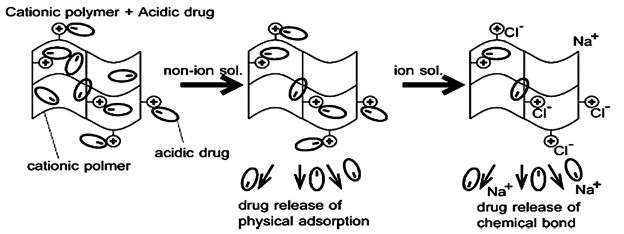


Fig. 45 The mechanism of ion-mediated drug release from ionic polymers at different ionic strengths. Adapted with permission from ref. 224. © 2002 Elsevier Science Limited.

pH 6.0, the pH value that is found in PEI-buffered endosomes enabling intra-cellular disassembly. This property arises from the PEI proton sponge ability and favors siRNA release from polyplexes when PEI exerts an osmotic pressure increase on endosomal membranes.[223]

3.1.3 Ionic-responsive cationic polymers. In many biological processes ions play a crucial role. Thus, the utilization of ionic sensitive cationic polymers could dramatically improve their therapeutic potential. Ionic-responsiveness of cationic polymers refers to their property of undergoing relatively large and abrupt physical or chemical changes in response to small external changes in the concentration of ions. The ionic concentration of solvents holds a key role in the interactions between polymeric chains and the solvents, and the final molecular conformation. Sutani *et al*. demonstrated that the copolymer of PDMAEMA-*co*-acrylic acid forms a stable ionic complex with ionic drugs metanil yellow and exhibits ionic sensitive drug release properties. Upon exposing this co-polymer to an isotonic sodium chloride solution the drug was released from the polymer and exhibited a constant drug release profile different from the release behavior in aqueous medium (Fig. 45).[224]

The amine moieties of the PDMAEMA chains are partly protonated in aqueous solution and the electrostatic repulsion among the repeating units of PDMAEMA chains leads to a more extensive conformation due to enhanced chain mobility. With the increase in ionic strength by the addition of NaCl, the repulsion is shielded and a more coiled conformation is assumed. These phenomena are clearly observed by ^{1}H NMR, where the peak intensity associated with PDMAEMA chains diminished remarkably with the increase of ionic strength compared to aqueous solution.[225] Cationic PDMAEMA-*g*-PEG hydrogel nanoparticles also possess ion-sensitive properties. The size of the cationic nanoparticles decreased with increasing ionic strength due to the decrease of the osmotic pressure with the polymeric networks. Further increase of the ionic strength led to the breaking of the hydrogen bonds of PEG and made the cationic hydrogel nanoparticles aggregate and thus larger in size (Fig. 46).[226]

3.1.4 Multiresponsive cationic polymers. Multiresponsive cationic polymers offer responsiveness to two or more external stimuli. This enables manipulation of polymeric systems to achieve better targeting and efficacy in complicated micro-environments or for other functions. Dual sensitivity was reported for a novel triblock copolymer consisting of PAA and PEG which was converted into an injectable pH and temperature-sensitive hydrogel.[204] PAAs with terminal vinyl

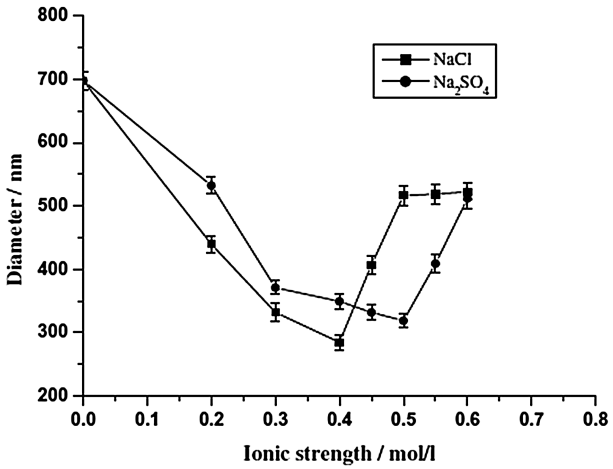


Fig. 46 Effect of ionic strength on swelling behavior of cationic (PDMAEMA-*g*-PEG) nanoparticles. Adapted with permission from ref. 226. © 2008 Elsevier Limited.

groups were first synthesized and then end-capped by 1-adamantylamine (ADA) (Fig. 47), which resulted in a hyper-branched polymer exhibiting both thermo and pH dependent properties.[227]

A series of novel temperature and pH responsive block copolymers composed of PLL and PNIPAM were synthesized using ROP.[228] The diblock copolymer was synthesized by the ROP of ε-(benzyloxycarbonyl)-L-lysine *N*-carboxyanhydride initiated by amine-terminated PNIPAM followed by an acidic deprotection step (Fig. 48). These PNIPAM-*b*-PLL copolymers self-assembled into micelle-like aggregates with PNIPAM as the hydrophobic block at acidic pH and high temperatures and with PLL as the hydrophobic block at alkaline pH and low temperatures.

Guo *et al*. prepared a cationic PDMAEMA-*g*-PCL copolymer exhibiting dual responsiveness to pH and temperature. The graft polymer was designed and prepared by ROP and ATRP methods using PCL-*co*-*g*-(2-bromo-2-methylpropionate)-3-caprolactone) as the macroinitiator. Nanoparticles prepared from this copolymer were able to simultaneously entrap hydrophobic paclitaxel and hydrophilic (negatively charged) DNA. The particles released paclitaxel faster in the acidic environment and showed excellent gene transfection efficiencies both in serum-free and serum-containing culture media.[211] Shen *et al*. prepared the first PAE dendrimers which showed both pH and temperature responsiveness. Using the thermo-responsiveness of the dendrimer, they loaded Dox by a solvent-free temperature-controlled method. The drug was released very slowly in a controlled way at 37 °C and physiological pH, but a faster release was observed under acidic conditions.[229] A triblock polymer of PAA and PEG was developed with pH and temperature responsiveness which exhibited bioadhesive properties when subcutaneously injected in rats.[204]

A pH and enzyme-responsive complex composed by self-assembly of Dox, CpG DNA fragments, cationic gelatin and a pH-sensitive alginate was developed by Dong *et al*. (Fig. 49).[230] The complexes of DNA with cationic gelatin notably enhanced the liver accumulation of Dox and caused serious hepatotoxicity which weakened the drug efficacy. To avoid

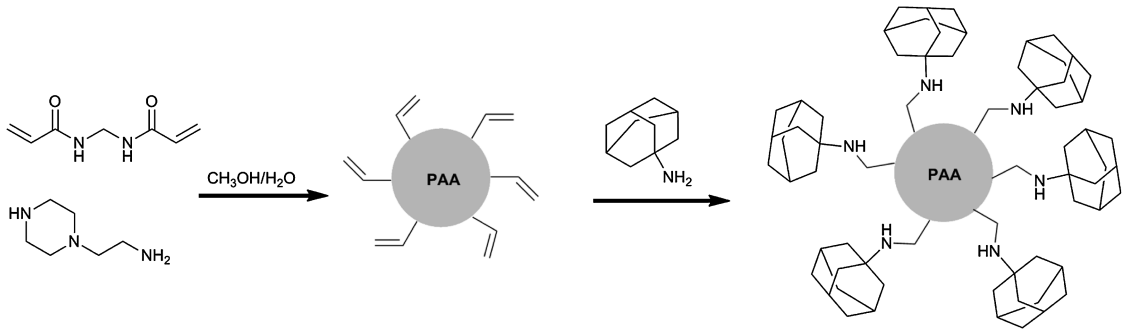


Fig. 47 The schematic procedure to synthesize hyper-branched PAA–ADA.

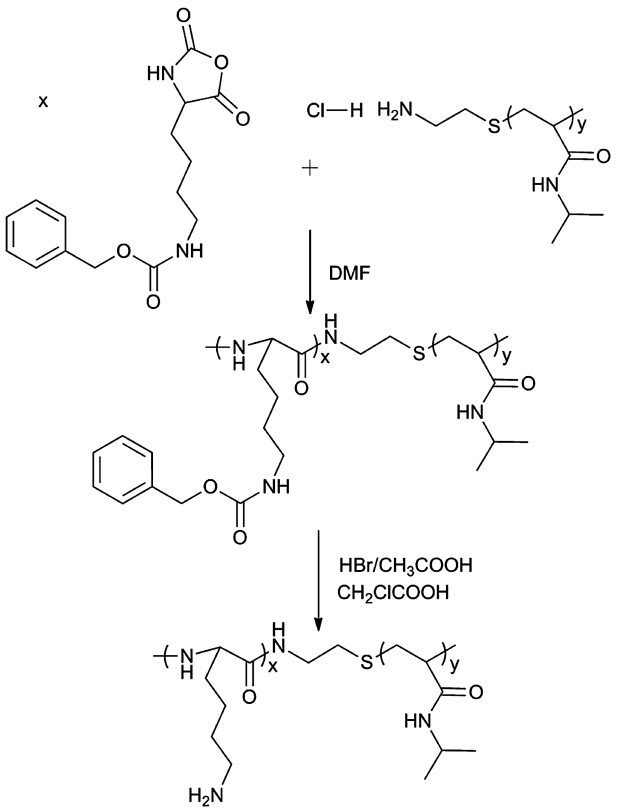


Fig. 48 Synthesis routes of PNIPAM-*b*-PLL.

this, pH-responsive PEGylated alginate was introduced into the system. Because of the PEGylation, there was an enhanced permeability and retention (EPR) effect that increased the distribution of the drug in the tumour and reduced liver accumulation.

3.2 Antimicrobial properties of cationic polymers

Infections by microbial strains are of great concern in many therapeutic devices, drugs, hygienic health care products. These infections can be the cause of some common or mild superficial infections such as candidiasis or thrush as well as lead to serious life-threatening diseases such as invasive aspergillosis. Statistics indicate that infectious diseases kill more people worldwide than any other single cause. The use of cationic polymers as potential antimicrobial systems helps to mitigate, combat or eradicate microbial causes for infections (Fig. 50). Cationic polymers are extensively and efficiently

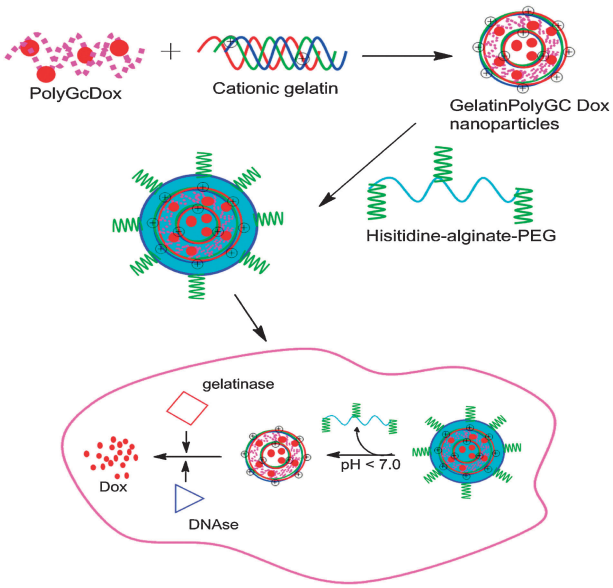


Fig. 49 Representation of the assembly and cellular pathway of cationic gelatin–DNA–Dox complexes.

employed in antimicrobial applications, which are gaining increased interest from both scientific and industrial communities.

Chitosan is one of the most extensively studied cationic polymers as an antimicrobial agent. Chitosan possesses inherent antimicrobial activity against many Gram-positive and Gram-negative bacteria and fungi at pH < 6.[231] Although the exact mechanism of the antibacterial activity of chitosan is not yet fully understood, several mechanisms have been suggested. One of them occurs *via* changes in the bacterial membrane permeability, breakdown of the cytoplasmic membrane barrier or the blockage of nutrient transport, resulting in cell lysis. Generally, the mechanisms of the inhibitory activity of chitosan may vary depending on M_W, DD, type of bacterium, the pH and the concentration of active compounds within chitosan.[232] *Escherichia coli* and *Staphylococcus aureus* are the most commonly employed bacteria for antibacterial studies and the minimal inhibitory concentrations (MIC) is the most commonly selected parameter to evaluate the activity of antimicrobial agents. Xiao *et al.* studied the antibacterial properties of chitosan with *N*-arginine substituents. They observed that chitosan with varying amounts of *N*-arginine substituents showed antibacterial activity in a concentration dependent manner. At a concentration

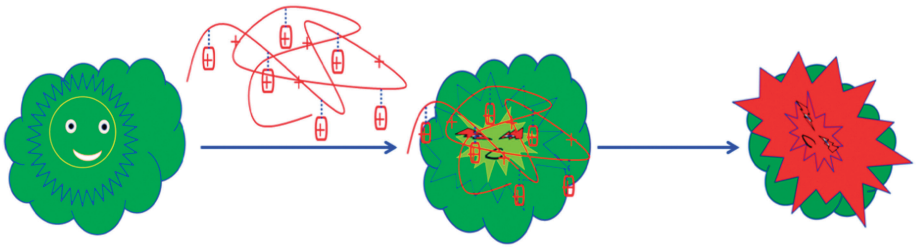


Fig. 50 Schematic representation of antimicrobial properties of cationic polymers.

higher than 150 ppm samples exhibited antibacterial activity whereas lower than 50 ppm, they were digested by microbes and absorbed as nutrients to promote the growth of microorganisms. The cationic derivative of chitosan, *N*-substituted chitosan, has been quaternized using CHPTA to react with the hydroxyl or primary amine groups of the glucosamine units in chitosan. The MIC showed higher values with higher extent of *N* substitution.[233]

Li *et al*. reported PEGylated quaternized chitosan derivatives as antimicrobial agents.[3] The chitosan derivatives were prepared by introducing a hydrophobic alkyl side chain and cationic charge through quaternization of the amino group along with the incorporation of hydrophilic PEG with six ethylene glycol repeats and a methacrylate functionality (Fig. 51). The hydrogels showed efficacy against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Fusarium solani* and the authors anticipated these hydrogels to have an 'anion sponge' like structure that interacted with regions of the anionic microbial membrane in the internal nanopores leading to microbial membrane disruption and subsequent microbial death.

Khalil *et al*. examined 10 families of antimicrobial agents alone and in combination with PEI, against a resistant clinical isolate of *Pseudomonas aeruginosa*. The attempt was aimed at minimizing the reduction in outer membrane permeability by co-treatment with permeabilizers such as PEI, thus allowing the increased uptake of antibiotics across the outer membrane.[234] The authors observed through this study that PEI at final concentration of 250 nM reduced the MICs of novobiocin, ceftazidime, cefotaxime, chloramphenicol, rifampicin, and norfloxacin by 1.5- to 40-fold. Quaternary ammonium PEI nanoparticles also inhibited *Staphylococcus aureus* growth at a concentration of 80 μg mL^{-1}.[235] The degree of alkylation and degree of methylation were the most influential factors affecting the antibacterial activity of the nanoparticles. Recently, a versatile living ROP based on metal-free organocatalysis was developed to synthesize cationic amphiphilic triblock polycarbonates that selectively killed various microbes while leaving mammalian red blood cells unaffected.[236] Cationic triblock polymers with three different compositions were designed and synthesized by the sequential ROP of 3-chloropropyl 5-methyl-2-oxo-1,3-dioxane-5-carboxylate followed by trimethylene carbonate initiated from a diol in the presence of a mixture of Lewis acid 1-(3,5-bis(trifluoromethyl)-phenyl)-3-cyclohexyl-2-thiourea and Lewis base 1,8-diazabicyclo-[5.4.0]undec-7-ene as the catalyst (Fig. 52). The polycarbonates

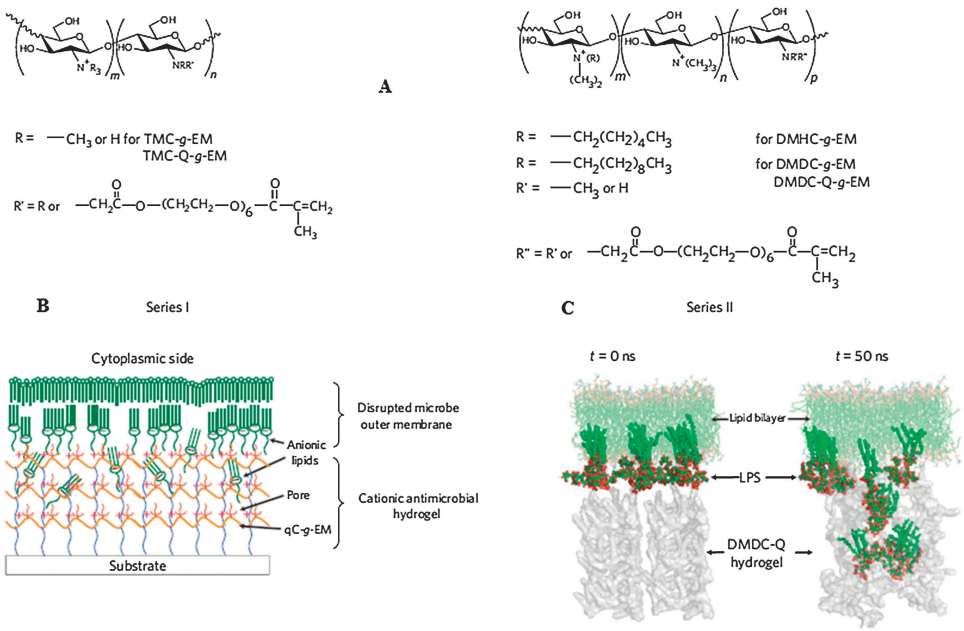


Fig. 51 (A) Structure of quaternized chitosan derivatives, (B) schematic diagram of the negatively charged bacterial membrane suctioned into the pores of cationic hydrogel and (C) computer simulation of the suctioning of the bacterial membrane into the cationic hydrogel at time 0 second and 50 nanoseconds. Adapted with permission from ref. 3. © 2011 Macmillan Publishers Limited.

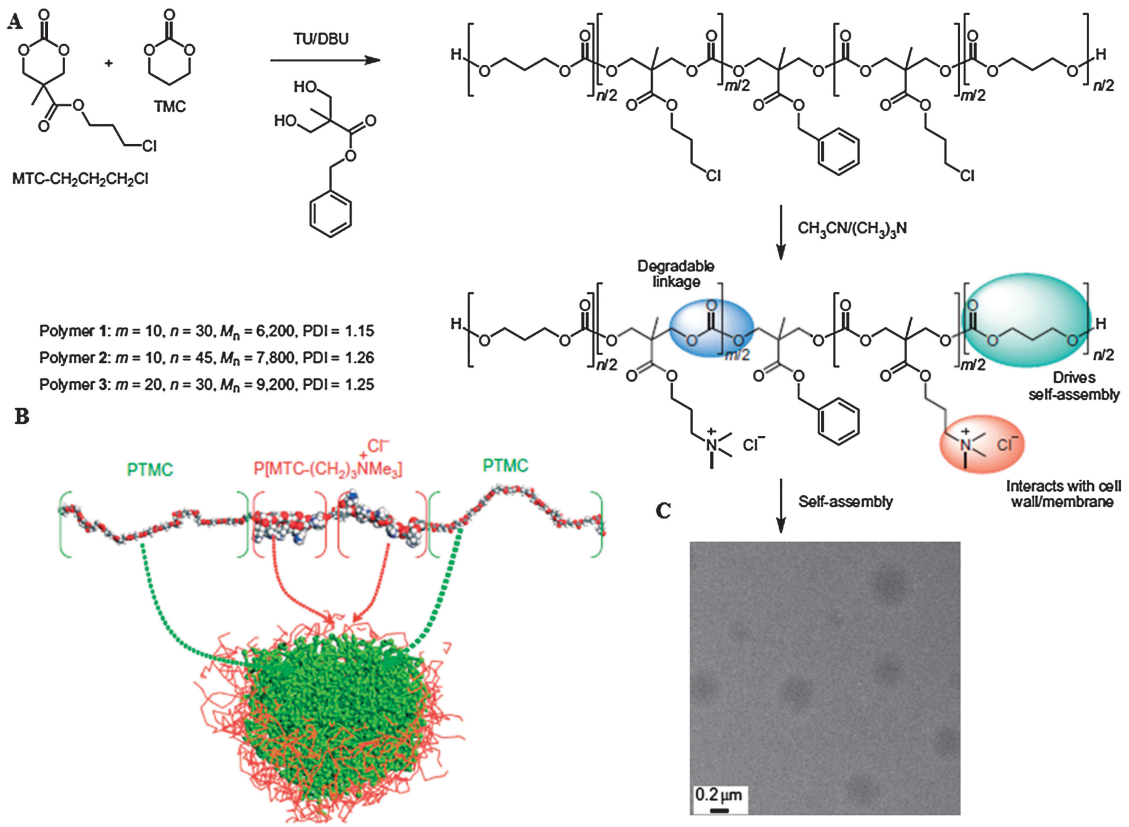


Fig. 52 Synthesis and micelle formation of cationic amphiphilic polycarbonates, (A) cationic amphiphilic polycarbonates (B) and (C) the formation of micelles as simulated by molecular modeling. Adapted with permission from ref. 236. © 2011 Macmillan Publishers Limited.

developed easily formed cationic micelles by simple polymer dissolution in water. The micelles formed could efficiently kill Gram-positive bacteria such as *Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus* and methicillin-resistant *S. aureus* and the fungus *Cryptococcus neoformans* without showing any significant haemolytic activity over a wide range of concentrations.

3.3 Antioxidant properties of cationic polymers

Antioxidants are compounds that inhibit or delay oxidation of cellular substrates. Their main role is to auto-protect the body against the damage, caused by reactive oxygen species (ROS) and degenerative diseases. The superoxide radical, hydrogen peroxide and hydroxyl radical metabolic byproducts are three major ROS that are generated continuously by the mitochondria in growing cells or are produced by exogenous factors such as sunlight, ultraviolet light and ionizing radiation. The roles of ROS are ambiguous, on the one hand, they prevent diseases by assisting the immune system in mediating cell signaling and playing an essential role in apoptosis. On the other hand, they are involved in a wide variety of pathologies, such as cancer, cardiovascular diseases, diabetes, and atherosclerosis.[237] These radicals are very unstable and react rapidly with many other substances in the body, leading to the cell or tissue injury. Oxidative stress can be generated by excess ROS, which are kept in check in a non-diseased state by endogenous cellular antioxidant mechanisms (Fig. 53).

The antioxidant activity of polymers has been ascribed to various causes such as the prevention of chain initiation, the binding of transition metal ion catalysts, the decomposition of peroxides, the reductive capacity and radical scavenging.[232] Naturally occurring cationic chitosan has shown appreciable antioxidant properties. The scavenging activity of chitosan is due to the strong hydrogen donating ability of chitosan. ROS can react with active hydrogen atoms in hydroxyl or amino groups of chitosan to form a stable macromolecular radical. Chitosan has high metal binding capacity due to the free amino groups. A low M_W and a higher concentration show a positive influence on the antioxidant activity of chitosan. Conversely, a higher M_W was found to be the most effective factor in reducing lipid oxidation. Feng *et al.* irradiated chitosan at 20 kGy, which exhibited high reductive capacity and expressed good inhibition of linoleic acid peroxidation.[238] They inferred that the active hydroxyl and amino groups are the cause of the scavenging ability of chitosan. Other factors that also contribute to the antioxidant properties of chitosan include M_W, DD and different substituting groups.

Liu *et al.* related the antioxidant properties of chitosan to the different forms of nitrogen atom containing functional groups such as primary amines, imines, secondary amines, and quaternary ammonium groups.[239] In a more detailed study of the relation between the antioxidant activity and the charge density of the cation in quaternized chitosan, they introduced more powerful electronegative groups and their reduction power and antioxidant activity against hydroxyl and hydrogen

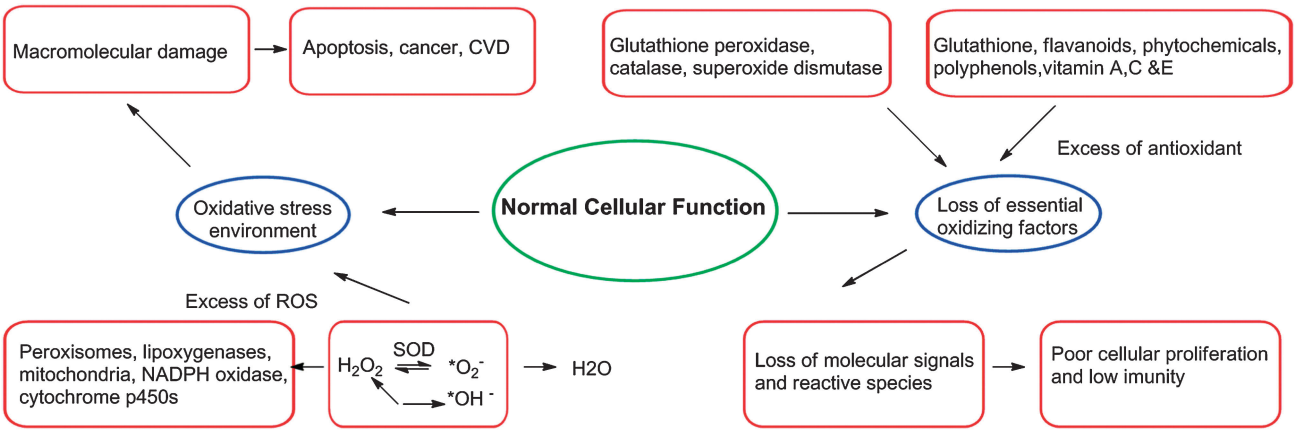


Fig. 53 Overview of cellular oxidative interactions.

peroxide were assessed *in vitro*. The results indicated that the antioxidant activities were influenced by the positive charges of nitrogen atoms in the quaternized chitosan. The charge density of the quaternized chitosan was strengthened by the substituted electronegative groups and they also exhibited much stronger hydroxyl and hydrogen peroxide scavenging activity than that of chitosan. In another study, PEI was linked to the dipeptide, L-carnosine, and its derivative, Boc-L-carnosine, which reduced the production of ROS and markedly increased transfection efficiency.[240] To synthesize PEI–peptide hybrid polymers, peptide moieties were linked *via* the primary amine groups of PEI. Modification of PEI–peptide conjugation reduced the oxidative damage within the cells, thereby resulting in higher cell survival and high transfection efficiency. The ROS levels were significantly lower in two primary cell types, adipose stromal cells and cardiac progenitor cells transfected with the hybrid polymers as compared to PEI.

3.4 Antitumor properties of cationic polymers

Physiological events regulating differentiation, apoptosis, proliferation and cell arrest modulate homeostasis and functionality of tissues. A disorder in these sequential events alters the ratio between cell death, cell differentiation and proliferation leading to an increase in the number of deregulated cells causing tumors. Advances in tumorigenesis and metastasis have provided opportunities to develop novel compounds that judiciously act on abnormal molecular and biochemical signals that lead to cancer. Macromolecular transport pathways across tumor vessels occur *via* open gaps, vesicular vacuolar organelles and fenestrations. The transport of an anticancer drug is governed by the physiological and also physicochemical properties of the interstitium and by the physicochemical properties of the molecule. Physiological barriers at the tumor level and at the cellular level in the body must be overcome to successfully deliver anticancer agents to tumor cells *in vivo*. The possibility of fine-tuning polymer properties has led to remarkable progress in their role as anti-tumour agent carriers or as conjugates with antitumor drugs. Polymeric nano-sized carriers have shown a high targeting ability to tumor tissues and are minimally found at healthy tissue sites.[241,242]

DMAEMA was co-polymerised with *N*-isopropylacrylamide (poly(NIPAM-*co*-DMAEMA)) and evaluated as nanoparticle carriers for the controlled release of a hydrophobic anticancer agent, 7-ethyl-10-hydroxy-camptothecin (SN-38).[243] The thermo-sensitive poly(NIPAM-*co*-DMAEMA) nanoparticles were synthesized by free radical polymerization as illustrated in Fig. 54. The antitumor efficacy was evaluated in a

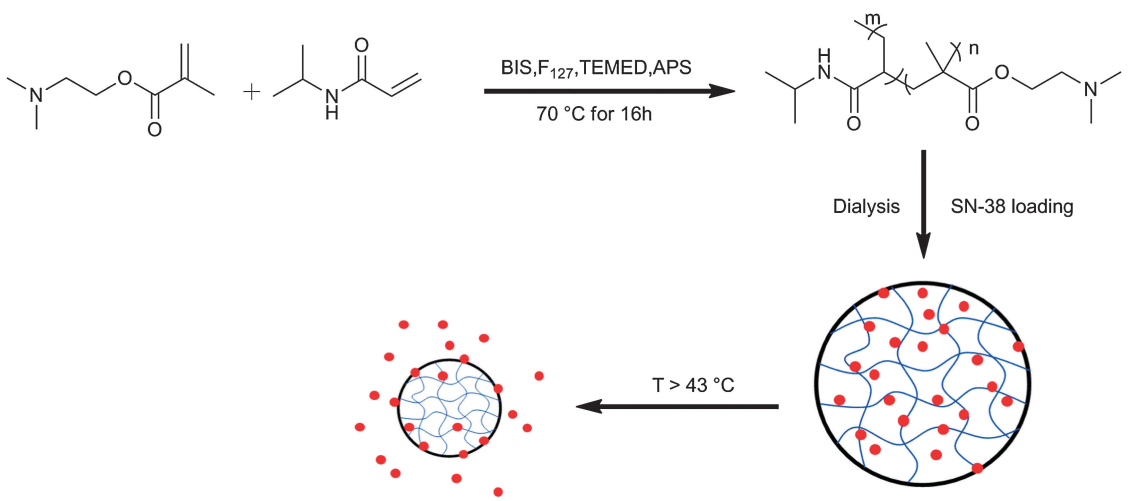


Fig. 54 Thermo-sensitive poly(NIPA-*co*-DMAEMA) nanoparticles and their release behavior.

C26 murine colon tumor model which showed that SN-38 loaded nanoparticles in combination with hyperthermia therapy efficiently suppressed tumor growth upon increasing the temperature above the polymer LCST.

Lu *et al.* prepared conjugates of β-CD and LPEI (M_W 600) to evaluate their efficiency as gene carriers in glioma cancer therapy.[59] The conjugates were comprised of the antitumor drug 5-fluoro-2′-deoxyuridine (FdUrd) with LPEI and β-CD. This conjugate was applied as a new bifunctional anticancer prodrug with improved therapeutic effects, while also showing good gene transfer efficiency. In the first step, CD-grafted PEI was first synthesized by reacting CDI activated CD with the primary and secondary amines of PEI. Next, CD-*g*-PEI-FdUrd was prepared by reacting CDI-activated FdUrd with CD-*g*-PEI (Fig. 55). The conjugates efficiently inhibited cancer cell migration and invasion in a time- and dose-dependent manner. PEGylated PLL dendrimers were prepared and conjugated with camptothecin, which is an enzyme that stabilizes and rejoins DNA breaks during replication.[135] In another study a saccharide-terminated G3 PAA dendrimer was synthesized as a drug carrier containing the drug methotrexate. The *in vitro* cytotoxicity was examined in the folate receptor expressing KB cell lines and the conjugate was observed to induce a dose-dependent cytotoxicity in the KB cells.[244]

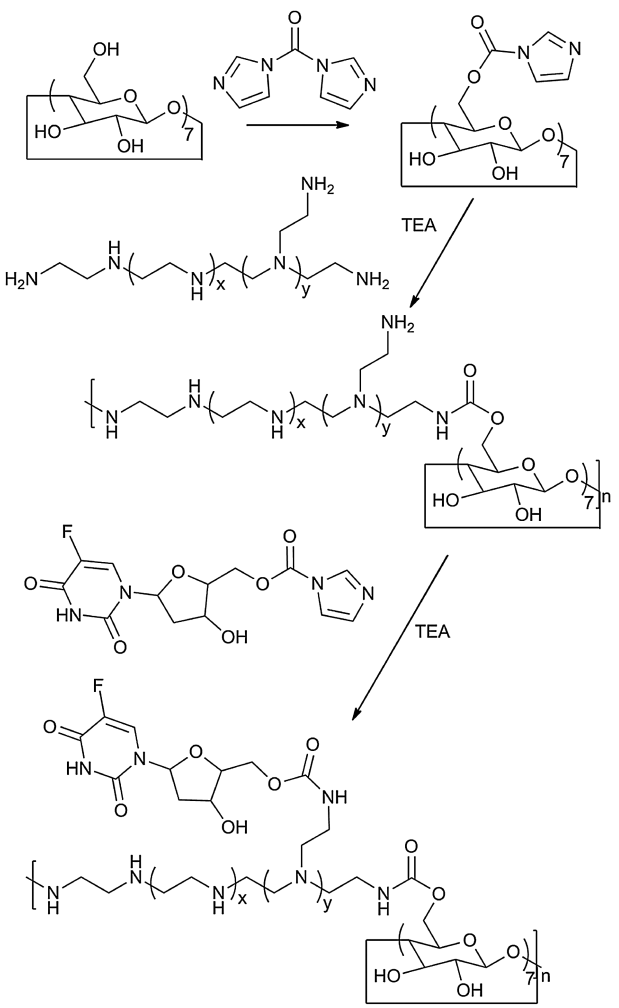


Fig. 55 Route of the synthesis of CD-*g*-PEI-FdUrd.

3.5 Anti-inflammatory cationic polymers

Inflammation is an immediate response of tissue towards infection or injury which is characterized by the accumulation of fluid and activated cells at the site of tissue destruction. Host defense against invading microbial pathogens is executed by the immune system, which consists of innate and acquired components. Detection and response to microbial infection by the immune system depend on a family of pattern recognition receptors called Toll-like receptors (TLRs). The action of TLRs is on the nucleic acids that are released by dead and dying cells. These extracellular nucleic acids can be internalized by inflammatory cells and activate multiple nucleic acid-sensing Toll-like receptors. The incorrect activation of these TLRs can evoke various inflammatory and autoimmune diseases (Fig. 56). Due to this mechanism, the neutralization of the pro-inflammatory effects of any nucleic acid independently from its sequence, chemistry or structure becomes obvious.[245] Lee *et al.* demonstrated that cationic polymers such as PAA dendrimer, 1,4-diaminobutane core-PAA-G3 dendrimer, PLL and β-CD can act as molecular scavengers and inhibit the ability of circulating immune stimulatory nucleic acids. The action of cationic polymers, which is irrespective of their sequence, chemistry, or architecture, involves the activation of a variety of nucleic acid-sensing TLRs and cytoplasmic pattern recognition receptors which can recognize and protect tissues from various harmful stimuli, such as pathogens and damaged cells.[246]

Howard *et al.* investigated the knockdown of tumor necrosis factor (TNF-α) expression in systemic macrophages by intraperitoneal administration of chitosan with small interfering RNA (siRNA) nanoparticles which downregulates systemic and local inflammation in mice.[247] Secretion of TNF-α by macrophages plays a predominant role in the development and progression of rheumatoid arthritis. Chitosan nanoparticles

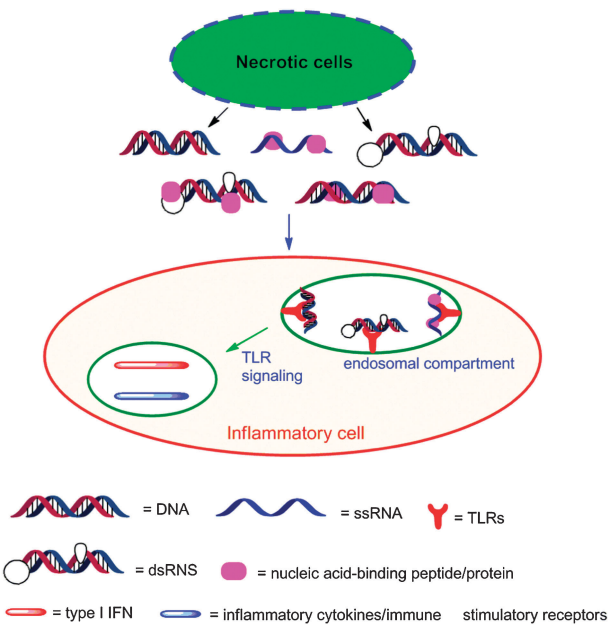


Fig. 56 Principle of release of nucleic acids by dead cells, which induce inflammatory responses and cause inflammatory diseases.

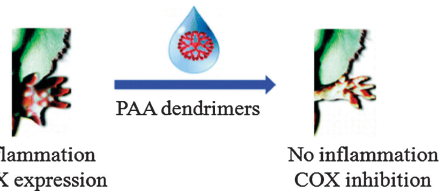


Fig. 57 Anti-inflammatory property of PAA dendrimers. Adapted with permission from ref. 248. © 2009 American Chemical Society.

containing an unmodified anti-TNF-α Dicer-substrate siRNA successfully mediated TNF-α knockdown (~66%) in primary peritoneal macrophages *in vitro*.

In another study, naked unmodified PAA dendrimers have showed significant and unexpected anti-inflammatory activity (Fig. 57). Long-term anti-inflammatory activity on rats was compared using three models including the carrageenan-induced paw edema model, the "cotton pellet test" and "adjuvant induced arthritis". The anti-inflammatory activity was dependent on the surface functionality, with amine surface groups being more efficient when evaluated on two factors, time frame after administration, and generation of dendrimers.[248]

4. Architecture and structure of cationic biomaterials

The chemical structure and architecture of biomaterials are of paramount importance for exploiting the full biomimetic potential and their designs have improved therapeutic functions. Cationic polymers have been framed in various architectures including hydrogels, scaffolds, membranes, fibers, nanogels, micelles, nanoparticles, and dendrimers. This section of the review provides an overview of the most common biomaterial architectures using cationic polymers, along with a brief description of their preparation, characteristic properties and recent advances in therapeutics relevant to biomaterial architecture.

4.1 Cationic polymers as hydrogels

Cationic polymer based hydrogels have been prepared by utilizing cationic monomers and/or polymers derived from both natural and synthetic origin. These types of biomaterials attract rapidly growing interest for various therapeutic applications. The therapeutic success of cationic hydrogels needs to seamlessly combine several strict, inter-related, requirements including biocompatibility, bioresponsiveness, antimicrobial activity, accelerating tissue regeneration and controlled release of biomolecules. The hydrophobicity or hydrophilicity of the cationic hydrogels strongly depends on the type of cationic groups selected. When the pH of the hydrogel environment is higher than its pK_a value, the gel becomes hydrophobic and possesses lower water content. Whereas at pH values lower than the pK_a, the hydrogels become hydrophilic and imbibe large amounts of water. The presence of charged groups on the polymer backbone also affects the osmotic balance between the hydrogel and the surrounding medium.[249] Cationic hydrogels play a significant role in the enhancement of cell adhesion,[250] and immobilization of heparin by the interaction with the negatively charged moieties. The most commonly used cationic polymeric hydrogels are composed of the cationic polymers,

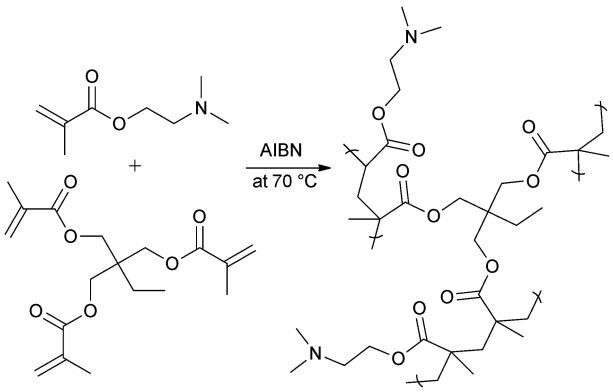


Fig. 58 Synthesis of cationic hydrogels based on DMAEMA and trimethylolpropane trimethacrylate.

chitosan, PEI, PAE, PAA, and PLL or combinations thereof. Among these, chitosan-based hydrogels are considered as the most popular for therapeutic applications. Chitosan shows strong hemagglutination by attracting the negatively charged sialic acid residues on red blood cells.[251] In addition chitosan has proven to enhance the function of leukocytes, macrophages and fibroblasts leading to enhanced granulation and tissue reconstruction.[252] Cationic gelatin hydrogels have been prepared and used as coatings on metal stents.[83] These hydrogels have demonstrated that Human pE-NTPDase gene transfer *via* cationic gelatin-coated stents inhibited subacute in-stent thrombosis and suppressed neointimal hyperplasia and inflammation without treatment with antiplatelet drugs. Satav *et al.* prepared PDMAEMA hydrogels as a feedback regulated drug delivery vehicle based on a toxicity biomarker strategy (Fig. 58).[253] Hawkins *et al.* performed a detailed study on hydrogels prepared by PAE. The developed hydrogels showed polymer dependent degradation profiles as well as pluripotent mesenchymal cells attachment directly onto the hydrogel surfaces.[254]

4.2 Cationic polymers as scaffolds

Cationic polymeric based scaffolds are 3D structural supports that provide an interconnecting porous network with suitable porosity and pore size and appropriate mechanical stability to withstand the contracting forces applied by adhering and proliferating cells, together with the property of being biocompatible and biodegradable at a controllable rate. These systems ideally should also closely mimic the complexities of the extracellular matrix (ECM) in order to maintain appropriate cell–matrix, cell–cell interactions and signaling pathways. Preparation of the above-mentioned systems might involve several techniques ranging from the more conventional porogen techniques to more sophisticated techniques like electrospinning and rapid prototyping technology with the final aim to obtain the desired 3D structure. Cationic polymers have been widely investigated in this respect for a variety of therapeutic applications. Chitosan has been widely investigated to fabricate scaffolds for various therapeutic applications.[255] Lee and Kim have developed highly porous and structurally stable 3D chitosan scaffolds by combining cryogenics and freeze-drying treatments (Fig. 59). MG-63 cells

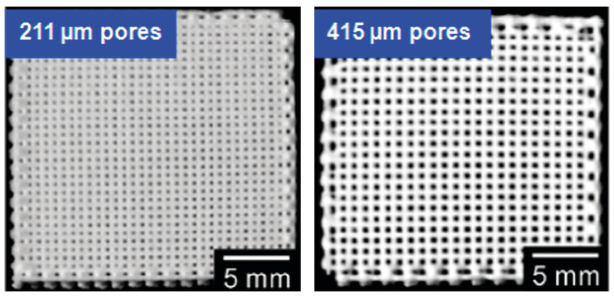


Fig. 59 Representative image of cryogenically 3D plotted chitosan scaffolds. Adapted with permission from ref. 256. © 2011 Elsevier Limited.

showed higher cell proliferation when cultured on the fabricated scaffolds, compared to the conventional spongy chitosan scaffolds.[256] Micro-fibers were obtained by wet-spinning chitosan as well as in blends with PCL for application in cartilage tissue engineering. Scaffolds prepared with chitosan and PCL ratio 3 : 1 supported neo-cartilage formation, based on an increase in glycosaminoglycan production and a homogeneous distribution of the ECM throughout the scaffold.[257]

PEI scaffolds have been used for the cultivation of bovine knee chondrocytes (BKCs).[258] PEI was bulk or surface cross-linked in combination with ternary polyethylene oxide, chitin and chitosan scaffolds. Keeping the concentration of PEI fixed, surface modification yielded higher chondrocyte affinity than bulk. Zhang *et al*. prepared PEI modified PEG scaffolds by electrospinning, wherein target DNA was adsorbed onto the electrospun nanofibers *via* electrostatic interaction between DNA and PEI–PEG. Electrospinning was employed by Khanam *et al*. who fabricated biocompatible PEI scaffolds cross-linked with succinic anhydride and 1,4-butanediol diglycidyl ether (Fig. 60). These non-woven were composed of 600–687 nm sized PEI fibres and were evaluated for their interaction with normal human fibroblast (NHF) cells. The PEI scaffolds supported the attachment and spreading of NHF cells.[259]

4.3 Cationic polymers as membranes

Cationic membranes are two dimensional (2D) crosslinked networks comprised of cationic polymer chains that swell in water or biological fluids and are considered as a viable approach prior to the fabrication of porous 3D scaffolds. Membranes are accepted as an interesting way to provide controlled delivery of drugs and biomolecules to the systemic circulation especially when applied subcutaneously. These have turned out to be appealing biomaterials for therapeutic applications including among others drug delivery to a specific target site, tissue engineering applications in 2D tissue constructs such as transdermal or articular cartilage, bioseparations and protein folding.[260] Membranes of chitosan with surface immobilized rhBMP-2 were prepared for guided tissue regeneration by the group of Chang *et al*.[261] The morphogen rhBMP-2 growth factor was covalently attached to the chitosan membrane using EDC-NHS chemistry. The rhBMP-2 enhanced MG-63 osteoblast cell adhesion, proliferation and differentiation, enabling bone formation in a rabbit critical-size defect model. A biodegradable chitosan membrane with

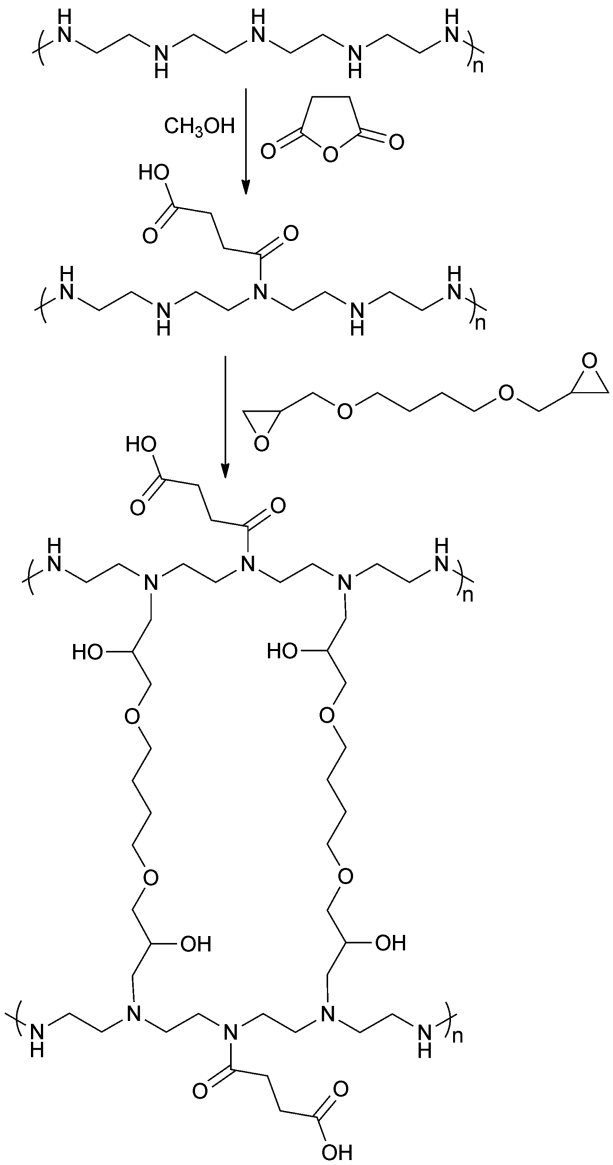


Fig. 60 PEI scaffolds cross-linked with succinic anhydride and 1,4-butanediol diglycidyl ether.

an asymmetric structure, seeded with fibroblasts, was prepared as a novel skin substitute.[262] The membranes were crosslinked using various genipin concentrations. Animal studies revealed that the asymmetric membrane promoted efficient re-epithelialization of the wound, indicating regeneration of damaged tissue.

4.4 Cationic polymers as fibers

In the last few decades, cationic fiber technology has gained global interest and has opened possibilities in various therapeutic applications including tissue engineering, drug and gene delivery. Fibers have been produced through different fabrication techniques such as self-assembly, electrospinning, wet spinning, dry spinning, melt spinning and template synthesis. Fibers are typically in the micron to nanometer range, which provides a high surface area to volume ratio, superior mechanical performance of individual fibers and a close resemblance with the structure and functions of the natural ECM.

In a recent study, Chen *et al*. developed electrospun cationic chitosan-*g*-PCL/PCL fibrous mats. The X-ray photoelectron spectroscopy and surface zeta-potential measurements showed the enrichment of amino groups on their surface. MTS assay and cell culture imaging confirmed that fibre mats with a moderate surface zeta-potential of 3 mV were the best in maintaining the cell morphology and promoting the cell attachment and proliferation with potential utilization for skin tissue engineering applications.[263] Chitosan/polyvinyl alcohol (PVA) membranes have also demonstrated excellent biocompatible potential support for lipase immobilization.[264] Tuzlakoglu *et al*. have demonstrated the utility of chitosan fiber mesh scaffolds for bone tissue engineering. They used wet spinning along with simple biomimetic spraying to develop homogeneous bone-like apatite coated chitosan fiber-based scaffolds. The cell adhesion and viability of these scaffolds were studied by using human osteoblast-like cell lines (SaOs-2) which indicated the bone-like apatite formation.[265]

Khanam *et al*. produced LPEI fibers and investigated 3-D attachment and spreading of NHF cells. LPEI was crosslinked with succinic anhydride and 1,4-butanediol diglycidyl ether. Cell growth was affected by the thickness of the 3-D scaffold. Fluorescence studies confirmed that NHF cells attached and spread throughout the cross-linked LPEI scaffold indicating that electrospun LPEI scaffolds support growth of NHF cells.[259] DNA was immobilized onto the PEG modified PEI fibres, *via* electrostatic adsorption. The obtained material exhibited high transfection efficiency.[266] LPEI was grafted with methacrylate functionality by reaction with glycidyl methacrylate. The microfibers were prepared by a reactive photo-electrospinning technology. The technique resulted in cross-linked L-PEI microfibers with significantly improved solvent resistance, thermal stability, and mechanical properties.[267]

Spasova *et al*. electrospun fibrous materials of stereo-complexes of high Mw poly(D or L)lactide (HMPDLA or HMPLLA) and diblock copolymers consisting of poly(L or D) lactide and PDMAEMA blocks, respectively (PLLA-*block*-PDMAEMA or PDLA-*block*-PDMAEMA). A significant number of adhered *S. aureus* cells were observed on the surface of the HMPLLA fibre mats. The morphology of the adhered cells was not altered, indicating that HMPLLA fibers are a favorable substrate for growth of pathogenic microorganisms, and a bacterial biofilm was formed on them. The fibers containing PDMAEMA led to a significant decrease in the number of adhered cells (Fig. 61) which was a result of the antibacterial properties of PDMAEMA leading to the inhibition of bacterial growth. The obtained results clearly indicated that the presence of the tertiary amino groups of PDMAEMA on the stereocomplex fibers imparts hemostatic as well as antibacterial properties to the novel fibrous materials.[268]

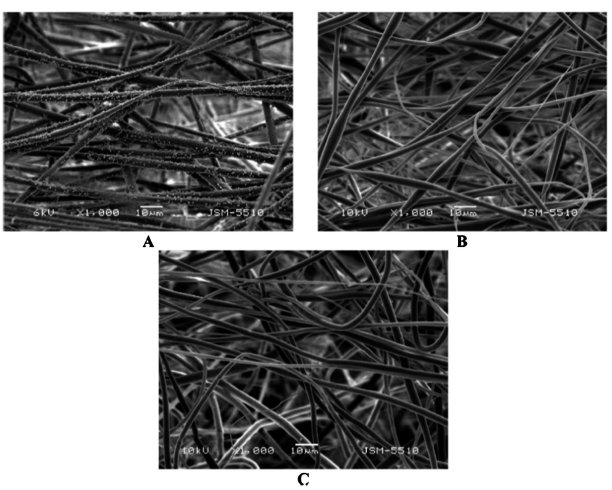


Fig. 61 SEM micrographs of fibrous mats after 24 h contact with *S. aureus*: (A) HMPLLA, (B) PLLA-*b*-PDMAEMA/HMPDLA mat, and (C) PDLA-*b*-PDMAEMA/HMPLLA mat. Adapted with permission from ref. 269. © 2010 American Chemical Society.

4.5 Cationic polymers as nanogels

Cationic nanogels are nanosized swollen cationic hydrogel particles composed of chemically or physically cross linked polymer networks. The highly hydrated properties and a tightly crosslinked core offer those systems superior colloidal stability, making them attractive and promising as biomaterials and carrier systems for the delivery of therapeutic cargos in comparison to the macroscopic hydrogels or nanoparticles. The cationic property of chitosan was utilized to prepare nanogels coated with hyaluronate and anionic photosensitizers. The prepared nanogels were applied as macrophage-targeting phototoxic materials for photodynamic therapy in an *in vivo* study in mice using the antigen-induced model of rheumatoid arthritis. The nanogels were suitable drug delivery systems for the targeted delivery of the encapsulated photosensitizers to macrophages and long-term retention of therapeutics in leaky inflamed articular joints.[269] A one step process was elaborated to prepare cationic PDMAEMA nanogel *via* the *in situ* formation of micelles by an amphiphilic trithiocarbonate macro RAFT agent (mPEG550-TTC). The nanogel possessed a size of about 10 nm in radii with a zeta potential of about +30 mV, showing potential as a gene delivery system.[270] Nanogels based on PEI were investigated for the delivery of nucleic acids. Kim *et al*. conjugated catechol groups to BPEI to produce nanogels with an average diameter of 110 nm (Fig. 62). The nanogels exhibited enhanced cellular uptake and promoted gene silencing efficiency.[271]

4.6 Cationic polymers as micelles

Micelles are composed of amphiphilic molecules that self-assemble due to the energy minimization with the surrounding solvent. When exposed to a hydrophilic solvent, the hydrophilic moiety of the molecule orients towards the solvent, while the hydrophobic moiety of the molecule orients towards the core and forms a cluster away from the solvent. In a similar manner, when amphiphilic molecules are exposed to hydrophobic solvent, they form micelles with hydrophobic moieties on the surface and hydrophilic moieties in the core. Micelles thus have a unique core–shell architecture composed of either hydrophobic or hydrophilic segments depending on the applied polymer chemical structure and the selected medium. The hydrophobic or hydrophilic core provides a reservoir for various water-soluble and water-insoluble drugs and protects them from decomposition in order to maintain their activity and stability.

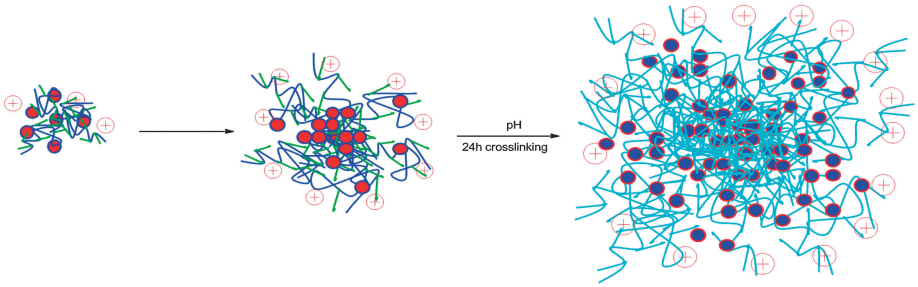


Fig. 62 Schematic diagram showing the preparation of catechol conjugated PEI nanogels.

Recently, Duan *et al*. prepared cationic micelles of chitosan-*g*-PCL as a carrier of 7-ethyl-10-hydroxy-camptothecin. The micelles showed prolonged drug release profiles, improved drug stability against hydrolysis under physiological conditions, and decreased cytotoxicity against L929 cell lines.[272] Chitosan with low M_W formed micelles that were used to deliver pVIVO2-mIL4-mIL10 plasmid encoding interleukin-4 (IL-4) and interleukin-10 (IL-10) in a low-dose streptozotocin induced diabetic mouse model.[273] The study indicated efficient expression of IL-4, and IL-10 when delivered intramuscularly which led to protection of the pancreatic beta cells from inflammation and insulitis. Song *et al*. prepared micelles of cationic cellulose as a drug delivery device for the water insoluble prednisolone acetate. Quaternary ammonium groups were introduced into cellulose as the hydrophilic moieties and attachment of the long alkyl chains provided the required hydrophobic character (Fig. 63). The resulting amphiphilic cellulose self-assembled into cationic micelles with an average hydrodynamic radius of 320–430 nm and demonstrated low cytotoxicity and better drug loading capacity at low concentrations.

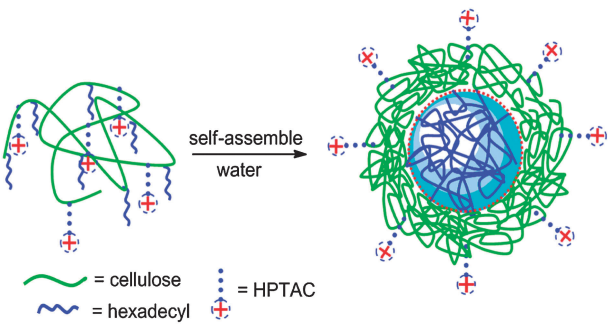


Fig. 63 Self-assembled hydrophobically modified quaternized cellulose micelles.

Cell viability increased slightly with the increase in the hydrophobic segment.[47]

Micelles of PAE copolymerized with PEG have shown dual response towards pH and a reductive environment. The copolymer with disulfide bonds in the backbone of PAE was synthesized *via* Michael addition polymerization from 2,2′-dithiodiethanol diacrylate, 4,4′-trimethylenedipiperidine, and methoxy PEG–NH_2–Dox. The copolymer self-assembled into micelles which are stable in a physiological environment, with the PAE constituting the core and the PEG the shell. The drug release was faster from micelles in a weakly acidic environment (pH 6.5) and in the presence of a higher concentration (above 5 mM) of the reducing agent dithiothreitol (DTT).[274] Biodegradable cationic micelles were prepared from PDMAEMA–PCL–PDMAEMA triblock copolymers with hydrophobic anticancer drug paclitaxel and siRNA for delivery into cancer cells (Fig. 64).[275] The micelles were capable of efficiently delivering paclitaxel into cancer cells, resulting in enhanced drug efficacy as compared to free paclitaxel. The co-delivery of VEGF siRNA and paclitaxel revealed improved gene knockdown efficiency.

A block copolymer of BPEI and PLGA was used to prepare cationic micelles for gene delivery applications. Adding a small amount of low M_W BPEI (1.8 kDa) completely shielded pDNA in the micelle–pDNA complexes and enhanced transfection efficiency by 50–100 fold for both fresh and reconstituted complexes without affecting the polyplex size. Low M_W BPEIs were introduced into the micelle–pDNA complexes which shielded any exposed pDNA onto the surface of the complexes through electrostatic layer-by-layer (LBL) attraction. In addition, this approach filled any free volume between the cationic micelles and pDNAs and induced tighter complexation. The micelle–pDNA–BPEI1.8 kDa system showed low cytotoxicity in MCF7 cells even with pDNA doses up to 20 mg while transfection levels increased linearly with increasing pDNA dose.[276]

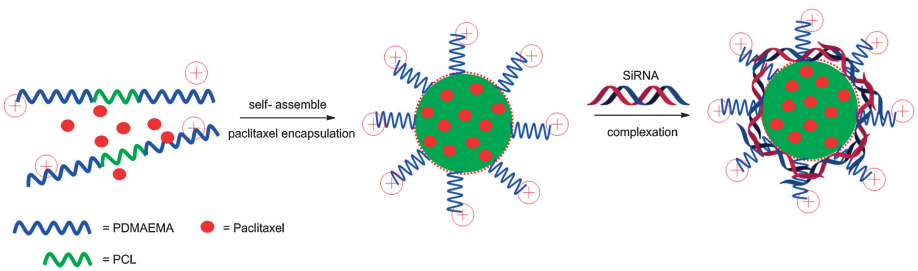


Fig. 64 Self-assembly of PDMAEMA-*b*-PCL-*b*-PDMAEMA cationic triblock copolymers into micelles containing hydrophobic paclitaxel and siRNA.

4.7 Cationic polymers as nanoparticles

Nanosized cationic polymeric particles emerged as a promising platform for a wide-range of therapeutic and diagnostic applications. These nanoparticles have a unique multi-functional character providing low toxicity, high surface area, extending the therapeutic agent life cycle and constituting more effective routes of administration. Nanoparticles can be easily prepared from a variety of natural as well as synthetic cationic polymers and copolymers with different positive charge density, varying molecular mass and hydrophilic/lipophilic properties. Chitosan as nanoparticles allows easy uptake by endosomes, thereby allowing the biomolecule to overcome the permeability barrier of the epithelia. In addition, nanoparticles of chitosan were able to control the release of genes or drugs in a sustained manner. Chitosan alone in the form of nanoparticles has been widely exploited with successful outcome and we will limit our discussion to modified chitosan nanoparticles. Yuan *et al.* prepared poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles by modifying their surface with positively charged chitosan (Fig. 65).[75] Nanoparticles were prepared by emulsion solvent evaporation with the particle size being dependent on the applied chitosan concentration. The resulting nanoparticles successfully delivered siRNA and effectively silenced GFP by inhibiting its expression in cultured cells.

Chitosan–PLGA nanoparticles prepared by Taetz *et al.* also showed effective results for the delivery of an antisense 2′-*O*-methyl-RNA directed against an RNA template of human telomerase.[277] Bilensoy *et al.* studied the influence of two cationic polymers, chitosan and PLL coated PCL nanoparticles. The developed nanoparticles showed favorable drug loading and release profiles as well as good cellular interaction and anticancer efficacy.[68] Zworiek *et al.* prepared cationic gelatin nanoparticles as a carrier to improve delivery of immunostimulatory CpG oligonucleotide. Particles were prepared using a two step desolvation method. Cationic gelatin nanoparticles enhanced the uptake and immunostimulatory activity of CpG oligonucleotide both *in vitro* and *in vivo* and increased the production of IFN-α, a key cytokine in both the innate and adaptive immune responses.[37] Recently Fernandez *et al.* developed cationic CDs that had the potential to complex and compact DNA into homogeneous nanoparticles of less than 70 nm. The CDplex-mediated transfection is a complex process simultaneously involving several cellular uptake mechanisms.[63] Quaternary ammonium β-CD nanoparticles were prepared by Gil *et al.* as drug delivery carriers for Dox across the blood–brain barrier. Nanoparticles were synthesized by a one-step condensation polymerization with 65–88 nm hydrodynamic radii. The cationic properties were controlled by adjusting the incorporated amount of quaternary ammonium groups in the final structure. The results indicated that endocytosis was the main mechanism for the permeability of nanoparticles across the blood–brain barrier.[278] PEI nanoparticles have also shown successful results in therapeutic applications ranging from drug delivery vehicles for anticancer drugs[279] to growth factors.[280] Abbasi *et al.* prepared Dox conjugated PEI nanoparticles activated by addition of human serum albumin. The particles were prepared by the ethanol desolvation technique with a size of about 130 nm. PEI-enhanced albumin nanoparticles illustrated a more potent cytotoxic effect on MCF-7 breast cancer cells over longer time spans.[279] Magnetic nanoparticles have been modified with PEI to investigate their usefulness as a potential vascular drug/gene carrier to brain tumors.[170] Self-assembled nanoparticles of glycol modified chitosan and PEI were developed by Huh *et al.* as an effective siRNA carrier system. Both glycol chitosan and PEI were modified with 5β-cholanic acid in order to form stable self-assembled nanoparticles. The particles presented tumor-targeting ability and significantly inhibited red fluorescent protein (RFP) gene expression in the cultured cell system combined with tumor targeting ability.[281] Quaternary ammonium PEI nanoparticles prepared by reductive amination and alkylation were tested against both G+ and G− bacteria. The results indicated that both the types of nanoparticles inhibited antibacterial growth with the alkylated particles being the most potent.[235] Groman *et al.* conjugated dextran-coated iron oxide nanoparticles with PLL to boost particle uptake by fibroblasts for use as imaging agents for fibroblasts. PLL was conjugated *via* reversible covalent Schiff base linkages. NIH-3T3 fibroblasts labeled with the conjugated nanoparticles demonstrated strong labeling with high localization of iron, suggesting the association of nanoparticles with cytoplasmic vacuoles while the nucleus of the cell was not labeled. As much as 25% of PLL conjugated iron nanoparticles were internalized by fibroblasts within 8 h in culture.[282] Recently, micro-RNA-10b-PLL particles were used to deliver anti-micro-RNA-10b molecules into the breast cancer cell line MDA-MB231. The resulting PLL–RNA nanoparticles delivered the anti-microRNA molecules into cytoplasm of breast cancer cells in a concentration-dependent manner.[283] Wang *et al.* have prepared hyperbranched PAA nanoparticles for gene delivery applications. The encapsulated DNA was protected by the nanoparticles from degradation for over 3 h and high gene transfection efficiency was achieved in COS7 and HEK293 cell lines. However slight cytotoxicity was observed in the study.[284]

PDMAEMA nanoparticles have also shown effective therapeutic applications. pH sensitive nanoparticles of PDMAEMA and hydroxy ethyl methacrylate (HEMA) were prepared for the triggered release of paclitaxel.[285] Nanoparticles were

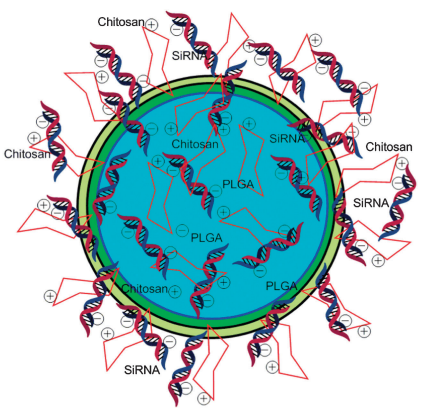


Fig. 65 Schematic representation of siRNA loaded cationic chitosan-PLGA nanoparticles.

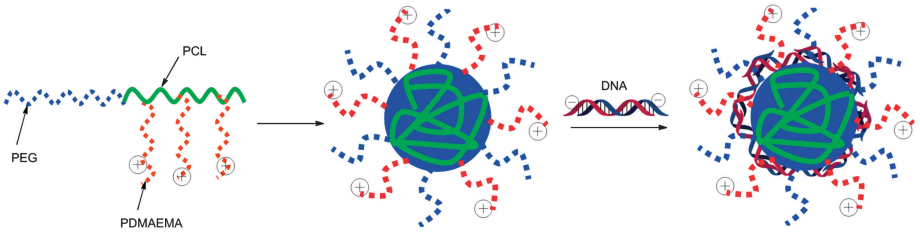


Fig. 66 Scheme representing the preparation of PEG-*b*-(PCL-*g*-PDMAEMA)-pDNA nanoparticles.

prepared by an oil in water emulsion method followed by photopolymerization, using tetraethylene glycol dimethacrylate as a crosslinker. The results indicate that low pH and low concentration were the key factors for the release of paclitaxel which occurred by a small pH change under the physiological conditions. Guo *et al.* prepared cationic polymers containing PEG-*b*-(PCL-*g*-PDMAEMA) to form nanoparticles which demonstrated effectiveness as gene carriers. The nanoparticles could bind pDNA to form polyplexes of 65–160 nm in size and showed much better transfection efficiency than lipofectamine 2000 and PEI in HepG2 cells (Fig. 66).[167]

4.8 Cationic polymers as dendrimers

Dendrimers are highly branched macromolecules possessing a large surface area to volume ratio with well-defined interior and exterior regions. A large number of terminal groups are present on the dendrimers, which play an influential role in their solubility and adhesive or conjugation properties. Cationic dendrimers form polyelectrolyte complexes with anionic biomolecules and can be highly informative for diverse cellular processes such as post-transcriptional RNA processing, protein synthesis, among others.[286–288] Cationic PAA dendrimers have been widely used for the delivery of biomolecules. Deng *et al.* used a facile click conjugation strategy to prepare dendronized chitosan derivatives for improved gene delivery. Propargyl focal point PAA dendron and 6-azido-6-deoxy-chitosan were synthesized and subsequently applied in the copper catalyzed azide alkyne cyclization reaction. The dendrimers showed better water solubility and buffering capacity when compared to native chitosan. The chitosan-*g*-PAA (G3) exhibited enhanced transfection efficiency and lower cytotoxicity in 293T and CNE2 cell lines when compared to commonly used PEI (25 kDa).[289] PAA dendrimers have been prepared using carboxylated methyl chitosan and glucuronyl-glucosyl-β-cyclodextrin for tissue engineering applications.[290,291] Aiming at an enhanced buffering capacity of PAA dendrimers under acidic conditions, Yu *et al.* conjugated histidine and arginine groups to PAA dendrimers (G4) (Fig. 67). The addition of histidine units into the established PAA-arginine polymer vectors improved their proton buffering capacity in the pH range 3.5–6. The PAA derivatives effectively condensed pDNA at a low charge ratio, and the transfection activity improved considerably when the number of histidine residues was increased.[292]

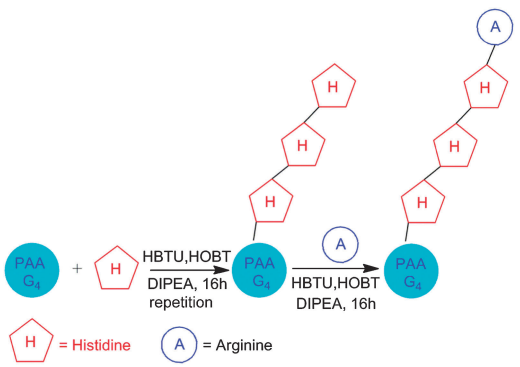


Fig. 67 PAA dendrimers with histidine and arginine groups.

Fluorescent labeled PAA dendrimers were developed as a gene carrier to deliver antisense oligonucleotides with the aim to inhibit either the p75 neurotrophin receptor or the nerve growth factor in rat C6 glioma cells. The oligonucleotides were electrostatically associated with the photoluminescent amino-terminated PAA dendrimer to yield fluorescent complexes at various N/P ratios.[293] In another study, PAA based dendrimeric hydrogels were prepared using a UV-cured PAA dendrimer (G3) tethered with three PEG and acrylate chains (Fig. 68). These dendrimer hydrogels were studied for the delivery of brimonidine and timolol maleate.[294]

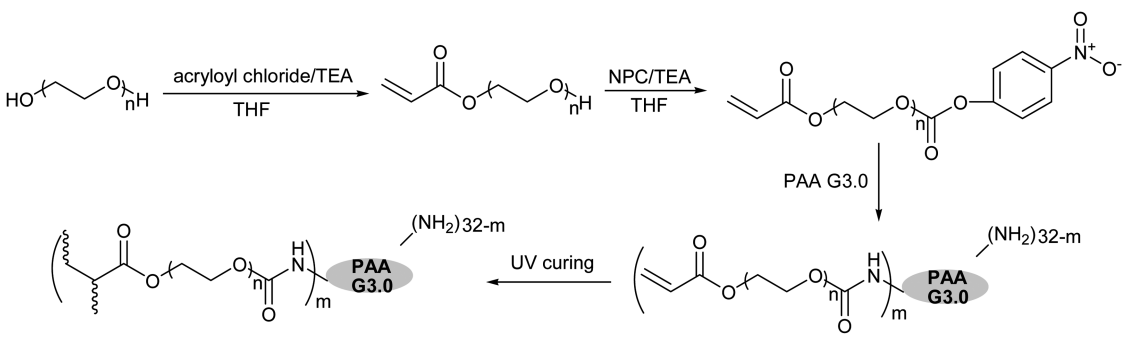


Fig. 68 Synthesis route of PAA–PEGacrylate conjugates.

Recently, a novel triblock nanocarrier, PAA-*b*-PEG-*b*-PLL, was designed to combine individual features of PAA dendrimers with the other constituting polymer blocks, PEG and PLL. PAA dendrimers provided tertiary amines for enhancing endosomal escape and electrostatic interaction with DNA. PEG protected the siRNA from enzymatic degradation while PLL offered cationic amine groups enabling electrostatic interaction with negatively charged siRNA. A three-step synthetic route was optimized for the preparation of the PAA-*b*-PEG-*b*-PLL nanocarrier (Fig. 69). In the first step, the PAA dendrimer was partially acetylated, the second step involved synthesis of PAA-*b*-PEG–COOH and in the third step, the terminal free acid group was reacted with PLL using EDC as a coupling reagent.[295]

Al-Jamal *et al.* described the intrinsic capacity of PLL (G6) dendrimers to display systemic antiangiogenic activity that could lead to solid tumor growth arrest.[296] The PLL dendrimers were reported to accumulate at the tumor site and exhibited intrinsic therapeutic anti-angiogenic activity. The developed polymer possessed the ability to bind electrostatically to the negatively charged heparin and thus neutralize its activity.[297] Yemul and Imae prepared PEI dendrimers using EDA as a core. Michael addition reaction was used for alkylation followed by Gabriel amine synthesis to produce amine-terminated dendrimers (Fig. 70). A strong fluorescence from PEI dendrimers was observed under acidic conditions. Emission intensities increased with increasing dendrimer generation from 1 to 3 and also showed an increasing pattern over time.[298]

Dendrimeric vectors of PEI were designed with various shells to improve transfection. The outermost oligocation shell was included with the aim to facilitate DNA release inside cells followed by a hydrophobic alkyl shell. The two layers were

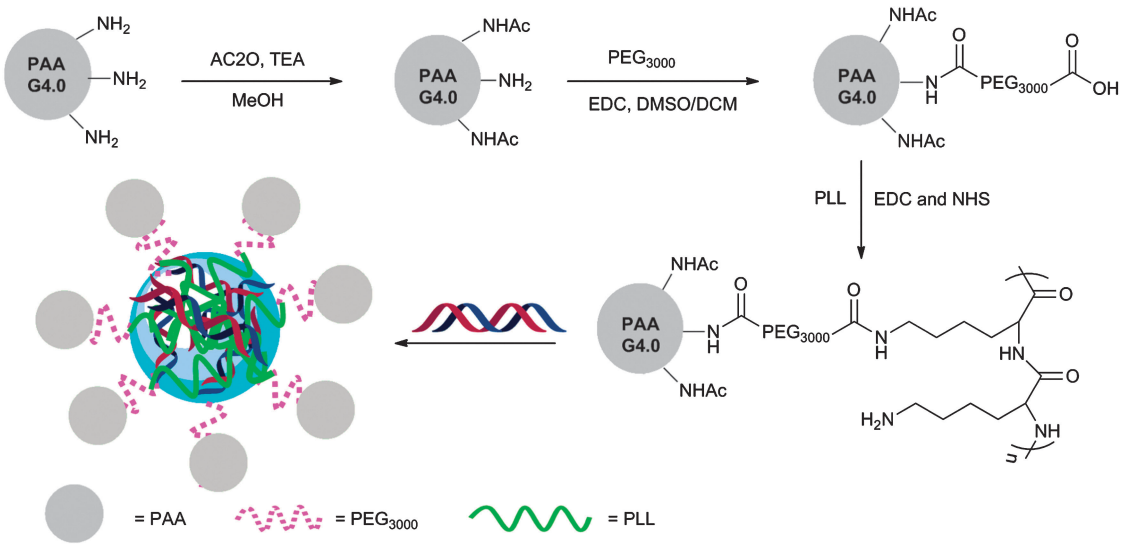


Fig. 69 Synthesis of PAA-*b*-PEG-*b*-PLL dendrimeric block polymers.

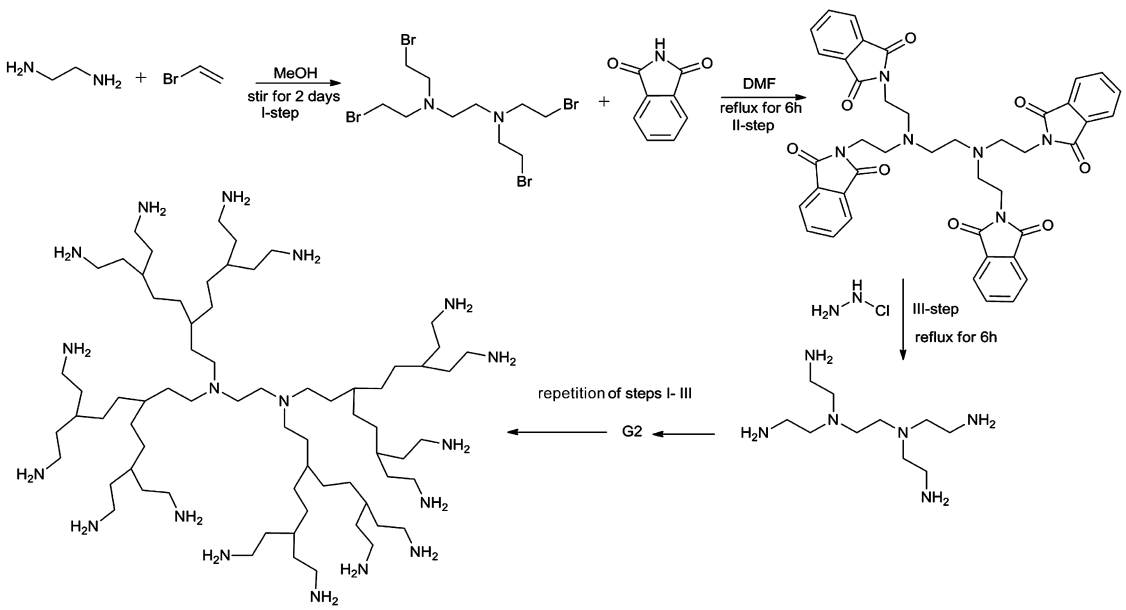


Fig. 70 Synthesis pathway of PEI dendrimers from EDA.

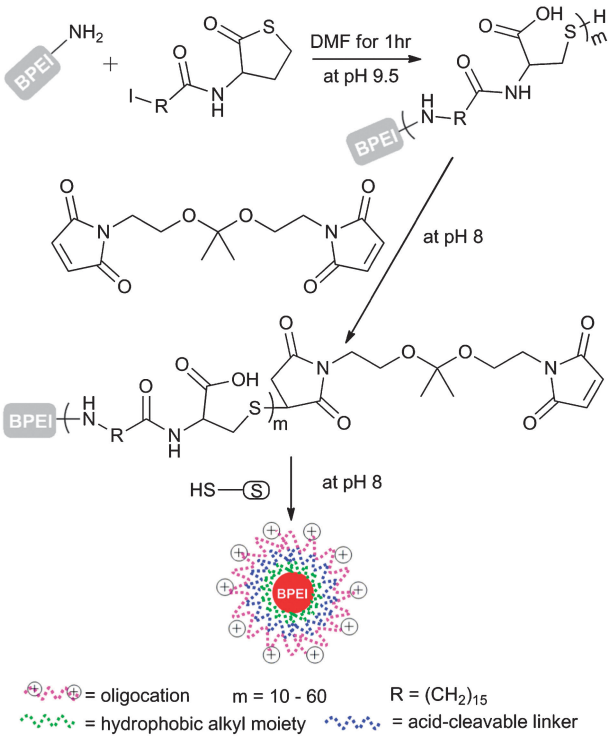


Fig. 71 Synthesis of dendrimeric alkylated polyethylenimine nanocarriers containing acid cleavable outer cationic shells.

attached to the polycationic core through an acid-cleavable linker (Fig. 71). The attachment of highly-charged oligocations to a PEI-based dendrimeric vector through an acid-cleavable linker increased the transfection efficiency without increasing cytotoxicity.[299]

5. Therapeutic applications of cationic polymers

5.1 Drug delivery potential of cationic polymers

Gradual progress has been made in modern drug delivery with the use of cationic polymer carriers for the release of therapeutics in both pulsatile dose delivery products and implanted reservoir systems. A useful cationic polymer for a drug delivery system must surpass many hurdles prior to clinical implementation such as addressing the need for specific targeting, intracellular transport, and biocompatibility while integrating properties enabling responsive behavior to physiological environments. Polycationic drug delivery systems have attracted much attention because of their unique characteristics, including good water solubility and high cellular uptake efficiency.

As cationic polymers, chitosan has been effectively used in drug delivery as a hydrogel system, drug conjugate, biodegradable release system, and polyelectrolyte complex for many components. Chitosan-based systems are used for the delivery of proteins/peptides, growth factors, anti-inflammatory drugs as well as antibiotics. In a recent study, Ma and Liu prepared protein loaded chitosan microspheres by applying a modified ionotropic gelation method combined with a high voltage electrostatic field. The resulting microspheres showed effective sustained delivery of protein.[300] In another study, the use of chitosan drug delivery to the inner ear across the "round window membrane" (RWM) was examined by Saber *et al.*[301] Three structurally different chitosans loaded with a tracer drug, neomycin, were injected into the middle ear cavity of albino guinea pigs ($n = 35$). After 7 days, the hearing organ was examined for hair cell loss and the RWM evaluated in terms of thickness. All chitosan formulations successfully released the loaded neomycin which diffused across the RWM, and exerted a concentration dependent ototoxic effect on the cochlear hair cells. Ghendon *et al.* demonstrated that intramuscular administration of soluble chitosan with monovalent and trivalent split inactivated influenza vaccine gave strong humoral and cell-immunity responses against different variants of A and B-type human influenza viruses.[302] Insulin-loaded chitosan nanoparticles enhanced nasal absorption of proteins to a greater extent than relevant chitosan solutions.[303] In another study Zhang *et al.* synthesized cationic β-CD complexed with insulin and then encapsulated the complex into alginate/chitosan nanospheres (Fig. 72). The positive charge of cationic β-CDs assisted the formation of complexes with insulin which protected them from degradation in the gastric compartment by shielding them within the core of the alginate/chitosan nanoparticle.[304]

Cationic gelatins have been investigated for the controlled release of peptide/protein drugs with different M_Ws and IEP.[38] In a study by Zwiorek *et al.* cationized gelatin nanoparticles were used as carriers to improve delivery of immunostimulatory CpG oligonucleotides both *in vitro* and *in vivo*.[37] Cationic surface charge on the nanoparticles avoided the undesired desorption of the CpG oligonucleotide from the carrier surface during the transport to the target cell. This formulation containing both antigen and immunostimulatory oligonucleotides could exhibit potential against cancer or viral infections.

A localized controlled release hydrogel system composed of cationic gelatin and chitosan was used to deliver an antisense oligonucleotide targeting murine TNF-α for the treatment of endotoxin-induced osteolysis. The gelatin-chitosan film with the oligonucleotide was placed over the calvarium in female SD rats and C57/BL6 mice. No obvious inflammatory

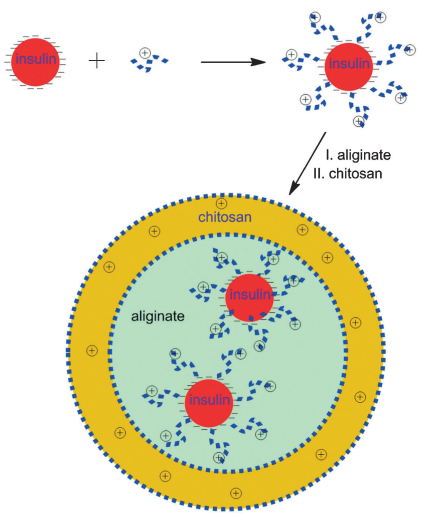


Fig. 72 Cationic β-CDs–insulin complexation and subsequent protection by alginate/chitosan nanoparticles.

symptoms were observed in the calvarial tissues and the film was degraded and absorbed within 10 weeks after application. The delivery of the oligonucleotide effectively suppressed the expression of TNF-α and subsequently the osteoclastogenesis *in vivo*.[305]

PAAs are also a class of cationic polymers widely investigated for drug delivery purposes. In a recent work, an effective intracellular protein delivery system was developed based on linear PAAs that form self-assembled cationic nanocomplexes with oppositely charged proteins.[306] PAAs were prepared by Michael-type polyaddition of 4-amino-1-butanol to CBA. The prepared PAAs are prone to fast degradation in the intracellular environment due to reductive cleavage of the disulfide linkages, thereby releasing the therapeutic payload and diminishing potential cytotoxicity of the polymer. The same group investigated two model proteins, β-galactosidase and human serum albumin with an IEP of 4.6 and 5.3, respectively, by simply mixing negatively charged protein and positively charged PAA at neutral pH. Self-assembled polyelectrolyte complexes were formed with nanosized dimensions (Fig. 73).

PAA dendrimers with disulfide linkages as drug delivery agents were investigated by Kurtoglu *et al.* *N*-acetyl cysteine was conjugated to PAA dendrimers (G4) and its release profile was studied in three media that induce disulfide exchange reactions: glutathione, cysteine and bovine serum albumin.[307] The results indicated that the prepared conjugate delivered 60% of its drug payload within 1 h at physiological pH and showed an increase in antioxidant activity compared to free drug. In addition to having disulfide linkages, the drug release characteristics of the PAA dendrimers using different ester, amide and peptide linkers have been investigated by the same group. Ibuprofen was directly conjugated to the NH_2-terminated dendrimer by an amide bond and the OH-terminated dendrimer by an ester bond. A tetra-peptide-linked dendrimer conjugate and a linear methoxy PEG–ibuprofen conjugate were also studied for comparison. Amide-linked conjugates were relatively stable against hydrolysis, whereas the ester-linked conjugates showed pH-dependent release and the extent of release varied with pH from 3% (pH 5) to 38% (pH 8.5) for 10-days study period. Direct amide and ester-linked conjugates did not release ibuprofen enzymatically whereas the methoxy PEG conjugate released 65% of its payload within 12 h in diluted plasma by esterase activity, and the peptide-linked dendrimer conjugate released 40% of its payload within 48 h by cathepsin B activity.[308] Erythromycin prodrug (EM-2′-glutarate) has been conjugated to the PAA dendrimer *via* an ester linkage and evaluated for sustained treatment of orthopedic inflammation. The conjugate demonstrated a high drug payload, improved the solubility of the drug, and enhanced activity. The conjugates released more than 90% of free drug over a period of 10 hours without any cytotoxicity towards RAW 264.7 macrophages.[309]

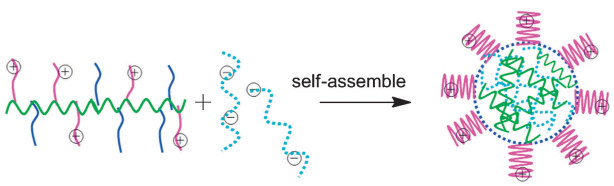


Fig. 73 Structure of PAA and self-assembled polyelectrolyte complexes.

In the case of anti-cancer drug delivery, PAA dendrimers have shown positive outcomes. Polyvalent conjugates of PAA dendrimers (G3) containing the anti-cancer drug methotrexate were internalized into folate receptor (FR)-expressing KB cells in a dose-dependent and receptor mediated fashion. The conjugates induced a dose-dependent cytotoxicity in the KB cells.[244] Li *et al.* further demonstrated that methotrexate functionalized PAA dendrimers (G5) effectively killed and targeted cancer cells serving as a dual-acting molecule.[310] Pang *et al.* prepared cationic drug carriers based on hyperbranched PAEs through proton transfer polymerization. Oxyanionic initiation of triethanolamine in the presence of the potassium hydride catalyst was used to polymerize both vinyl and epoxy groups of the glycidyl methacrylate monomer. Chlorambucil was conjugated to the hyperbranched PAE as a model anti-cancer drug which exhibited the growth inhibition against MCF-7 cells.[311]

PEI was used as a cationic delivery system for the immobilization of etidronic acid that is used as a model bis(phosphonate) drug.[312] The immobilization of etidronic acid was achieved by ionic cooperative interactions between the cationic polymeric matrix and the deprotonated etidronate. Stable and slow release was obtained with the faster release occurring at pH 5 in comparison to pH 3 and 4. In another interesting study by Kim *et al.*, PEI conjugated with pluronic F-127 was designed for conjugation of diazeniumdiolates which are a source of nitric oxide. The controlled release of nitric oxide is important due to its potential to inhibit the proliferation of vascular smooth muscle cells in restenosis, the apoptosis of vascular endothelial cells, and aggregation of platelets. In this study BPEI provided a large amount of secondary amines as conjugation sites for the diazeniumdiolates and pluronic gave the thermosensitive property to the polymer. The prepared polymer gels displayed slow and prolonged release of nitric oxide with an increase of endothelial cell proliferation and reduction of smooth muscle cell proliferation in comparison to the non-nitric oxide releasing control (Fig. 74).[313]

5.2 Tissue engineering with cationic polymers

Tissue engineering is a highly interdisciplinary field that combines the principles and methods of life sciences and

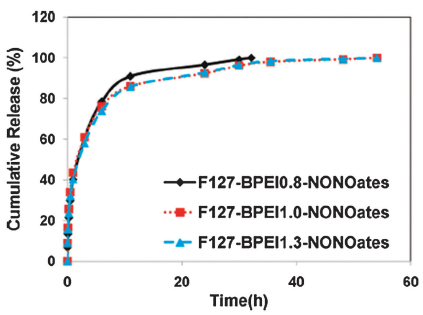


Fig. 74 Cumulative NO release (%) from three types of F127-BPEINONOates at 37 °C. Adapted with permission from ref. 313. © 2011 American Chemical Society.

engineering to utilize structural and functional relationships in normal and pathological tissues to develop biological substitutes intended for restoring, maintaining, or improving biofunction of the damaged organ or tissue. The role of cationic polymer chitosan in tissue engineering has also been reported. Chitosan can be molded into porous structures which is advantageous for osteoconduction in bone tissue engineering. Zhao *et al.* used chitosan scaffolds with calcium phosphate cement. The scaffolds improved resistance to fatigue and fracture, for the delivery of stem cells aimed at bone tissue engineering.[314] Similarly, in cartilage tissue engineering, chitosan was chosen as a scaffolding material in articular cartilage engineering due to its structural similarity with various GAGs found in articular cartilage. The cartilage-specific ECM components such as type II collagen and GAGs play a critical role in regulating expression of the chondrocytic phenotype and in supporting chondrogenesis *in vitro* and *in vivo*.[13] A chitosan hydrogel was prepared for chondrocyte cells to reconstruct tissue engineered cartilage and repair articular cartilage defects in a sheep model.[315] The *in vitro* tissue-engineered cartilage reconstructions were prepared by mixing sheep chondrocytes with a chitosan hydrogel. Cell survival and matrix accumulation analysis were characterized after 3 weeks in culture. To enable *in vivo* repair, the reconstructions were cultured for 1 day and transplanted to the freshly prepared defects of the articular cartilage of sheep. The cartilage defects were repaired completely within 24 weeks.

PLL revealed special efficacy in cartilage tissue engineering.[316] PLL was grafted to polyethylene oxide/chitin/chitosan scaffolds and shown to be effective in producing cartilaginous components. In another study the low shrinkage, ability to promote cell adhesion and good biocompatible properties of PLL were suitable for the development of 3D scaffolds for cardiac tissue engineering.[316] Electrospun nanofibres of polyaniline modified by hyperbranched PLL showed biocompatibility and cardiomyocyte proliferation.[317] PLL is known to attract neurons and promote neurite outgrowth because of its positive charge and high hydrophilicity. Chitosan/glycerol phosphate functionalized with PDL is an excellent *in vitro* substrate and scaffold for cortical cells, and is envisioned to be useful for neural tissue engineering as an injectable scaffold.[318] PDL immobilised onto chitosan *via* azidoaniline photocoupling (Fig. 75) showed improved neuron survival.

PLL was coated onto PLGA microspheres encapsulated with retinoic acid. Neural differentiation was induced by using mouse embryonal carcinoma cells by treatment of cells with encapsulated retinoic acid in the biodegradable PLGA microspheres.[188] The histological investigation of the microspheres clearly indicated that PLL facilitated cell attachment and growth along with the MTT assay which also confirmed the results and showed that the number of cells on coated microspheres increased in comparison with uncoated microspheres.

PLL was covalently incorporated into PEG diacrylate (PEGDA) hydrogels to improve their bioactivity by providing positive charges. The number of pendent PLL chains in the hydrogels was varied by photo-cross-linking PEGDA with different weight compositions of PLL. The PLL-grafted hydrogels with an optimal weight percent of 2% demonstrated the ability to promote neural progenitor cells (NPCs) viability

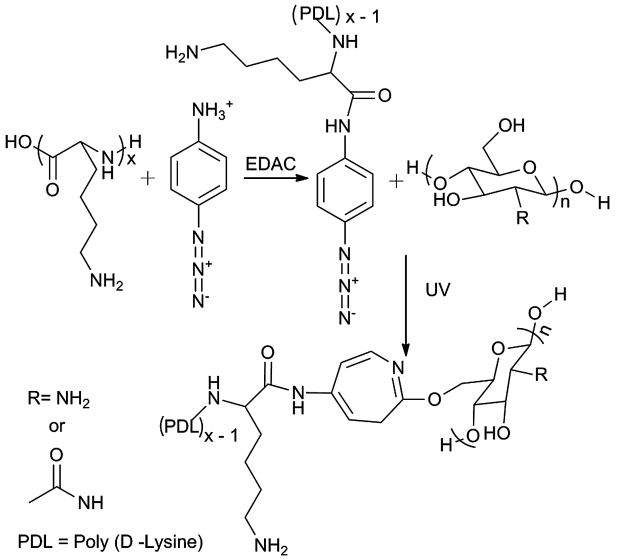


Fig. 75 Development of PDL-chotosan graft polymers by azidoaniline photocoupling reaction.

after encapsulation, attachment, proliferation, differentiation and neurite outgrowth. The size and number of neurospheres on the hydrogels showed their dependence on the weight percent of PLL. The distinct morphologies of single NPCs with typical neurite outgrowth on hydrogels with different weight percent of PLL was observed by fluorescence imaging (Fig. 76). NPC differentiation into different lineages demonstrated a parabolic or non-monotonic dependence on the weight percent of PLL.[187]

Studies were also performed with PEI which increased cell adhesion and improved the electrospinnability of PCL. Morphologies of fibroblasts on these matrices suggested that the PCL–PEI electrospun nanofibers provided a better environment for cell adhesion, proliferation and distribution as compared to the PCL indicating its potential as a scaffold for tissue engineering.[319] Moreover biocompatible PEI based scaffolds were prepared using electrospinning in the presence of succinic anhydride and 1,4-butanediol diglycidyl ether. The PEI scaffolds supported the growth and spreading of the human fibroblast cells.[259]

An investigation of the *in vivo* antibacterial effect of quaternized PEI nanoparticles incorporated in a resin composite to treat the formation of an intra-oral biofilm was performed by Beyth *et al.* The resultant resin composite showed a strong

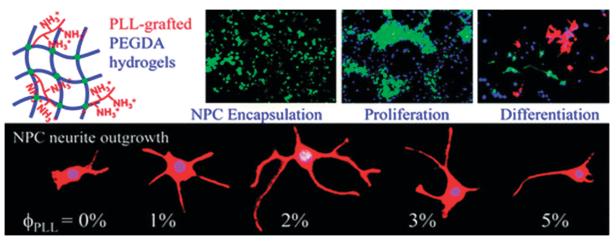
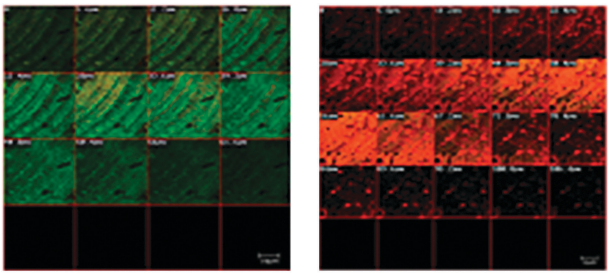


Fig. 76 Fluorescence images of NPC neurite outgrowth on the neutral and PLL-grafted PEGDA hydrogels after 7 days of culture in differentiation media. Adapted with permission from ref. 187. © 2012 American Chemical Society.



(A) (B)

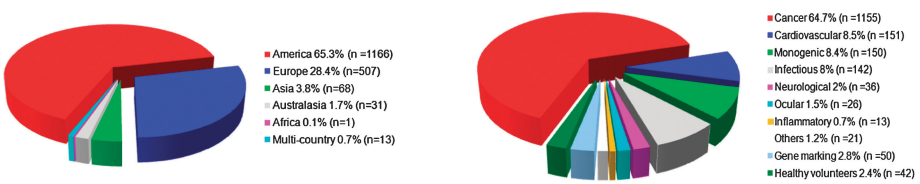
Fig. 77 Biofilms formed on resin composite incorporating quaternized PEI nanoparticles and unmodified resin composites. Confocal images of biofilms formed on (A) resin composites and (B) resin composites with incorporated QPEI nanoparticles. Adapted with permission from ref. 320. © 2010 Proceedings of National Academy of Science.

antibacterial and antibiofilm effect. Analysis of the samples collected from all 10 volunteers demonstrated a significant reduction of the viable bacteria in the biofilm formed on the surface of the resin composites with incorporated quaternized PEI nanoparticles (Fig. 77). With the results of the study, the authors claimed that more than 50% of the bacteria in the biofilm formed on the surface of the modified resin composites incorporating QPEI nanoparticles were non-viable, even in the outer, more remote parts of the biofilm.[320]

5.3 Gene delivery applications of cationic polymers

Gene therapy is based on methodologies to deliver a therapeutic nucleic acid into targeted cells. The therapeutic nucleic acid can be encapsulated with nonviral vectors such as DNA, siRNA, peptide nucleic acid (PNA) or a single-stranded oligonucleotide. In recent years, a variety of cationic polymers have been designed and developed specifically for gene delivery, and their structure–function relationships studied in detail. To date substantial research on gene therapy has entered into clinical trials with some ongoing or in the process of approval worldwide. Fig. 78A shows the statistics on gene therapy research ongoing in several countries worldwide while Fig. 78B indicates the various diseases tackled in gene therapy research. Due to the net positive surface charge of cationic polymers, nucleic acids readily attach to the cell surface *via* charge–charge interactions, thereby facilitating internalization by different endocytic mechanisms. Therapeutic nucleic acids can be either noncovalently complexed or covalently conjugated to the polymeric carrier. Release of the nucleic acid at the target site from the polymer may proceed by exchange processes against polyions, by polymer degradation, or by cleavage of the nucleic acid from the polymer attachment sites. In addition to electrostatic interaction, hydrogen bonding[321] and hydrophobic polymer interactions are also prevelant.[322]

In recent years, chitosan-based carriers have become one of the non-viral vectors of interest as a safe delivery system for genes, including pDNA, oligonucleotide and siRNA. However, gene delivery efficiency of chitosan is significantly influenced by formulation related parameters. Interaction between the positively charged chitosan backbone and negatively charged DNA/siRNA leads to the spontaneous formation of nano-size complexes (polyplexes) in the aqueous milieu. Under neutral or alkaline conditions, where chitosan is partially charged, gel electrophoresis studies showed that secondary (non-electrostatic) interactions, such as hydrogen bonding and hydrophobic interactions, could be responsible for the binding between chitosan and DNA.[323] In addition, DNA/siRNA can also be encapsulated in the chitosan matrix by using traditional methods for nanoparticle preparation. High M_W chitosans have shown to be superior to those compared to their low M_W in increasing the stability of complexes, beneficial for the protection of DNA in the cellular endosomal/lysosomal compartments, but they slow down the release of DNA once inside the cells resulting in low or delayed expression.[324] Low M_W on the other hand form complexes that are not sufficiently stable and cannot provide effective protection for DNA but provide an early release of the DNA. Hence, a balance is needed between extracellular DNA protection and efficient intracellular DNA release in order to obtain high levels of transfection.[325] Weecharangsan *et al.* found that chitosan M_W 20, 45 and 200 kDa had effective transfection efficiencies in CHO-K1 cells, whereas M_W 460 kDa had slightly higher transfection efficiency than naked DNA.[14] Another influencing factor is the DD of chitosan, higher DD results in increased positive charge per polymer chain enabling a greater DNA binding capacity and cellular uptake. Structural modifications on chitosan have also yielded appreciable results for gene delivery, Gao *et al.* observed that chitosan linked to arginine through the NH_2 group could improve its water solubility and enhance its gene transfection efficiency in HEK 293 and COS-7 cells.[326] As discussed earlier, PEI is often used as a vector modifier commonly used to improve the solubility and transfection efficiency of chitosan and its derivatives, Jiang *et al.* prepared chitosan-*g*-PEI by an imine reaction between periodate-oxidized chitosan and low M_W PEI. They reported that the copolymer possessed low cytotoxicity in three different cell lines, compared to 25 kDa PEI while the transfection efficiency of the copolymer was higher than that of 25 kDa PEI in the



(A) (B)

Fig. 78 Application of gene therapy in clinical trials (A) by continent and (B) by disease type (The Journal of Gene Medicine, © 2012 John Wiley and Sons Limited, http://www.wiley.com//legacy/wileychi/genmed/clinical/).

293T, HeLa and HepG2 cells.[327] Chitosan–DNA nanoparticles expressing pneumococcal surface antigen A (psaA) were used to immunize intranasally BALB/c mice. The results indicate that mucosal, systemic, and cellular immune responses were induced with an enhanced level by intranasal immunization with chitosan-*psaA* vaccines. Nasopharyngeal carriage was also decreased in mice indicating that nasal vaccination using chitosan could be used as an effective noninvasive route for the delivery of DNA vaccines against pneumococcal infections.[328]

Delivery of chitosan/siRNA nanoparticle aerosols into the lungs of mice has proved to be a simple noninvasive method for improved administration of exact siRNA dosage.[329] The ability of the aerosolized chitosan/siRNA nanoparticles to silence genes at pulmonary sites could have potential in pulmonary RNAi-based therapies. Utilizing the improved and selective binding of cyclic Arg-Gly-Asp (RGD) peptide with a$v\beta3$ integrin, which is overexpressed in a wide range of tumors, RGD containing polymeric formulations have been widely investigated and used in tumor targeted gene delivery. RGD labeled chitosan nanoparticles were fabricated for targeted silencing of multiple growth-promoting genes.[330] The RGD peptide was conjugated to chitosan *via* thiolation reaction using *N*-succinimidyl 3-(2-pyridyldithio)-propionate. siRNA loaded RGD-chitosan polyplexes were efficiently and selectively delivered to ovarian cancer cells in orthotopic animal models. Howard *et al.* showed that the knockdown of TNF-α expression in systemic macrophages by intraperitoneal administration of chitosan/siRNA nanoparticles in mice downregulated systemic and local inflammation.[247]

Recently, Zhang *et al.* developed cationic cellulose derivatives as gene delivery agents. Quaternized cellulose condensed DNA efficiently and displayed relatively low cytotoxicity as compared to PEI. Quaternized cellulose–DNA complexes exhibited effective transfection compared to the naked DNA in 293T cells.[51] Later they extended their study to quaternized celluloses with various M_Ws and with different degrees of substitution. The level of transfection efficiency was significantly influenced by M_W and degree of substitution of quaternized celluloses. At a similar degree of substitution, lower M_W quaternized celluloses had better transfection efficiency and less cytotoxicity compared to those with higher M_W. Cationized dextran was internalized into MSCs by using a sugar-recognizable receptor to enhance the expression level of plasmid DNA.[90] It was investigated whether transplantation of MSCs transfected using the adrenomedullin plasmid DNA enhanced the therapeutic efficacy of MSCs for a rat model of myocardial infarction. Cationic dextran was prepared by chemically introducing spermine into the hydroxyl groups of dextran using the CDI activation method. Complexation of pDNA with spermine–dextran decreased the apparent size of the complexes small enough to support cell internalization and interact with the negative charge of the cell surface promoting the transfection of plasmid DNA and enhancing the level of gene expression.

PEI has a privileged place among the components of non-viral gene delivery, due to its superior transfection efficiency in a broad range of cell types compared to other systems described later and is often referred to as the gold standard. PEI polymers are able to successfully complex DNA molecules, leading to polyplexes. Studies showed that LPEI with low M_W are the most efficient in transfection and the least cytotoxic. Kunath *et al.* indicated that low M_W PEI can transfect cell lines more effectively than the high M_W counterparts only if higher PEI amine/DNA phosphate (N/P) ratios are used.[331] Non-protonated amines with different pK_a values give PEI a buffering ability in a wide range of pH levels. This buffering capacity allows PEI polyplexes to avoid lysosomal trafficking and degradation once inside the cell due to the proton sponge effect. However, a decade ago it was observed that the high amount of positive charges and the non-biodegradability resulted in fairly high toxicity of PEI polymers *in vivo*.[332] In order to balance the transfection efficiency and toxicity, researchers have attempted to make some modifications using PEI as starting material. Höbel *et al.* systematically analyzed and determined optimal DNA and siRNA complexation conditions with regard to various parameters including buffer concentration, ionic strength, pH and incubation time. In their study it was shown that LPEI performs DNA transfection and siRNA gene targeting with identical efficacies in the presence of serum.[333] PEI derivatives with reductively cleavable cystamine periphery were designed in order to reduce carrier associated cytotoxicity as well as to enhance further the transfection activity. The derivatives were synthesized by Michael-type conjugate addition of 25 kDa PEI with *N-tert*-butoxycarbonyl-*N′*-acryloyl-cystamine (Ac-Cys-^{t}Boc) and *N-tert*-butoxycarbonyl-*N′*-methacryloyl-cystamine (MAc-Cys-^{t}Boc) in methanol, followed by deprotection (Fig. 79A). The modification reaction transforms one primary amine into one primary and one secondary amine group or transforms one secondary amine into one primary and tertiary amine group. Hence, the key features of 25 kDa PEI as gene vectors including water solubility, DNA condensation and buffer capacity are not altered significantly by the modifications. *In vitro* gene transfection in HeLa and 293T cells enhanced *in vitro* transfection under both serum-containing and serum-free conditions at an N/P ratio of 10/1(Fig. 79B).[181]

In a very recent study Jan *et al.* demonstrated that PEI–DNA complexes have potential to express human telomerase reverse transcriptase (hTERT) to transfect hair follicle stem cells and produce adequate hTERT to stimulate hair growth. The transfection efficiency was evaluated in the hair bulge region of dorsal skin in a rat model. hTERT expression was observed in the DNA–PEI-treated group compared with non-treated control. The expression of hTERT activates hair follicle stem cells mainly in the bulge region promoting a transition from telogen to anagen, which results in hair growth (Fig. 80).[334]

Dexamethasone-conjugated LPEI was characterized and evaluated as a gene carrier in various cells.[121] LPEI–dexamethasone formed stable complexes with pDNA more efficiently than LPEI alone. The conjugate showed higher transfection efficiency than LPEI, BPEI 25 k, and Lipofectamine 2000. LPEI–dexamethasone also showed the highest transfection efficiency among the carriers in A7R5 cells. Heparin was conjugated to low M_W PEI to form nanoparticles and evaluated their potential as non-viral gene carriers. These nanoparticles were used to deliver plasmid-expressing mouse survivin-T34A to treat C-26 carcinoma *in vitro* and *in vivo*. According to the

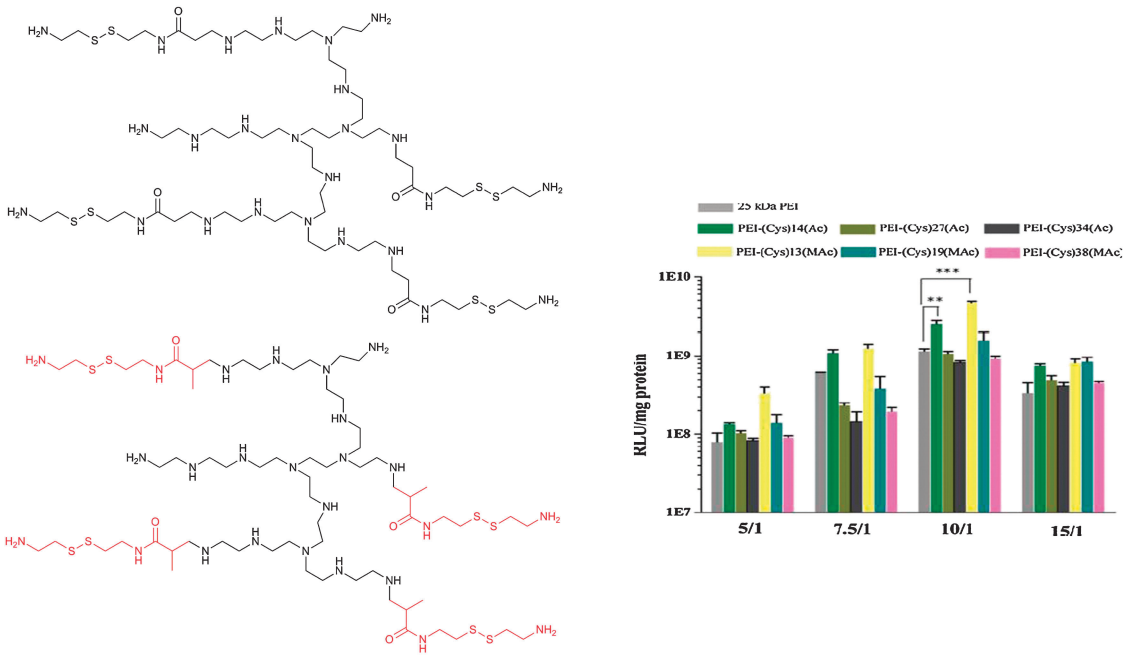


Fig. 79 (A) Reductively cleavable PEI-Cys conjugates, (B) transfection efficiencies of PEI-Cys polyplexes in HeLa cells at N/P ratios of 5/1, 7.5/1, 10/1, and 15/1 in serum-free media. 25 kDa PEI was used as a control. Adapted with permission from ref. 181. © 2011 American Chemical Society.

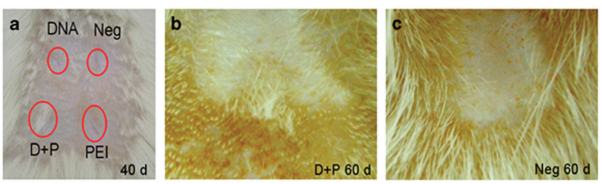


Fig. 80 Effect of hTERT–DNA–PEI complexes on (A) morphology of rat dorsal skin at day 40 after wounding, (B) apparent hair growth on day 60 after wounding (C) non-transfected negative control. Adapted with permission from ref. 334. © 2012 Macmillan Publishers Limited.

in vitro studies, the nanoparticles could efficiently transfect the pGFP reporter gene into C-26 cells, with a transfection efficiency of 31%. Intra-tumoral injection of HPEI nanoparticle-mediated mouse survivin-T34A significantly inhibited growth of subcutaneous C-26 carcinoma *in vivo* by induction of apoptosis and inhibition of angiogenesis.[335] While PEI is the gold standard for pDNA delivery it is less effective for siRNA delivery.[336] The reduced efficacy of PEI in the latter case is due to the short length of siRNA. The weak electrostatic attraction between the negatively charged siRNA and the cationic PEI polymer plays a crucial role in dissociation of PEI–siRNA complexes at the anionic cell surface. In addition to this behaviour the cytotoxicity of these systems becomes a major concern. Introduction of hydrophobic moieties into the PEI-based polymeric vector has been investigated as a feasible approach to address the cytotoxicity. Oskuee *et al.* incorporated alkylcarboxyl groups into 25 kDa BPEI to impart hydrophobicity as well as to neutralize the positive charge.[337] Investigation of various formulations having varied degree of alkyl carboxylation revealed that at low degree of carboxylation (<20%) endosomal escape operated efficiently. Interestingly, an increase of the hydrophobic alkyl chain length generally resulted in improved complex stability with siRNA.[322]

Zintchenko *et al.* prepared a number of nontoxic derivatives of BPEI through modification of its amines using ethyl acrylate, acetylation of primary amines, or introduction of negatively charged propionic acid or succinic acid groups into the polymer structure. The resulting PEI derivatives showed high efficiency in siRNA-mediated knockdown of target gene. In acrylate and propionic acid modified series, the primary amines of PEI were transformed into the secondary amines by Michael addition. In acetyl and succinic acid series, the primary amines were acetylated to amides. Among all derivatives, PEI-succinic acid could mediate knockdown at siRNA concentrations as low as 50 nM. However, the stability of polyplexes did not correlate with the efficiency.[338]

LPEI-*co*-PEG prepared by Tsai *et al.* substantially enhanced the siRNA release. The LPEI-*co*-PEG synthesized by synchrotron X-ray irradiation showed photoluminescence and was useful for intracellular trafficking. These polymers exhibited an intense green photoluminescence in the cytoplasm, localized to the endosome and/or lysosome after cell uptake, indicating that PEI-*co*-PEG was able to cross the cell membrane (Fig. 81A). For *in vivo* studies, the siRNA–PEI-*co*-PEG complexes were used to directly silence the mRNA of VEGF, which is a well-known protein in stimulating the development of new blood vessels during the tumor growth. The siRNA–PEI-*co*-PEG complexes significantly suppressed tumor growth (Fig. 81B).[119] Pegylated PEI was developed to improve the transfection efficiency of siRNA to T lymphocytes.

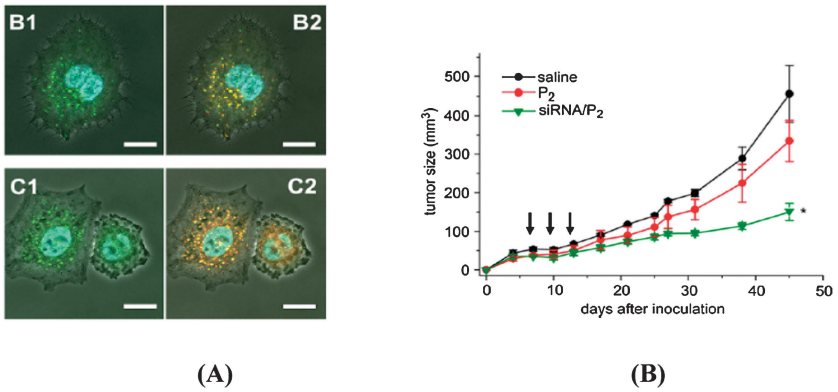


Fig. 81 (A) Confocal images of A549 and H460 and (B) tumor growth suppression of LPEI-*co*-PEG. Adapted with permission from ref. 119. © 2011 Elsevier Limited.

The PEI–PEG–siRNA polyplexes were observed to knock-down glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression and inhibit HIV replication when administered repeatedly over the time span of several weeks.[339]

Jere *et al*. prepared chitosan-*g*-PEI copolymers composed of LPEI for the delivery of siRNA. The chitosan-*g*-PEI carrier formed stable complexes with siRNA with compact spherical morphology. The polyplexes delivered EGFP-siRNA and silenced EGFP expression nearly 2.5 fold higher than PEI 25 kDa at 50 pM EGFP-siRNA concentration. Cell viability was 2 fold higher with chitosan-*g*-PEI carrier than PEI 25 kDa. The chitosan-*g*-PEI carrier efficiently delivered Akt1 siRNA and thereby silenced the oncoprotein Akt1 which significantly reduced the lung cancer cell survival and proliferation. In addition, Akt1 protein knock-down decreased A549 cell malignancy and metastasis.[340]

PNA is an attractive antigene agent which still requires a delivery system. PEI, which is an efficient candidate for DNA and siRNA delivery, still lacks applicability for PNA complexation owing to the inherent neutral charge of PNA. PEI is commonly used for cellular transfection of DNA and RNA complexes, but is not readily applicable for PNA due to its inherent charge neutrality. However, efficient PNA cellular delivery was achieved *via* chemical conjugation with PEI as shown by Berthold *et al*.[113] PEI was treated with the amine-reactive heterobifunctional linker agent *N*-succinimidyl-3-(2-pyridyldithio) propionate (with and without a PEG spacer moiety) and further reacted with a cysteine-PNA (Fig. 82). PEI was shown to be an efficient cellular delivery agent for PNA that did not require any additional lysosomolytic activity.

PEI in conjugation with myristic acid was used to deliver genes to the brain for the treatment of glioblastoma. The *ex vivo* images of the brain (Fig. 83) showed that the Rhodamine B isothiocyanate fluorescent signal was observed in the brain of the myristic acid–PEI group, in comparison to the control.[341]

Laemmli was the first to demonstrate in 1975, the exceptional ability of PLL to condense DNA.[342] Since then several groups have assessed the various structure–property relationships of PLL for gene delivery. Akinc and Langer highlighted the pH environment of PLL–DNA complexes following cellular uptake by covalently double-labeling DNA with fluorescein, a pH sensitive fluorophore, and Cy5, a pH insensitive fluorophore. The average pH surrounding PLL after cellular

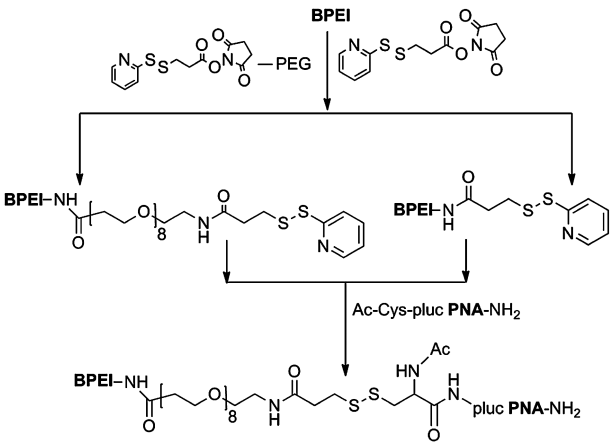


Fig. 82 Reaction pathway for the synthesis of PEI–PNA conjugates.

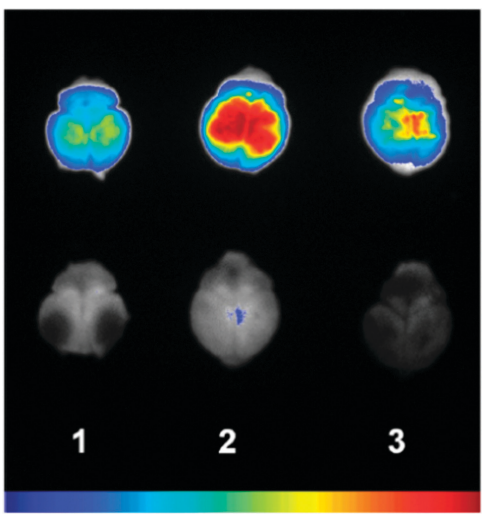


Fig. 83 The brain uptake of MC-PEI10 K/RITC–DNA (upper panel) and PEI10 K/RITC–DNA (lower panel). 1, 2 and 3 represent images at 2 h, 4 h and 8 h, respectively. Adapted with permission from ref. 341. © IOP Publishing 2012.

uptake was found to be between 4.0 and 4.5, indicating that most of the polyplex is contained in the lysosomal trafficking pathway as opposed to being released into the cytoplasm.[343] PLL with M_Ws > 3 kDa effectively condensed DNA to form

stable complexes but exhibited relatively high toxicity. To overcome such issues, the focus was on conjugation strategies with PLL or PLL based dendrimers. A triblock copolymer of PEG, poly-[(3-morpholinopropyl)aspartamide], and PLL was synthesized by Fukushima *et al.* The triblock copolymer was synthesized by the successive ROP of the *N*-carboxyanhydrides of β-benzyl-L-aspartate and ε-(benzyloxycarbonyl)-L-lysine initiated by the $-NH_2$ group of α-methoxy-ω-amino PEG (M_W 12 kDa), followed by the aminolysis using 4-(3-aminopropyl)morpholine (Fig. 84). The polyplexes showed significantly enhanced transfection activity against HeLa cells through the buffering capacity of the poly[(3-morpholinopropyl)aspartamide] segment, while efficient pDNA compaction was realized through the PLL segment.[344]

Kim *et al.* synthesized ester-linked PLL–PEG multiblock copolymers with various ratios of histidine residues to promote buffering capacity. The compounds exhibited reduced cytotoxicity and improved gene transfer efficiency as compared to underivatized PLL. *In vivo* biodistribution data of intact complexes revealed a blood circulation time of up to 3 days, suggesting that the PEG chains provide steric stabilization to the complexes *via* dysopsonization by which certain serum proteins shield the complexes from the reticuloendothelial system.[345] A ternary copolymer of PLL, LPEI and PEG was prepared and studied for the delivery of a therapeutic gene, *i.e.* the TNF-related apoptosis-inducing ligand gene. Multi-step reactions were performed to synthesize the gene delivery agent whose gene transfection was evaluated using a reporter gene assay with pEGFP-N3 in three cell lines HepG2, U251 and BHK21 (Fig. 85). Among the three cell lines, the first two were human carcinoma cells while the last one was baby hamster kidney cells. Gene delivery efficiency of the ternary polymer batches was further elevated by conjugating a targeting ligand folate that could induce specific interactions with target cells. The results showed remarkable tumor cell apoptosis and growth inhibition when the folate-encoded ternary copolymer was used to transfer a tumor apoptotic gene.[346]

Another triblock polymer of PAA–PEG–PLL was designed, synthesized, and evaluated for the delivery of siRNA.[295] PLL provided cationic primary amine groups for electrostatic interaction with negatively charged siRNA, the PAA dendrimer offered necessary tertiary amine groups for the proton sponge effect; while PEG conferred nuclease stability in blood serum. The cell viability measurements after incubation with the nanocarriers at different concentrations showed a relatively low cytotoxicity (Fig. 86A). The cellular uptake of naked and complexed fluorophore-labeled siRNA was studied in living cells using confocal microscopy. A2780 human ovarian cancer cells were incubated with free siRNA and PAA–PEG–PLL–siRNA complex and were subjected to confocal microscopy. It was found that siRNA complexed with a PAA–PEG–PLL cationic nanocarrier provided excellent cellular uptake (Fig. 86B). PLL complexation with an apoprotein E derived

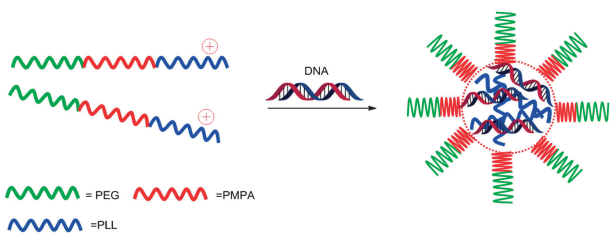


Fig. 84 Chemical structure of PEG-*b*-PMPA-*b*-PLL triblock copolymers and schematic illustration of the hypothesized three-layered polyplex micelles with spatially regulated structure.

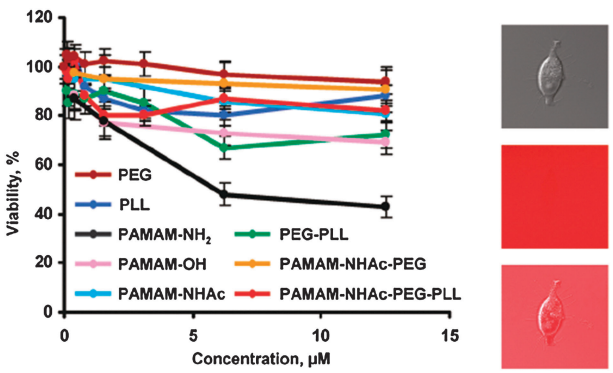


Fig. 86 (A) Viability of human cancer cells incubated with the indicated carriers and (B) cellular uptake and intracellular localization of naked siRNA and PAA–PEG–PLL–siRNA complexes. Adapted with permission from ref. 295. © 2011 American Chemical Society.

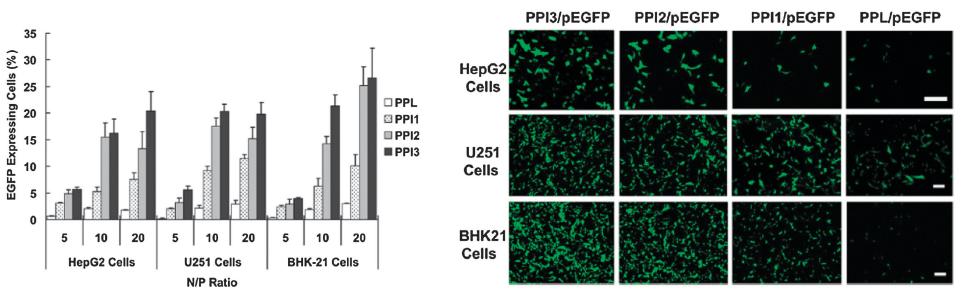


Fig. 85 PEG-*b*-PLL-*g*-LPEI (A) transfection efficiency of complexes as determined by flow cytometry in HepG2, U251s, and BHK-21 cells at various N/P ratios (5, 10, and 20) and (B) fluorescent images of EGFP expression obtained in HepG2, U251, and BHK-21 cells transfected with different polymer–DNA complexes at different N/P ratios. Adapted with permission from ref. 346. © 2010 Elsevier Limited.

peptide that targets LDL receptors to transport molecules across the blood–brain barrier showed its ability to deliver DNA to brain cells *in vivo*. PLL dendrimers have also shown to be effective gene carriers. Yamagata *et al.* studied the structure–activity relationships of PLL dendrimers for gene delivery into cells.[347] High transfection efficiency was observed in these PLL dendrimers which had a cubic octa(3-aminopropyl)-silsesquioxane core. The more globular nature promoted better DNA compaction. PLL with lipid chains was prepared by Florence *et al.* to improve biodegradability.[140] These dendrimers formed polyplexes with DNA and exhibited a sustained release.[348]

PAAs with hydroxyl and histidine showed transfection efficiency of relevant polyplexes with reduced cytotoxicity.[349] Random and block copolymers containing histidine and tertiary amine terminated side chains also improved transfection efficiency.[145] Haartmen *et al.* prepared PAA conjugates with PEO for double-stranded plasmid DNA condensation with different combinations of the three amine functionalities.[350] The group of Peng *et al.* developed flexible polycationic PAA dendrimers with triethanolamine as the core, for siRNA delivery.[351] They observed formation of strong siRNA–dendrimer complexes at neutral pH and the efficient internalization of the siRNA molecules into the cytoplasm. Structurally flexible PAA dendrimers (G7) with the triethanolamine core were also effective in delivering heat-shock protein 27 (Hsp27) siRNA into human prostate cancer (PC-3) cells, leading to specific silencing of the Hsp27 gene and a pronounced caspase-dependent apoptosis-induced anticancer effect.[352]

Bioreducible PAAs have been prepared by introducing disulfide bonds in the main chain. The random copolymers of bioreducible PAA with various ratios of histamine and 3-(dimethylamino)-1-propylamine (DMPA) were synthesized by Michael-type addition between N-CBA and a mixture of histamine and DMPA. The PAA copolymers were able to transfect COS-7 cells *in vitro* with high efficiency.[353] The same group went further to investigate the PAAs with disulfide linkages and various oligoamines in the side chain. These PAAs with aminoethylene units in the side chains showed high buffer capacities and lead to high levels of gene expression. They observed that the elongation of the alkyl spacer between the amino groups in the side chain from ethylene to propylene resulted in lower transfection efficiencies.[145] Peist and Engbersen investigated the effects of variation in charge density and hydrophobicity on the gene delivery properties of these polymers by varying the degree of acetylation and benzoylation in disulfide containing PAAs with aminobutyl side chains. For both the acetylated and benzoylated derivatives well-defined nanosized polyplexes could be formed with pDNA. Transfection efficiencies in COS-7 cells in the presence and absence of serum showed that the benzoylated derivatives gave much higher transfection efficiencies than the acetylated derivatives (Fig. 87).[354]

The modification of disulfide-containing PAA *via* grafting with dendritic PAA was performed by Xue *et al.*, for highly efficient gene delivery. The advantage of introducing dendritic PAA side chains into disulfide-containing PAA combined the biodegradation of disulfide-containing PAA and high DNA binding ability, high buffer capacity and low cytotoxicity of dendritic PAA. The polymers condensed DNA into small sized polycation–DNA complexes, which degraded and released the incorporated DNA under reductive conditions.[355] Hydrophobic modification of PAA dendrimers was accomplished by the group of Santos *et al.* They functionalized the outer shell using hydrophobic alkyl chains that varied in length and number for efficient gene delivery to mesenchymal stem cells.[356]

Langer *et al.* conducted the first investigations into PAE for gene delivery. A library of PAE showed members with gene transfer efficiencies comparable to PEI, PLL, and Lipofectamine 2000 in both COS-7371 and HUVEC cell lines.[357] Zugates *et al.* also prepared a library of PAE and assessed their utility as gene delivery vehicles. Polymers were synthesized using a rapid, two-step approach that involves initial preparation of an acrylate-terminated polymer followed by a post-polymerization amine-capping step to generate end-functionalized polymers. Variations in the terminal amine structure were shown to affect many properties that are important for effective polymeric gene delivery. These modifications

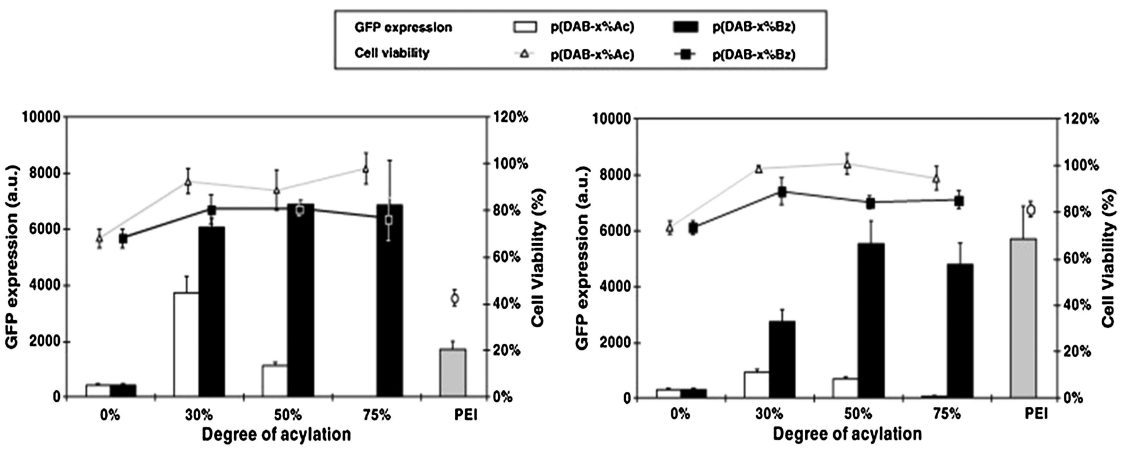


Fig. 87 Effect of acylation on transfection efficiencies in COS-7 cells measured by GFP expression in the absence (A) and presence (B) of serum. Acetylated polymers (white) are compared with benzoylated polymers (black). Adapted with permission from ref. 354. © 2011 Elsevier Limited.

effectively decreased the polymer–DNA nanocomplex size and increased cellular uptake.[358] Kim *et al*. evaluated two biodegradable ester-bonded polymers synthesized by double-monomer polycondensation for a nonviral gene delivery system. The backbone was constructed to include inner tertiary amines and outer primary amines. The transfection efficiency was investigated by the firefly luciferase reporter gene expression in two cell lines, HepG2 and C2C12. Self-assembly with DNA resulted in the production of regularly nano-sized spherical polyplexes with good transfection efficiency, especially in the presence of serum. The polymers showed relatively slow degradability and the complex was stable up to 7 days under physiological buffer conditions.[359] Later Lee *et al*. prepared a multi-component nanoparticulate system using PAEs as a delivery enhancer, and gold nanoparticles to assemble siRNA strands into cells. To verify cellular transfection of the PAE-siRNA gold nanoparticles, gene knockdown was evaluated in a modifed HeLa cell line, where the HeLa cells were genetically engineered to express both firefly luciferase and Renilla luciferase.[208]

Due to its inherent cationic charge, PDMAEMA offers significance as a gene transfer agent. The mechanism of gene transfer for methacrylate polyplexes proceeds by both clathrin- and caveolae-dependent pathways.[360] Dubruel *et al*. varied various percentages of the ammonium groups of PDMAEMA to pyridine and imidazole functionalities to improve endosomal escape. Combination of DMAEMA with pyridine containing methacrylate (*N*-(2-hydroxyethyl) nicotinamide methacrylate, HENIMA) significantly reduced transfection efficiency, while imidazole derivatization (4-methyl-5-imidazoyl methyl methacrylate, HYMIMMA) eliminated transfection.[157,164] Introduction of PEG moieties onto PDMAEMA suppressed aggregate formation while decreasing cytotoxicity. PEGylated PDMAEMA was prepared by ATRP and the effects of PEGylation on transfection efficiency *in vitro* delivered with a GFP expression plasmid were reported. The immunogenicity *in vivo* after complexation with a HIV gag gene DNA vaccine was also studied. For *in vivo* studies, mice were intranasally vaccinated with the polymer formulation containing plasmid expressing HIV-1 CN54 Gag and intramuscularly boosted with recombinant Tiantan vaccinia vector expressing the identical immunogen. It was found that PEGylated PDMAEMA used as DNA delivery vector significantly improved the priming effect and thereby increased the immunogenicity of DNA vaccine through intranasal administration.[166]

Block polymers of methoxy PEG-*b*-(PCL-*g*-PDMAEMA) were synthesized by combining ROP and ATRP methods to form nanoparticles to study their transfection efficiency. The polymeric nanoparticles could effectively condense DNA to form compact complexes with sizes ranging from 65–160 nm. The *in vitro* gene transfection studies using HeLa and HepG2 cells showed that polymeric nanoparticles had better transfection efficiency compared to PEI and Lipofectamine 2000 at low doses. However, *in vitro* gene transfection efficiency showed a dependency on the type of cell lines, the M_W of PDMAEMA grafts and N/P ratios of carriers.[167] Benito *et al*. extensively contributed in the area of cyclodextrins for gene delivery applications. A detailed review was published in 2010 which highlights the developments with cyclodextrin as a gene delivery agent.[361] Díaz-Moscoso *et al*. prepared cationic cyclodextrin with fine-tuned properties such as density of cationic groups, flexibility, and the presence of additional hydrogen-bonding functionalities while keeping a C7-symmetric disposition to be served as gene delivery agents. Transfection efficiencies of these polymers were tested on BNL-CL2 and COS-7 cell lines and the results indicated comparable transfection efficiencies to PEI and JetPEI with low cytotoxicity.[62] The same group incorporated a triazole-thiourea by a ''dual click'' approach, through sequential CuAAC; thiourea-forming reactions, that provided scope for adjustment of the molecular topology to optimize pDNA complexation and delivery. Yang *et al*. prepared star polymers by conjugating multiple OEI arms with chain lengths ranging from 1 to 14 ethylenimine units onto a α-CD core to investigate them as non-viral gene delivery vectors. The star polymers showed excellent gene transfection efficiency in HEK293 and Cos7 cells. The transfection efficiency increased with an increase in the OEI arm length and branching.[57] Cyclodextrin polymers have also been developed as siRNA delivery agents. Heidel *et al*. used cyclodextrin–transferrin nanoparticles to study the effects of siRNA delivery on the immune system of cynomolgus monkeys.[362] Intravenous delivery of siRNA targeting the M2 subunit of ribonucleotide reductase was delivered in escalating doses, and it was further established that multiple, systemic doses of targeted nanoparticles containing non-chemically modified siRNA could be safely administered to non-human primates. Cyclodextrin–transferrin nanoparticles demonstrated efficacy in knocking down luciferase and ribonucleotide reductase genes in mice.[363,364]

6. Concluding remarks and future perspectives

The central aim of this review was to bring forward some of the recent advances in the field of cationic polymer-mediated therapeutic applications. In summary, various strategies have been assembled to synthesize cationic polymers with desired properties, including the development of biodegradable cationic polymers with reduced toxicity, incorporation of cell targeting and additional transport domains for effective and specific delivery, and improved endocytosis. The impressive developments with this class of polymers are closely linked to the broad range of properties they offer and the possibility of tenability leads to many areas of applications for therapeutic needs. Continued studies in multi-disciplinary areas of cationic polymers, such as their role in cellular processes and establishment of structure–function relationships, will provide a better guideline for further designs. Studies to overcome subcellular barriers, including endosomal escape and nuclear translocation, should be carefully considered when designing cationic polymers.

However, the success of cationic polymers has been traditionally hindered by the non-degradability and toxicity associated with some formulations. In the past few years, modifications to commonly applied delivery systems have been made and novel carrier systems have been developed to overcome these drawbacks. The toxic effects are also linked somewhat to biodegradable ability. Cationic polymers with structural modifications lead to polymers that can be degraded

and are less toxic, and this can be considered as an efficient approach for several therapeutic applications. Cationic polysaccharides and cationic natural polymers have displayed biodegradability and low toxicity, and may be widely used in the future. In summary, all these modifications have been highlighted in the present review along with the structural properties of the cationic polymers and their therapeutic applications. This review will contribute to a further understanding of the various breakthrough research studies to date.

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