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Review Article

Macromolecular Gene Delivery Systems: Advancing Non-viral Therapeutics with Synthetic and Natural Polymers

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Abstract

Macromolecular-based gene delivery systems have emerged as viable alternatives to non-viral vectors for gene therapy due to their versatility, biocompatibility, and capacity to efficiently deliver therapeutic cargo. These systems, primarily based on synthetic and natural polymers, offer significant advantages in terms of safety, controlled gene release, and targeted delivery. This review explores the design and synthesis of macromolecular carriers, focusing on their chemical and physical architectures, which play a key role in improving gene delivery. Catanionic polymers and their derivatives (comb, brush, and star polymers) have been extensively researched for their capacity to condense and protect genetic material. Furthermore, natural polymers like chitosan and hyaluronic acid have been modified to enhance gene delivery capabilities. These macromolecular carriers are engineered to boost circulation time, increase cellular uptake, and facilitate the controlled release of genetic material at the target site. Strategies such as incorporating targeting ligands, stimuli-responsive elements, and reducing cytotoxicity are being pursued to improve the overall efficiency and specificity of these systems. This review highlights the current state of macromolecular gene delivery systems, their applications, and the ongoing research aimed at overcoming existing challenges, paving the way for more effective non-viral gene therapies.

Introduction

Genetic disorders present significant challenges to human health, often leading to chronic conditions with limited treatment options. Conventional therapies relying on small molecules or protein-based drugs have shown only partial success, frequently addressing symptoms without providing lasting solutions or cures. Gene therapy has emerged as a revolutionary approach to overcoming these challenges by directly targeting the underlying genetic abnormalities.[1,2] By introducing, repairing, or modifying specific genes, this strategy holds the potential for durable therapeutic effects and, in some cases, curative outcomes. However, the efficiency of gene therapy depends heavily on the safe and efficient delivery of therapeutic genes to the target cells or tissues.[3]

Delivering genetic material faces several biological barriers, including enzymatic degradation, rapid clearance from the bloodstream, and cellular membrane impermeability. Naked DNA or RNA introduced directly into the body is typically subject to rapid clearance and transient expression, limiting therapeutic efficacy.[4] To address these issues, researchers have developed various delivery systems that encapsulate and protect genetic material while facilitating its targeted transport. Although viral vectors such as retroviruses and adenoviruses have demonstrated high transfection efficiency due to their natural ability to invade cells, their drawbacks—including immunogenicity, limited cargo capacity, and risks of insertional mutagenesis—have driven the pursuit of alternative strategies.[5]

Non-viral gene delivery systems, particularly those based on macromolecular carriers, have gained traction as promising alternatives to viral vectors.[6] Synthetic macromolecules, such as polymers, lipids, and peptide-based carriers, are being extensively studied for their ability to condense, protect, and transport nucleic acids to target cells with reduced immunogenicity and enhanced tunability. These materials offer numerous advantages, including scalability, design flexibility, and potential for functionalization to improve targeting, biocompatibility, and controlled release.[6,7]

Despite substantial progress, a significant gap remains in the clinical translation of macromolecular gene delivery systems. Many polymer-based carriers demonstrate high efficiency *in vitro* but face challenges such as poor stability, limited biodistribution, inefficient endosomal escape, and suboptimal gene expression *in vivo*. Moreover, a lack of systematic comparisons among various

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Table 1. Comparison of the advantages and disadvantages of gene delivery carrier systems

Carrier type	Advantages	Disadvantages
Viral vectors	Powerful *in vivo* transfection efficiency. Long-term transgene expression	Immune response. Inefficient transduction. Size limitations. Low gene loading capacity[10]
Lipid-based nanocarriers	High load capacity. Degradability. Easy to modify structure and charge	Toxicity at high dose[11,12]
Natural polymer-based nanocarriers	Biocompatible and biodegradable. Minimal immunogenicity. Efficient condensation and protection of genetic material. Stimuli-responsive degradation	Limited gene loading capacity. Lower transfection efficiency compared to viral vectors. Variable batch-to-batch consistency. Susceptibility to enzymatic degradation *in vivo*[13]
Synthetic polymer-based nanocarriers	Powerful gene chelation ability. Improved endosomal escape	Cytotoxicity. Complex preparation[5–8]

macromolecular systems, regarding their *in vivo* performance, toxicity profiles, and long-term safety, hinders the field. Bridging this gap will require deeper insights into the structure–function relationships of these materials, as well as comprehensive preclinical studies that closely replicate physiological conditions.

This review focuses on the use of macromolecules in DNA-based gene delivery, highlighting their physicochemical properties, mechanisms of action, and therapeutic potential. It examines key synthetic polymers such as polyethyleneimine (PEI), chitosan, and dendrimers, alongside lipid-based systems and hybrid nanomaterials. By addressing both the advancements and ongoing challenges in this area, this review aimed to provide insight into the potential of macromolecular systems to revolutionize gene therapy and improve the treatment of genetic disorders.

Role of macromolecules in gene delivery systems

Biotechnological advancements in recent decades have significantly contributed to the rapid growth of pharmaceutical research based on polymeric DNA, RNA, peptide, and protein molecules.[8] These biopolymers are critical components of drug delivery, functioning as carrier materials, active pharmaceutical ingredients, and targeting agents. The U.S. Food and Drug Administration categorizes polymers into several groups, including vaccines, therapeutic cell preparations, allergenic extracts, DNA therapeutic preparations, blood products, and infectious pathogen detection reagents.[9] Their unique properties—such as high affinity, target specificity, and multifunctionality—have positioned macromolecules as promising therapeutic options, particularly for treating complex diseases like cancer, which remains a leading global health concern.[10] A summary of the advantages and disadvantages of gene delivery vectors is provided in Table 1.[5–8,10-13]

Non-viral gene delivery technologies have attracted considerable attention due to their greater biocompatibility and lower immunogenicity compared to viral vectors. These approaches enable the safe and efficient transport of genetic material using a variety of materials, including lipids, cationic polymers, and plasmid-based complexes.[11] Physical non-viral delivery techniques, such as gene guns, microinjection, sonoporation, and electroporation, facilitate the direct introduction of genetic material into cells.[12] For example, microinjection allows precise delivery of genetic material into specific cells, while ballistic DNA injection involves shooting gold-coated DNA particles into target tissues. Other techniques, including electroporation, sonoporation, and photoporation, use electrical, sound, or laser pulses to temporarily permeabilize the cell membrane. Advanced techniques such as magnetoporation and hydroporation enhance delivery by utilizing magnetic fields or hydrodynamic forces to drive nucleic acids into cells.[13]

Chemical non-viral gene delivery methods employ synthetic or natural materials to form vectors that promote gene transfer via endocytosis (Fig. 1). Two major types of chemical vectors are liposomes and polymers. Liposomal vectors form lipoplexes, which encapsulate and protect genetic material while enhancing cellular uptake. Similarly, polymer-based vectors form polyplexes by interacting with DNA, promoting efficient gene transfer. These non-viral strategies offer scalable, tunable, and safer alternatives to viral vectors, and they are driving innovation in therapeutic gene delivery.[12,14]

Targeting strategies of macromolecules for non-viral gene therapy

Functional polymers have emerged as promising tools for enhancing the efficiency and specificity of gene transfer. Their versatility supports the development of innovative non-viral delivery systems designed to address critical challenges in gene therapy (Table 2).[15–24] By incorporating targeting ligands or moieties, polymers and their resulting polyplexes can be engineered to selectively bind to specific cell types and surface receptors, thereby reducing off-target effects and improving therapeutic outcomes. Moreover, these polymers can help direct genetic material to precise intracellular locations, such as the nucleus, facilitating effective gene expression.[25,26]

Natural polymers

Natural polymers have gained significant attention for gene transfer applications due to their biodegradability and low toxicity.[27] These polymers often contain functional groups that can be chemically modified to enhance their physicochemical properties. Cationic polymers, whether linear or branched, typically possess amine groups that can be protonated under acidic conditions. The number of protonatable groups varies among cationic polymers, resulting in a distribution of positive charges along their main chains and branches.[28] Chitosan and pullulan are notable natural polymers known for their high biodegradability and low toxicity. Similarly, synthetic polymers can be engineered to improve degradability and biocompatibility, making them attractive candidates for gene therapy. Polysaccharides can adopt helical forms and are classified as either neutral (e.g., dextran) or cationic (e.g., chitosan).[29]

Chitosan

Chitosan, a poly-D-glucosamine derived from the deacetylation of

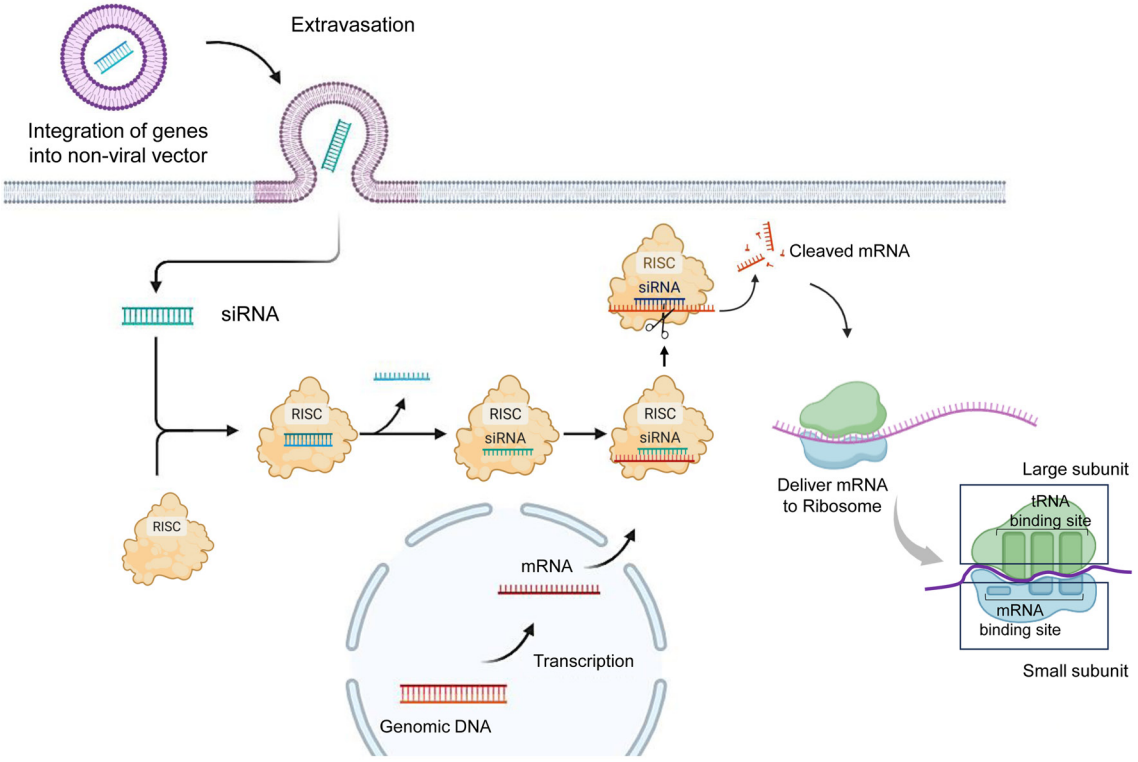

Fig. 1. A schematic representation illustrating the key steps involved in non-viral gene delivery. mRNA, messenger RNA; RISC, RNA-induced silencing complex; siRNA, small interfering RNA; tRNA, transfer RNA.

chitin, has received considerable attention as a gene carrier due to its unique structural and functional properties. As a cationic copolymer of D-glucosamine and N-acetylglucosamine, chitosan is well-known for its biodegradability, biocompatibility, mucoadhesiveness, and antibacterial properties. Several factors influence its transfection efficiency, including the degree of deacetylation, molecular weight, plasmid concentration, amine-to-phosphate charge ratio, serum concentration, pH, and cell type.[29] One of chitosan's distinguishing traits is its pH-sensitive behavior, attributed to its pKa of approximately 6.5, which allows for a reversible

Table 2. Various polymeric materials used in non-viral gene delivery

Polymer type	Polymers example	Properties	Applications in gene delivery	Ref
Cationic polymers	Polyethylenimine (PEI), Poly(L-lysine) (PLL)	High positive charge density, facilitates DNA/RNA condensation, high transfection efficiency	Delivery of plasmid DNA, siRNA, and mRNA. Effective in endosomal escape via the proton sponge effect	15,16
Comb polymers	Polysiloxanes, Poly(methacrylates)	Unique architecture with linear backbone and branched side chains, customizable functionality	Surface modification for cell targeting, improved stability and efficiency in complexation with nucleic acids	17
Natural polymers	Chitosan, Hyaluronic acid, Dextran	Biodegradable, biocompatible, inherently low toxicity, functionalizable	Delivery of siRNA and plasmid DNA, especially in regenerative medicine and localized therapeutic applications	18,19
Hyperbranched polymers	Poly(amidoamine) (PAMAM)	High surface functional groups, tunable molecular weight, efficient gene complexation	Efficient gene complex formation, targeted delivery systems, multifunctional nanocarriers	20
Brush polymers	PEG-grafted polymers, Polyoxazolines	Dense polymer brushes, steric stabilization, tunable surface properties	Enhancing circulation stability, reduced immunogenicity, and targeted delivery of therapeutic genes	21,22
Star polymers	Polylysine-based star polymers	Multiple arms radiating from a central core, high drug-loading capacity	High gene loading, improved cellular uptake, and specific delivery to cancer or diseased tissues	23,24

mRNA, messenger RNA; PEG, polyethylene glycol; siRNA, small interfering RNA.

soluble–insoluble transition around pH 6.0–6.5. This characteristic improves its applicability in tissue engineering, drug delivery, and gene transfection. Its mucoadhesive properties also make it a promising candidate for nucleic acid-based therapies delivered via oral and nasal routes.[30] Various chemical modifications have been developed to enhance its gene delivery efficiency. Common techniques include methylation, PEGylation, and histidination. These modifications improve polyplex stability, facilitate endosomal escape, and enhance cellular uptake. Notably, PEGylation increases water solubility and polymer half-life, significantly boosting chitosan's effectiveness as a gene delivery vehicle.[29,30]

Nguyen *et al*.[31] encapsulated miR-33 in polyethylene glycol–chitosan nanoparticles using sodium tripolyphosphate. This delivery system effectively targeted mouse macrophages, inhibited ATP-binding cassette transporter A1 expression, and reduced liposterol export, thereby influencing cholesterol metabolism.[31] Similarly, Zhou *et al*.[32] developed trimethylchitosan nanoparticles modified with the arginine–glutamic acid–aspartic acid–valine peptide and polyethylene glycol (PEG) to deliver miR-126 to vascular endothelial cells. This approach enhanced cell proliferation and reduced ischemic myocardial necrosis.[32] Kritchenkov *et al*.[33] investigated both hydrophilic and hydrophobic covalent modifications of chitosan, demonstrating that hydrophilic modifications, such as PEG conjugation, significantly improved chitosan's solubility. PEGylation increased solubility, reduced nanoparticle size, and lowered zeta potential, while maintaining small interfering RNA (siRNA) binding capacity. However, excessive PEGylation diminished cellular uptake and transfection efficiency, highlighting the need for optimization. Other hydrophilic modifications explored for gene delivery include conjugation with dextran and polyvinylpyrrolidone.[34]

Dextran

Dextran, a non-immunogenic polysaccharide, is widely used in gene transfection and drug delivery due to its excellent water solubility and low toxicity. Its structure comprises α-D-glucose units linked by α-(1→6)-glycosidic bonds, with occasional branching via α-(1→4) or α-(1→3) linkages. Dextran is biosynthesized by Gram-positive bacteria such as *Leuconostoc and Streptococcus*, which utilize sucrose as a substrate. To improve its transport qualities, dextran can be chemically modified through etherification, esterification, amidation, and oxidation.[35] Although inherently neutral, dextran can be functionalized with positively charged groups, such as diethylaminoethyl dextran or aminoethyl methacrylate, to enable electrostatic interactions with genetic material.[36] A significant application involves combining docetaxel, chloroquine, and autophagy related 5-targeting siRNA within a delivery system composed of carboxymethyl-β-dextran and the triple-drug carrier protamine sulfate. This platform, which leverages both hydrophobic and electrostatic interactions, demonstrated potent anticancer efficacy *in vitro* against triple-negative breast cancer (MDA-MB-231) cells and *in vivo* in a mouse xenograft model. The system significantly suppressed tumor growth while maintaining biological safety, making it a promising approach for treating triple-negative breast cancer.[37]

A pH-sensitive, biocompatible dextran-based nanocarrier has also been developed for prostate cancer gene delivery. Urea-linked ligands on the nanocarrier bind to Prostate-Specific Membrane Antigen. This system uses a 40 kDa dextran backbone modified with acetal-linked amine groups, which undergo pH-triggered cleavage in the acidic endosomal environment to release encapsulated siRNA. The delivered siRNA effectively downregulated programmed cell death ligand 1 and membrane cofactor protein (CD46), two key genes implicated in cancer cell immune evasion.[38,39] Furthermore, dextran and chitosan nanoparticles have been developed for microRNA (miRNA) delivery. These systems incorporate a redox-responsive polyelectrolyte complex made of thiolated dextran, chitosan, and miR-145, a tumor-suppressing miRNA. To improve targeting efficiency, the nanoparticles are functionalized with the anti-nucleolin aptamer AS1411 (apt-PEC), allowing for more precise delivery and enhanced intracellular expression of miR-145.[40,41]

Hyaluronic acid (HA)

HA is a linear, anionic polymer composed of non-sulfated glycosaminoglycan chains containing repeating disaccharide units of N-acetyl-D-glucosamine and D-glucuronic acid. It exhibits a broad range of molecular weights, from 5 kDa to 20,000 kDa, each with distinct physicochemical properties. Low-molecular-weight HA is highly water-soluble, whereas HA molecules above 200 kDa exhibit excellent water-binding capacity, making them crucial for hydration processes.[42,43] Commercially available HA with molecular weights over 1.8 MDa is particularly noted for its biocompatibility, biodegradability, and lack of inflammatory, toxic, or immunogenic responses.[44,45] HA and its derivatives are widely applied in healthcare, including in viscosity supplements, ocular surgery, and drug delivery. They facilitate the transport of various therapeutic agents such as antibiotics, antiglaucoma drugs, vasodilators, cytokines, and enzymes in both *in vitro* and *in vivo* settings. Furthermore, HA is essential for cell adhesion, proliferation, and migration, underscoring its multifaceted significance in biomedical applications.[46]

HA has a high affinity for the CD44 receptor, which is overexpressed in many tumor cells, making it an ideal targeting ligand for nanoparticle systems.[47] This receptor-specific binding enhances cellular uptake, while HA's intrinsic negative charge extends circulation time and protects against degradation by reactive oxygen species and hyaluronidase, even under extreme pH conditions. HA nanoparticles are widely employed as carriers in drug delivery systems, either independently or in combination with copolymers. These systems have proven effective in targeting tumors and delivering xenobiotics, genes, and prodrugs, overcoming drug resistance in cancer therapy.[48] Kim *et al*.[49] developed HA–chitosan nanoparticles loaded with plexin domain containing 1 siRNA for targeted delivery to CD44 receptors on tumor endothelial cells. This novel antiangiogenic strategy for ovarian cancer successfully delivered siRNA to cancer-associated endothelial cells, protected the siRNA during circulation, silenced the target gene, and inhibited tumor angiogenesis, thereby reducing tumor cell migration and invasion.[49] HA has been extensively studied for various applications, including its physicochemical characteristics, receptor interactions, industrial production methods, degradation pathways, biosynthesis, and cosmetic use.[50] Knopp-Marques *et al*.[51] conducted a comprehensive review that highlighted the versatility of HA and its derivatives in biomaterials, particularly for hydrogels and coatings designed for controlled cytokine release in implantable devices. These innovations aim to minimize immune responses and promote tissue regeneration.[51]

Catatonic polymers

Cationic polymers play a crucial role in gene delivery by forming electrostatic complexes with negatively charged genetic materials such as DNA or RNA, resulting in stable polyplexes. These polymers enhance cellular uptake via endocytosis, protect nucleic acids from enzymatic degradation, and facilitate endosomal escape (Fig.

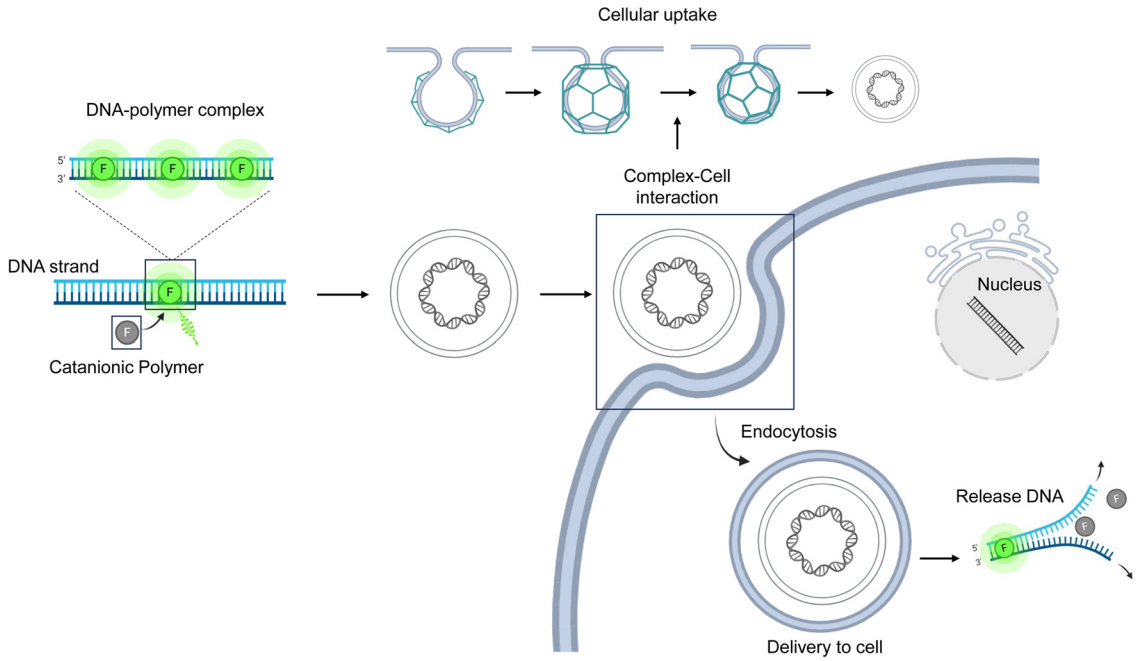

Fig. 2. Schematic diagram of gene delivery using catanionic polymers.

2). Examples include PEI and poly-L-lysine (PLL), both widely used due to their efficiency and tunable properties (Table 3).[52–58]

PLL

PLL is a synthetic, linear polypeptide composed of repeating L-lysine residues. Its strong affinity for negatively charged DNA enables the formation of stable complexes suitable for gene transfer, particularly with DNA molecules larger than 3,000 Da. Despite its potential, PLL faces several practical challenges, including serum instability, limited endosomal escape, and inherent cytotoxicity. To overcome these issues, chemical modifications, such as PEGylation, have been investigated to improve serum stability and reduce toxicity. Additionally, the incorporation of reducing or pH-sensitive groups has been shown to enhance transfection efficiency and cell targeting.[52,59]

Both high molecular weight PLL and its low molecular weight analogue, oligolysine, have been extensively studied for their ability to condense DNA into nanoparticles. These nanoparticles exhibit diverse morphologies, including toroids, spheroids, cubes, and rods, as demonstrated by Nayvelt *et al*.,[60] highlighting PLL's adaptability for designing custom delivery systems. Furthermore, Korolev *et al*.[61] showed that PLL–DNA interactions occur under both salt-dependent and salt-independent conditions, offering insight into its binding mechanisms in physiological environments. Recent developments have focused on improving the efficacy and safety of PLL-based systems. For instance, modifying PEI with PLL has significantly enhanced transfection efficiency while reducing cytotoxicity, as shown in studies involving HeLa cells. One notable application includes the combinational suicidal gene therapy strategy for glioblastoma, developed by Malik *et al*.,[62] which employed genetically engineered mesenchymal stem cells.

Kodama *et al*.[63] synthesized dendrigraft poly-L-lysine that forms a ternary complex with γ-PGA and DNA, achieving high transfection efficiency across various tissues. Copolymerization strategies have also shown promise; for instance, Yu *et al*.[64] grafted PLL onto chitosan, combining PLL's strong DNA-binding capability with chitosan's biocompatibility and biodegradability. This copolymer demonstrated improved transfection efficiency and reduced cytotoxicity compared to its individual components. Moreover, PLL's positively charged amino groups facilitate the formation of replication particles at physiological pH, which co-adsorb plasmid DNA and support the development of cross-linked PLL-based gene delivery systems.[64] These advances underscore PLL's versatility and continued potential in non-viral gene delivery platforms.

PEI

PEI is a cationic polymer containing secondary amino groups and ethylene moieties (-NH-CH_2CH_2-), frequently used as a transfection agent and nanocarrier in drug delivery systems.[65] Its utility arises from the high positive charge density resulting from the protonation of amine groups, which enables strong interactions with negatively charged DNA or RNA.[66] However, PEI is often limited by its inherent cytotoxicity, necessitating chemical modifications to improve biocompatibility and broaden its therapeutic applications.[67,68] For example, Zhou *et al*.[69] developed a cyclic amine-modified PEI derivative that demonstrated low toxicity and inhibited CXCR4-mediated tumor cell invasion, showcasing the polymer's oncological potential. Another innovation, Glc-PEG-PEI, created by Gupta *et al*.,[70] is a liver-targeted gene delivery system comprising galactose, PEG, and PEI. This formulation exhibited superior transfection efficiency in hepatocytes compared to unmodified PEI, making it a promising candidate for liver-specific therapies.[70]

Further breakthroughs include PEI-based copolymers, such as those developed by He *et al*.,[71] who created poly(5-methyl-5-allyloxycarbonyl trimethylene carbonate) (hereinafter referred to as PMAC) and enriched it with PEI to generate *PMAC-g-PEI*. In 293T cells, this modified polymer showed enhanced transfection efficiency and reduced cytotoxicity. A subsequent modification for differentiated thyroid cancer (hereinafter referred to as DTC)

Table 3. ***In vitro*** **and** ***in vivo*** **biological targets for modified polymer for gene delivery**

Modifications	Polymer	*In vitro* targets	*In vivo* targets	Outcome	Ref
PEG, lactose, targeting peptides and antibodies, folic acid, mannose, arginine, cholesterol, targeting peptides and proteins	Dendrimers (PAMAM); Polyamidoamine	Mesenchymal stem cells, cytokine-activated primary human saphenous vein endothelial cells, various cell lines	Intramuscular gene silencing in mouse, gene therapy in tumors	Efficient gene delivery and silencing in muscle and tumor tissues	53
Poly(DMAEMA), arginine, cyclodextrin, PEI, chitosan, targeting peptides/proteins, lipid carriers, PAMAM dendrimers, adenovirus, folic acid	PEG	Brain capillary endothelial cells, Kupffer cells, primary smooth muscle cells, macrophages, various cell lines	Gene therapy in tumor-bearing mice/Wistar rats, intramuscular gene silencing in mouse, reporter gene expression in organs	Multi-organ gene expression and tumor gene therapy with systemic and local effects	54
PEG (branched/crosslinked), thiol-reactive side chains, spermine	PLL	Embryonic/adult stem cells, adipose-derived stromal cells, neuronal cells, various cell lines	Reporter gene expression in mouse muscle, wound healing, and tumor gene therapy	Targeted gene expression in tissue regeneration and cancer	55
PEG, disulfide linkage, PAMAM dendrimer	Poly(β-aminoesters)	Various cell lines	Rabbit injured vessel	Gene delivery to vascular injury site, useful in cardiovascular therapy	56
PLL, arginine, guanidylated, PEG, histidine, cysteine, glutathione, glutamic acid, galactose, targeting peptides/proteins, biotinylated, chondroitin sulfate, chitosan nanobubbles, PEI, lipid shells, spermine	Chitosan	Macrophages, adipose-derived mesenchymal stem cells, various cell lines	Anti-apoptotic Bcl-2 gene knockdown in mice, autoimmune diabetes, aerosol lung delivery, topical and endovascular gene expression in rats/rabbits	Versatile platform for gene knockdown, respiratory, topical, and vascular delivery	57
Cyclodextrin, targeting peptides, succinylation, disulfide linkage, deacylation, Jeffamine®	PEI	Rat brain endothelium, embryonic neurons, mesenchymal stem cells, macrophages, various cell lines	Newborn mouse brain, tumor-bearing mice (local/IP), reporter gene expression in organs	CNS-targeted and systemic gene therapy with broad cell transfection potential	58

Bcl-2, B-cell follicular lymphoma; CNS, Central nervous system; IP, intraperitoneal injection; PEG, polyethylene glycol; PEI, polyethyleneimine; PLL, Poly(L-lysine).

resulted in *P(MAC-co-DTC)-g-PEI*, which shown additional potential as a gene delivery vector.[71] In a different application, Yoshitomi *et al*.[72] discovered that PEI enhanced the generation of reactive oxygen species in *Haematococcus pluvialis* cells, promoting the accumulation of astaxanthin, a potent antioxidant. This finding highlights PEI's broader potential in metabolic engineering and biosynthesis regulation.[72]

Chen *et al*.[73] designed a high-performance gene delivery vector by conjugating the carboxyl groups of graphene oxide (GO) with the amino groups of branched PEI to create a PEI-GO composite. Compared to traditional 25 kDa PEI, this modification significantly reduced cytotoxicity while improving transfection efficiency. The incorporation of GO also leveraged its unique properties, such as a high surface area and enhanced cellular uptake, making it a compelling candidate for gene delivery applications.[73] In another study, Cook *et al*.[74] employed a thiol-yne reaction followed by acid hydrolysis to synthesize hyperbranched poly(ethyleneimine-co-oxazoline). This structure significantly reduced cytotoxicity, a major limitation of conventional PEI. However, it exhibited slightly lower transfection efficiency, highlighting the need for further optimization to balance reduced toxicity with high gene delivery performance. Potential enhancements may include functionalization with targeting ligands or the integration of stimuli-responsive elements to improve specificity and efficiency.[74]

Poly(β-amino ester) (PβAE)

PβAEs are promising cationic polymers for nucleic acid delivery. They are synthesized via the Michael addition reaction between acrylates and amines. Their biodegradability, biocompatibility, and pH-responsive nature make them ideal candidates for gene therapy applications.[75] Under physiological conditions, the polymer backbone's ester linkages hydrolyze into non-toxic byproducts such as bis(β-amino acids) and diols, which are harmless to mammalian cells. Triacrylates and amines have been used to develop highly branched PβAEs with multiple reactive sites, resulting in diverse and enhanced delivery strategies. PβAEs distinguish themselves from traditional cationic polymers like PEI due to their high transfection efficiency and low cytotoxicity. For example, PβAEs form stable polyplexes with nucleic acids, facilitating efficient gene transfer. In one study, PβAEs demonstrated superior plasmid DNA delivery compared to PEI, underscoring their potential in gene therapy.[76]

Beyond plasmid DNA, PβAEs have also been employed to transfect primary cells, supporting their applicability in therapeutic gene transfer. Their versatility includes delivery of RNA molecules, such as messenger RNA and siRNA. These applications have shown encouraging results in protein expression and gene silencing studies, reinforcing PβAEs' role in advancing RNA-based therapies. The integration of PβAEs into gene delivery platforms highlights their promise as next-generation polymers for safe and effective nucleic acid delivery. Their tunable structures, biocompatibility, and degradation into benign byproducts position them as significant contributors to the evolution of gene therapy technologies.[77,78]

Dendritic polymers

Dendrimers are highly branched, three-dimensional polymers with well-organized morphologies and nearly perfect symmetry, making them ideal carriers for drug delivery, including biologics such as genes. Their unique structure is often likened to that of a snowflake, with a central core from which branches radiate in a repetitive and orderly manner, resulting in a precise and uniform framework.[79]

The surfaces of dendrimers can be readily functionalized with ligands to enhance target specificity or with polymers to improve biocompatibility and reduce toxicity. Their multivalent surface also facilitates strong interactions with nucleic acids, making dendrimers suitable candidates for non-viral gene delivery. Dendrimers have demonstrated significant potential for delivering therapeutic genes and plasmid DNA with high efficiency, aided by their nanoscale size, which enhances cellular uptake and tissue penetration.[80]

Poly(amidoamine) (PAMAM)

Dendritic macromolecules are ideal scaffolds for targeted gene therapy due to their precise structure and numerous functional chain ends. Among them, PAMAM dendrimers have shown particular promise.[79]

Mastorakos *et al*.[81] revealed that amine-functionalized, hydroxyl-terminated PAMAM dendrimers could effectively compact plasmid DNA, a key step in forming stable polyplexes for gene delivery. The study also demonstrated that adding triamcinolone acetonide to the dendrimer-gene complex significantly improved nuclear localization, leading to enhanced cellular uptake and transfection efficiency.[81] While high-generation PAMAM dendrimers exhibit superior gene transfection efficiency due to their abundance of functional groups and surface amines, their clinical use is limited by inherent cytotoxicity and high production costs. To address these limitations, innovative conjugation strategies have been developed. For example, conjugating reactive oxygen species-responsive polypropylene sulfide to PAMAM dendrimers produces a cost-effective amphiphilic structure that maintains high transfection efficiency while significantly reducing cytotoxicity. These modified dendrimers also demonstrate improved DNA condensation and controlled release properties.[82]

In drug delivery, Najlah *et al*.[83] developed *PAMAM-G3* and *PAMAM-G0* dendrimers modified with diethylene glycol and lauroyl chains to improve the pharmacokinetics of naproxen and reduce cytotoxicity. This surface modification enhanced solubility and biocompatibility and promoted naproxen transport across Caco-2 cell monolayers. The use of diethylene glycol, lauroyl, and pyrrolidone derivatives effectively balanced reduced cytotoxicity with improved delivery efficiency, illustrating their value in drug delivery systems.[84]

Li *et al*.[85] demonstrated that incorporating epidermal growth factor (EGF) ligands into PAMAM dendrimers led to the self-assembly of *PAMAM/DNA/EGF* polyplexes. *In vitro* studies showed these complexes were less cytotoxic than unmodified PAMAM dendrimers. Furthermore, EGF absorption via non-covalent interactions was found to be less toxic than covalent binding, suggesting a promising direction for future research, particularly in investigating the ability of EGF covalently conjugated to dendritic structures for greater targeted specificity.[85] The targeting capability of EGF-modified polyplexes was confirmed *in vivo* using *EGFP+ MDA-MB-231* breast cancer models. When transfected with a luciferase-encoding plasmid, these polyplexes demonstrated enhanced tumor-specific uptake. *In vitro* cellular uptake and *in vivo* biodistribution studies using the near-infrared dye LSS670 revealed significant accumulation of EGF-centered dendriplexes at tumor sites following tail vein injection, confirmed via bioluminescence imaging.[86] These findings underscore the utility of EGF-modified dendrimers in targeting epidermal growth factor receptor-overexpressing cells. However, challenges remain, such as the need for detailed organ-specific distribution studies and addressing potential off-target effects. Moreover, since the interaction of EGF with the dendrimer was achieved through electrostatic binding, suggesting that alternative methods such as covalent binding could further optimize targeting potential.[85,86] Different strategies for gene vector modification are shown in Table 4.[87–91]

Conjugation-based polymer

Gene delivery is evolving with a focus on designing improved carriers capable of condensing DNA into nanocomplexes, stabilizing circulation, and ensuring successful gene transfer to target sites.[92] Kataoka and colleagues developed polyplex micelles utilizing the multifunctional block catiomer PEG20C, composed of PEG20kDa-poly[N′-[N-(2-aminoethyl)-2-aminoethyl]aspartamide] (hereinafter referred to as PEG-PAsp(DET))-cholesterol. This design features a hydrophobic core stabilized by cholesteryl residues and a PEG shell that increases circulation time compared to standard PEG-PAsp(DET) conjugates. To prevent toxicity, the formulation was carefully designed to eliminate residual free cationic structures, ensuring safety in HeLa, HuH-7, and HUVEC cell lines, as demonstrated by Cell Counting Kit-8 assays. PEG20C micelles outperformed other micelles in terms of transfection efficiency, measured using a luciferase reporter gene. Targeting integrins αvβ3 and αvβ5 using cyclic oligopeptides improved tumor-specific accumulation and anticancer efficacy. In a BxPC3 pancreatic cancer model, PEG20C micelles delivering plasmid DNA encoding the sFlt-1 gene reduced tumor growth threefold and significantly decreased vascular density compared to controls, demonstrating the efficacy of integrin-targeted methods.[93] Professor Kataoka *et al*.[94] also investigated a redox-sensitive PEG-releasable gene delivery system employing P-[Asp(DET)] polymer, which boosted human tumor necrosis factor-alpha gene expression *in vivo* and showed antitumor efficacy in a pancreatic cancer mouse model. These redox-sensitive polyplexes exhibited preferential tumor accumulation *in vivo* while reducing off-target effects, underscoring their potential to maximize target specificity and minimize systemic toxicity.[94]

Star polymers

Star polymers have emerged as a versatile platform for gene transport due to their well-defined structure, ease of modification, and enhanced transfection efficiency.[95] These branched polymers are characterized by linear polymer chains covalently linked to

Table 4. Common strategies for gene vector modification

Modification strategy	Carrier	Complexation	Outcome	Advantages	Ref
PEGylation (Polyethylene Glycol)	PEG-modified liposomes	polycation-plasmid DNA complexes (polyplexes)	Prolong circulation time; reduce immune clearance	Increased stability, reduced opsonization, prolonged half-life	87,88
Lipid Modification	Lipid-conjugated siRNA; cationic lipid nanoparticles	Lipid-siRNA complex	Improve membrane fusion and endosomal escape	Enhanced cellular delivery and endosomal escape	89
Polymer Coating (e.g., chitosan, poly(L-lysine))	chitosan-DNA nanoparticles	Chitosan-coated DNA complexes	Improve gene condensation and protect genetic material	Improved stability; protection from nucleases	90
Surface Charge Modulation	Zwitterionic coatings; charge-reversal polymers	zwitterionic polymer complex	Control biodistribution and cellular uptake	Reduced nonspecific interactions; controlled delivery	91

PEG, polyethylene glycol; siRNA, small interfering RNA.

a central core, creating a single branching point per polymer.[96] Their straightforward synthesis, ability to achieve high molecular weights, adaptability, and unique properties have made them increasingly attractive for gene delivery applications. Several star-shaped polymers featuring polycationic arms have been developed for this purpose. However, their practical use is often limited by concerns over toxicity.[97] Research has demonstrated that the cytotoxicity of these polycations is strongly influenced by their chemical structure and molecular weight.[98] The synthesis of star polymers typically involves blocking the polymer arms via electron transfer atom transfer radical polymerization. For instance, poly(butyl acrylate-tert-butyl acrylate) was synthesized by converting a linear block polymer into a multi-arm star block polymer, then crosslinking the end groups using divinylbenzene.[99] Zhang *et al*.[100] employed the arm-first method to fabricate multi-arm star polymers with a 70% yield in two steps. They utilized a dual-styrene-functionalized tetraphenylethene core with aggregation-induced emission properties, while the arms were composed of polystyrene, polyethylene, or polyethylene-b-polycaprolactone.[100]

Cho *et al*.[101] reported that PEG-functionalized star-shaped polymers effectively delivered DNA and siRNA to S2 cells *in vitro*, offering a reliable platform for gene transfer research. Their studies demonstrated the efficacy of star- and sun-shaped polymers with hyperbranched cores as vectors for genetic material delivery. A polypeptide-PEG miktoarm star copolymer demonstrated excellent cellular uptake and transfection efficiency in A549 lung cancer cells while exhibiting minimal cytotoxicity. Remarkably, these miktoarm copolymers achieved 68% luciferase gene silencing at a siRNA dose of 150 nM, while also enabling intracellular trafficking visualization, combining gene delivery with bioimaging capabilities. Furthermore, star-shaped polymers containing *P(DMAEMA-co-OEGMA-OH)* arms showed the potential to efficiently transfer DNA and messenger RNA, making them a versatile platform for a variety of genetic material delivery applications.[96]

Huang *et al*.[91] further advanced this field by employing grafting techniques to develop a novel star-shaped polymeric amide epoxy polymer. This structure combined low molecular weight PEI in the core with low molecular weight lysophosphatidic acid ethanol amide in the arms. The modified polymeric amide epoxy demonstrated significantly enhanced gene transfection efficiency in adipose-derived stem cells, surpassing the performance of standalone PEI and lysophosphatidic acid ethanol amide by factors of 264 and 14,781, respectively, while also reducing cytotoxicity.[91] Additionally, multi-branched star-PEI-g-PEG polymers have been proposed as polycationic gene carriers for non-viral retinoblastoma gene therapy. These *PEI-g-PEG*polymers successfully condensed genetic material into cationic nanocomplexes with a PEG shell, optimizing uptake efficiency and minimizing toxicity by fine-tuning their compositional ratios.[102] Collectively, these studies highlight the transformative potential of star-shaped polymers in both gene delivery and bioimaging applications.

Comb polymers

Comb polymers are characterized by a primary backbone, commonly referred to as the shaft, and pendant functional groups, or "teeth", which form the repeating units. Due to their excellent transfection efficiency and low cytotoxicity, these polymers have gained considerable attention as promising gene transfer vehicles. These comb-like structures, typically composed of a hydrophobic backbone and oligolysine side chains, form stable polyplexes with DNA, facilitating efficient gene transport and protection.[103] Studies have revealed that comb polymers outperform many commercial transfection reagents in terms of efficiency while maintaining high cell viability. Their hydrophobic backbones reduce DNA interaction, lowering binding free energy and improving transfection performance. Modifications such as the incorporation of zwitterionic components further enhance colloidal stability and reduce cytotoxicity. Notably, comb polymers grafted with poly(2-dimethylaminoethyl methacrylate) showed exceptional transfection efficiency and cell viability in human T cell transfection.[104] To improve *in vivo* efficacy, researchers have explored combining comb polymers with physical delivery techniques such as sonoporation. Owing to their unique architecture and functional versatility, comb polymers represent a promising platform for rapid and safe cellular transfection.[105] Polylactic acid (PLA), an aliphatic polyester, is synthesized via the ring-opening polymerization of lactide monomers. Methacrylate-functionalized PLA macromonomers are homopolymerized using reversible-deactivation radical polymerization to produce PLA comb polymers with a polymethacrylate backbone. A variety of synthetic strategies have been employed, including surface grafting, chain extension with small monomers, and copolymerization with other macromonomers. Researchers

have compared these compact polymers with their linear counterparts to evaluate structural effects.[106] Wu *et al*.[107] synthesized a triblock copolymer *(PEO-b-PHEMA-g-PLA-b-PNIPAM*; Đ = 1.35) through reversible addition-fragmentation chain transfer polymerization for use in murine (mouse-derived) cell line (LA5MA). This was achieved using a polyethylene oxide-based macro-chain transfer agent, followed by chain extension with N-isopropylacrylamide.[107] PLA macromonomers bearing methacrylate ω-end functionality were prepared using functional initiators during ring-opening polymerization. Subsequent radical polymerization of the brush polymer backbone allowed decoration of α-end functionalities on the side chain termini.[107]

PLL-grafted-PEG (PLL-g-PEG)

Comb-type polymers such as *PLL-g-PEG* function like a comb with bristles, where the PEG "bristles" prevent entanglement with other molecules. Studies show that PEGylation of PLL, at levels of 10–20%, effectively reduces the formation of large protein aggregates in the bloodstream, akin to detangling hair to avoid knots.[108] Similarly, Gref *et al*.[109] found that a PEG chain length of 5 kDa acts as an effective shield, preventing plasma protein adsorption onto PLA nanoparticles, much like an invisible raincoat repels water. The ε-amino groups of *PLL-g-PEG* are positively charged under physiological conditions, allowing them to electrostatically pair with negatively charged molecules and form polyion complexes, comparable to how magnets attract and form stable structures.[110] In synthetic gene delivery, PLL and other cationic polymers are used to form complexes with nucleic acids. These polymers function like efficient couriers, leveraging their positive charge to tightly bind and deliver genetic cargo to target cells.[111]

Poly(propyleneimine) and methacrylate polymers

Multidrug resistance (MDR) in cancer is primarily driven by the overexpression of ATP-binding cassette transporters, such as P-glycoprotein (P-gp/MDR1), which actively expel chemotherapeutic agents from cancer cells. This reduces intracellular drug concentrations and limits cytotoxic efficacy, making P-gp inhibition a critical strategy for enhancing drug retention and therapeutic outcomes.[112] Polypropyleneimine dendrimers combined with Pluronic P123 have been developed as a strategy to overcome MDR. This formulation not only stabilizes nanocomplexes to promote cellular uptake but also inhibits P-gp activity, enhancing intracellular drug retention. In a pivotal study, anti-CD44 antibodies were conjugated to these dendrimer-based nanoplexes to selectively target CD44+ MDR cancer cells, resulting in a 2.5-fold increase in transfection efficiency compared to non-targeted formulations. Co-delivery with doxorubicin (DOX) led to a sixfold reduction in tumor size in an MCF-7 breast cancer mouse model compared to free DOX.[113]

Gene delivery to the central nervous system faces significant challenges due to the restrictive nature of the blood-brain barrier. While earlier methods utilized intracerebral injection of deactivated viral vectors, recent advancements have shifted toward non-viral polymeric carriers. Qian *et al*.[114] developed diblock copolymers, *methoxy-PEG-PDMAEMA* and *maleimide-PEG-PDMAEMA*, functionalized with a 12-amino acid blood-brain barrier-targeting oligopeptide. These targeting oligopeptide-modified polyplexes achieved a threefold increase in transfection efficiency compared to non-targeting polyplexes. However, substantial enrichment in off-target organs such as the heart and lungs highlights the need for further purification and optimization before clinical application.[114] These examples highlight the critical importance of targeted delivery systems in overcoming physiological barriers, enhancing therapeutic efficacy, and minimizing adverse effects.

Brush polymers

Brush-like structures, when combined with CD-based star polycations, exhibit significantly greater gene transfer efficiency in COS-7 and 293T cells compared to standalone star polycations or PEI25K. These architectures have been utilized to develop multifunctional carriers capable of co-delivering DOX and a p53-encoding plasmid.[115] Additionally, PEG-based brush polymers containing disulfide linkages have shown promise in facilitating targeted siRNA delivery to cancer cells.[116] These systems demonstrated enhanced nuclease stability, improved cellular uptake, and favorable siRNA distribution, which translated to superior *in vitro* transfection efficiency and post-transfection cell survival rates. Notably, these brush polymers achieved a 19-fold increase in blood elimination half-life, underscoring their anti-tumor efficacy and protective potential *in vivo*. PEG bottlebrush polymers have thus emerged as promising candidates for siRNA silencing therapy, acting as environmentally friendly, long-circulating carriers. Blum *et al*.[117] highlighted the critical role of mobile, penetration peptide "fingers" within brush polymers, emphasizing their contribution to enhanced gene delivery and protein transfection efficiency.

In addition, Ahern *et al*.[118] demonstrated that the hydrophobicity and charge density of polymer arms significantly influence cytotoxicity and the overall effectiveness of polymer-mediated transfection. Given the high biocompatibility of natural materials, Ni *et al*.[119] developed organic brush polymers for gene transfer by grafting polymethacrylic acid onto heparin side chains. These findings reinforce the importance of diverse side-chain modifications, which can modulate key properties of brush polymers, including cytotoxicity, mitochondrial sequestration avoidance, and nuclear localization, thereby tailoring them for specific gene delivery applications.[119] Table 5 summarizes key macromolecules, highlighting their structural features, common chemical modifications to enhance functionality, and specific biological targets relevant to gene delivery systems.[120–131]

Limitations and future prospectives

Despite remarkable progress in polymer-based gene delivery, several key limitations continue to hinder their clinical translation. Compared to viral vectors, these systems often suffer from lower transfection efficiency and may induce cytotoxicity, immunogenic responses, or inconsistent gene expression. Additional challenges such as the instability of polymer–gene complexes under physiological conditions and variability in polymer synthesis complicate large-scale production and reproducibility. Addressing these issues requires ongoing efforts to refine polymer structures, enhance biocompatibility, and develop standardized, scalable manufacturing processes.

The future of gene delivery is expected to center around the development of smart polymers that respond to the dynamic conditions of the cellular environment. These polymers can be engineered to release genetic material, such as plasmid DNA or small RNAs like siRNA and miRNA, in response to stimuli such as pH, temperature, or redox changes, allowing for precise spatiotemporal control of gene transfer. Such stimuli-responsive systems enhance transfection efficiency while minimizing off-target effects and systemic toxicity by ensuring cargo release only within the desired intracellular compartment.[132]

Table 5. Structural and functional overview of macromolecules in gene delivery

Name	Structural characteristics	Chemical modifications	Targets	Ref
Poly(L-lysine) (PLL)	Linear polycation with primary amines that condense DNA into compact nanoparticles	PAMAM dendrimer, PEG, and disulfide linkage	Rabbit injured vessel	120
Polyethyleneimine (PEI)	Linear or branched structures with high charge density; strong interaction with DNA via electrostatic forces	deacylation, succinylation, disulfide linkage, Cyclodextrin, targeting peptides and Jeffamine®	Newborn mouse brain, local and/or intraperitoneal injection in tumor-bearing mice and reporter gene expression in vital organs	121,122
Poly(β-aminoesters)	Biodegradable and positively charged at physiological pH for DNA binding	PEG, branched and/or crosslinked, thiol-reactive side chains and spermine	Reporter gene expression in mouse muscle, and excisional wound and gene therapy in tumor-bearing mice	123,124
Poly(ethylene glycol) (PEG)	Neutral hydrophilic polymer often used for surface modification to enhance biocompatibility	Poly(DMAEMA), cyclodextrin, PAMAM dendrimers, PEI, chitosan, targeting peptides and proteins, arginine, lipid carriersadenovirus and folic acid	Gene therapy in tumor-bearing mice and Wistar rats, intramuscular gene silencing in mouse and reporter gene expression in vital organs	125,126
Dendrimers (PAMAM)	Highly branched, multivalent structures with abundant surface functional groups	PEG, lactose, targeting peptides and antibodies, folic acid, mannose, arginine, cholesterol, targeting peptides and proteins	Intramuscular gene silencing in mouse, gene therapy in tumors	127,128
Chitosan	Natural polysaccharide with amino groups for ionic interaction with nucleic acids	PLL, arginine, guanidylated, PEG, histidine, cysteine, glutathione, galactose, PEI, lipid shell, stargeting peptides and proteins, glutamic acid, biotinylated, chondroitin sulfate, chitosan nanobubbles, and spermine	Anti-apoptotic Bcl-2 gene knockdown in mice, autoimmune diabetes, aerosol delivery to mouse lung, reporter gene topical delivery to rat and reporter gene expression in endovascular rabbit organs	129,130,131

Bcl-2, B-cell follicular lymphoma; DMAEMA, 2-(Dimethylamine)ethyl methacrylate; PAMAM, Poly(amidoamine).

An emerging strategy in polymer-based gene delivery involves the integration of multifunctional components into the carrier systems. By conjugating targeting ligands, such as antibodies or small molecules, to the polymer matrix, researchers aimed to achieve cell- and tissue-specific delivery, particularly for challenging targets like tumor tissues or neural cells. For example, targeting CD44, a glycoprotein often overexpressed in cancer cells, can significantly enhance tumor accumulation and improve therapeutic outcomes.

Natural polymers, including chitosan, HA, and pullulan, are gaining attention for their intrinsic biocompatibility and biodegradability, which contribute to reduced immunogenicity and long-term safety. These natural macromolecules can be chemically modified or blended with synthetic polymers to create hybrid systems that combine the biological functionality of natural carriers with the structure and release characteristics of synthetic polymers. Such platforms are particularly well-suited for delivering fragile molecules like small RNAs, which require protection from nucleases and precise control over their release profile.

Nanotechnology-driven innovations further enhance gene delivery potential by enabling the development of nanocarriers that can bypass physiological barriers such as the blood-brain barrier. Functionalized nanoparticles and engineered protein-based delivery vehicles can facilitate the transport of small RNAs to the brain and other inaccessible tissues. Additionally, nanoparticles protect genetic material, including siRNAs and miRNAs, from enzymatic degradation and improve cellular uptake.[133]

Conclusions

Polymer-based gene delivery systems represent a promising class of non-viral vectors, offering significant advantages in safety, structural versatility, biodegradability, and the potential for targeted and controlled gene release. Both natural and synthetic polymers, as well as their hybrid forms, have been extensively explored to improve cellular uptake, endosomal escape, and transfection efficiency. Strategies such as surface modification with targeting ligands and integration of stimuli-responsive features have expanded their therapeutic potential.

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Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Author contributions

Study concept and design, writing of the manuscript (PP, SRG, DB, GS, RM), literature collection and analysis, and approval of the manuscript (PP). All authors reviewed and approved the final manuscript.

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