Design of polymers for siRNA delivery: Recent progress and challenges

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Abstract
RNA interference with the ability to specifically silence target genes has shown great potential to treat various diseases. The primary challenge for RNA interference is to effectively and selectively deliver genetic materials such as small interfering RNA (siRNA) to targeted tissues and cells in vivo. Numerous efforts have been made to overcome the extracellular and intracellular barriers during siRNA delivery. In this review, we focused on most recent advances in the design of functional polymers to achieve efficient siRNA delivery via addressing the various parameters, including siRNA binding, serum stability, specific targeting, tissue penetration, cellular internalization, endosomal escape, and intracellular siRNA release. The beneficial roles of assembled polymer nanostructures and polymer-based hybrid nanocomposites in siRNA delivery were also reviewed. Finally, the challenges and perspectives in the future development of polymer-based siRNA delivery systems will be discussed.

KEYWORDS
cancer therapy, gene therapy, nanoparticles, polymers, RNA interference, siRNA

1 | INTRODUCTION
RNA interference (RNAi) refers to a cellular process that is specifically silencing of gene expression. The phenomenon was first discovered in 1998 by Andrew Fire and Craig Mello.¹ RNAi is actually posttranscriptional gene silencing that degrades messenger RNA (mRNA). Double-stranded RNA (dsRNA) can be cut into multiple small fragments (21-23 bp, small interfering RNA [siRNA]) in the cytoplasm by the endonuclease Dicer² (Figure 1). siRNA then combines with helicase to form a silencing complex (RNA-induced silencing complex [RISC]) that induces the degradation of mRNA with complementary sequence to the antisense strand of siRNA. The phenomenon of RNAi is highly conservative and has been discovered in many eukaryotes. Theoretically, siRNAs are able to silence the expression of essentially any target gene by switching the sequences.³ The specificity, efficiency, and simplicity of RNAi make siRNA quickly become the most popular gene regulation tool. In 2001, Tuschl et al showed that...
siRNAs are capable of triggering RNAi in mammalian cells that proved the possibility of siRNA in clinical applications. In 2004, the first clinical trial of siRNA drug for wet age-related macular degeneration started, and a list of siRNA drugs was then evaluated for the treatment of various diseases. Although siRNA drugs suffered setbacks due to the challenges in delivery and off-target effects, these therapeutics have recently renewed great attentions. In 2018, the first siRNA drug patisiran developed by Alnylam Pharmaceuticals was approved by FDA for the treatment of polynuropathy of hereditary transthyretin (TTR)-mediated amyloidosis (hATTR). Another siRNA drug givosiran was approved in 2019 for the treatment of acute hepatic porphyria (AHP). It is believed that the era of RNAi drugs has arrived.

The efficient delivery of siRNA to targeted tissues and cells is the major challenge during the development of siRNA-based therapeutics. siRNA faces a series of barriers including rapid renal clearance, internalization by phagocytic cells, aggregation with serum proteins, and easy enzymatic degradation by endogenous nucleases. When reaching the target cells, the siRNA needs to be transported into the cytoplasm for RNAi. However, due to the relatively large molecular weight, polyanionic and hydrophilic nature of siRNA molecules, inefficient uptake, and endosome escape are the obstacles for efficient siRNA delivery. Therefore, the success of siRNA drugs depends on the development of siRNA delivery systems to safely and efficiently transfer siRNA into desired organs or cells. Generally, two strategies were adopted to deliver the
synthetic siRNA to target cells: direct conjugation of targeting or membrane-anchoring ligands to siRNAs, and the use of siRNA delivering carriers. Soutschek et al reported the effectiveness of cholesterol-conjugated siRNA in silencing apolipoprotein B in mice after intravenous administration.\textsuperscript{10} After that, various hydrophobic ligands were conjugated to siRNAs for therapeutic purpose.\textsuperscript{11–15} These conjugates can fuse with cell membranes and enter into the cells via bulk-phase pinocytosis. Besides, biologically active molecules such as aptamers,\textsuperscript{16–19} peptides,\textsuperscript{20–22} and sugars\textsuperscript{23,24} were conjugated to siRNA. An RNAi drug based on N-acetylgalactosamine (GalNAc)-conjugated siRNA is now being evaluated in phase III clinical trials.\textsuperscript{25} GalNAc is reported with high selectivity toward asialoglycoprotein receptors (ASGPR) on the surface of liver cells, and conjugating of siRNA with GalNAc enables liver targeting.\textsuperscript{25} These modifications on siRNA have shown improved activity and specificity in gene silencing, and reduced the risk of carrier toxicity.\textsuperscript{26} Despite these promising advances, ligand-siRNA conjugates are limited by their poor endosome escape ability.\textsuperscript{27} Modification of siRNA onto cationic polymers provides a solution to this issue;\textsuperscript{28,29} however, stimuli-responsive linkages should be incorporated to ensure efficient siRNA release in the cytoplasm.\textsuperscript{30–32}

To date, several kinds of siRNA carriers have been developed. Lipid nanoparticles represent one of the most widely used materials for siRNA delivery among the nonviral vectors. Lipid-based siRNA carriers have gained the great success in translation from bench to clinical trials.\textsuperscript{33} Both patisiran and givosiran, the first and second FDA-approved siRNA therapeutics, are based on lipid nanoparticles. The main problem for lipid-based carriers is their relatively low siRNA loading capacity. They often require additional cationic materials to facilitate the condensation of siRNA payloads into the core of lipid nanoparticles.\textsuperscript{3,34,35} Polymers are another most widely used materials for gene delivery.\textsuperscript{36–42} Cationic polymers bind negatively charged siRNA through electrostatic interactions and reversibly package siRNA into nanoparticles. Besides, the materials are easy to be synthesized and modified, and thus, could be conveniently designed to meet the requirements of siRNA delivery. This review particularly focused on the most recently reported strategies in the design of polymers to address the multiple barriers during siRNA delivery including siRNA binding, serum stability, cell selectivity, cellular uptake, endosome escape, and intracellular siRNA release. Considering the paradox between transfection efficiency and toxicity for cationic polymers in siRNA delivery, the strategies to achieve both highly efficient and low-toxic siRNA delivery were also discussed.

2 | POLYMERS IN ADDRESSING THE BARRIERS IN siRNA DELIVERY

2.1 | siRNA binding

Effective siRNA binding is the necessary prerequisite for cationic polymer to achieve successful siRNA delivery. Cationic polymers could form nanoscale polyplexes with siRNA, protect it from enzymatic degradation, and favor its cellular uptake. However, the efficient condensation of siRNA by cationic polymers is challenging due to the relatively rigid structure and low charge density of siRNA. Polymers with high charge densities and/or molecular weights are often required for efficient siRNA binding, as they can make the formed polyplexes less prone to destabilization by biological polyanions.\textsuperscript{43} However, an excess amount of positive charges on the polymer may lead to high toxicity and rapid clearance by the reticuloendothelial system (RES) after intravenous administration. Considerable efforts have been made through the design of polymers to achieve efficient siRNA binding without bringing extra positive charges.

2.1.1 | Amphiphilic polymers

Amphiphilic polymers can condense and stabilize siRNA via a combination of ionic and hydrophobic interactions. These polymers allow efficient siRNA condensation at a relatively low positive charge density.\textsuperscript{44–46} By incorporation of hydrophobic segment into the polymer backbone, high molecular weight polymers with extremely low charge density could achieve efficient siRNA delivery both \textit{in vitro} and \textit{in vivo}.\textsuperscript{47} For example, a library of ter-polymers synthesized through enzyme-catalyzed copolymerization of lactone (15-pentadecanolide [PDL]), diester, and diethanolamine was synthesized and used as carriers for siRNA delivery. The polyplexes with high PDL contents (50-90\%) led to more hydrophobic polymers that could form solid core nanoparticles in the presence of siRNA, supporting the hypothesis that hydrophobicity modulates siRNA condensation and stabilization. The polymer with the PDL composition of 70\% successfully delivered siRNA to suppress the major histocompatibility complex (MHC) class II molecule expression on donor endothelial cells and protected the allograft from acute rejection after transplant. Meanwhile, the biodegradable ester bond in the backbone ensures efficient polymer degradation after siRNA delivery, and these polymers were reported with low cytotoxicity during siRNA delivery.\textsuperscript{48–51}
2.1.2 Supramolecular polymer assemblies

Low molecular weight polymers can be fabricated into nanostructures via a supramolecular strategy. The polymers could be associated with each other in aqueous solutions via hydrophobic, hydrogen-bonding, fluorophilic, and host-guest interactions. The assembled polymer nanostructures can form stable polyplexes with siRNA in extracellular environment, and further disassemble into low molecular weight polymers after internalization or siRNA release. For example, low-generation polyamidoamine (PAMAM) dendron bearing one or two aliphatic chains at the focal point exhibited the advantageous features of both lipids and polymers in siRNA delivery.\cite{52-55} These lipid-dendron conjugates could assemble into spherical micelles or dendrimersomes, exposing a highly positive surface for siRNA binding.\cite{52} The hydrophobic core not only contributes to siRNA binding, but also enables encapsulation of hydrophobic drugs for the codelivery of siRNA and drugs.\cite{56-57} A supramolecular polymer assembled from 1,3,5-benzenetricarboxamide (BTA) was proposed as the carrier for siRNA delivery.\cite{58} BTA monomers including nonfunctional, positively charged, and fluorescently labeled ones were synthesized and formulated into multicomponent supramolecular nanofibers (Figure 2A).\cite{59} siRNA molecule was efficiently loaded onto the supramolecular polymers via electrostatic interaction, while hydrophobic drugs were encapsulated in the hydrophobic core of nanofibers. Both siRNA and the hydrophobic drug were successfully delivered into living cells for combination therapy. Similarly, host-guest chemistry was adapted to fabricate supramolecular polymer assemblies for siRNA delivery. Cationic \(\beta\)-cyclodextrin (\(\beta\)-CD) derivatives were decorated to cholesterol-modified poly(ethylene glycol) (PEG)-poly(vinyl alcohol) (PVA) via host-guest interactions, and the yielding assemblies enable the compaction of siRNA into stable nanoparticles.\cite{60} The complexes exhibited comparable gene knockdown efficiency but three orders of magnitude lower cytotoxicity compared to high molecular weight polyethyleneimines (PEIs). Similarly, the host-guest interactions between \(\beta\)-CD and adamantane were used to cross-link low molecular weight PEIs for siRNA delivery.\cite{60}
2.1.3 | Preassembly of siRNA

Another strategy to increase the siRNA condensation by polymers is preassembly of siRNA into structures with a high charge density like plasmid DNA. For example, oligomerized siRNA was obtained via self-assembly of siRNAs with short complementary A(5′-8)/T(5′-8) 3′ overhangs by annealing to each other, the clustered siRNA could be efficiently delivered by cationic PEI with much improved transfection efficiency.[61] In another work, Y-shaped siRNA was fabricated by assembly of three individual single-stranded DNA/siRNA chimeras, and subsequently served as a building block to construct dendrimeric siRNA.[62] The dendrimeric siRNA could be effectively complexed with a low molecular weight polymer (poly(β-amino ester)) (PBAE) to form stable complexes, exhibiting higher gene knockdown efficiency than native siRNA/PBAE complexes.

Rolling circle transcription (RCT) is a facile strategy to obtain multimeric siRNA. The multimeric siRNAs were assembled into sponge-like microspheres, which efficiently protected siRNA in a crystalline form (Figure 2B).[63,64] The high negative charge density on the siRNA microsponges allows efficient condensation into nanoscale particles by a cationic polymer such as PEI. In vitro gene silencing results showed that the siRNA microsphere/PEI formulation exhibited high gene silencing efficiency at an extremely low siRNA concentration, which is three orders of magnitude lower than that for commercial nanoparticles.[64]

Polymerization of siRNAs by chemical cross-linking is another strategy to construct multimeric siRNA. Both sense and antisense siRNA were designed with a thiol group at the 3′-end, and then reacted with the trimeric cross-linker tri-[2-maleimidodiethylene]-amine (TMEA) or a dimeric cross-linker 1,8-bis(maleimidodiethylene) glycol [BM(PEG)₂] to generate tri- or dimeric siRNAs.[65,66] After annealing, multimeric siRNA was obtained via complementary base pairing. The multimeric siRNA formed stable and compact polyplexes with linear PEI in the presence of polyanion competitors and serum proteins, and exhibited high gene silencing efficiencies both in vitro and in vivo. Various structures of multimeric siRNA can be obtained via rational design of the cross-linkers.[65] These multimeric siRNA also can be prepared by first annealing of the thiol-modified sense and antisense strands, followed by polymerization via oxidation of the thiol groups.[67,68]

Preassembled siRNA was also fabricated by directly complexation of siRNA with nanoparticles[69] or small molecules (Figure 2C).[70–73] Natural polyphenols were reported with high binding affinity with nucleic acids and proteins via multiple hydrogen bonds, π–π stacking, and hydrophobic interactions.[74–78] Based on this, epigallocatechin gallate (EGCG), the major component of green tea, was able to form a uniform and negatively charged nanoparticle with siRNA. These anionic nanoparticles were coated with low molecular weight cationic polymers to yield core-shell structured nanoparticles, termed “green” nanoparticles (GNPs) for efficient siRNA delivery (Figure 2C).[73,79] The presence of EGCG successfully facilitated the condensation of siRNA molecules by various types of low molecular weight polymers including linear and dendritic polylysine (PLL), linear and dendritic PEI, PAMAM dendrimer, and poly(propylene imine) (PPI) dendrimers. The complexes exhibited high gene knockdown efficiency even at an siRNA dose of 0.1 nM. The GNPs efficiently downregulated the target gene in vivo and ameliorated intestinal inflammation in a dextran sulfate sodium (DSS)-induced intestinal injury model. In another study, preassembled siRNA was fabricated by mixing of siRNA with doxorubicin hydrochloride via electrostatic interactions (Figure 2C).[71] The interaction transformed siRNAs from a hydrophilic nature to a hydrophobic one, which enabled their efficient encapsulation by polymeric micelles. An amphiphilic polymer PEG-b-poly(D, L-lactide) (PEG-b-PLA) exhibited 41.16±0.47% encapsulation efficiency for the modified siRNA. The formulation allowed efficient codeelivery of both siRNA and doxorubicin. Moreover, this strategy can be extended to other hydrophobic drugs or cationic lipids[70] (Figure 2C).

2.1.4 | High siRNA binding ligands

Functionalization of polymers with high siRNA binding ligands allows efficient siRNA binding using low molecular weight cationic polymers or noncationic polymers. Inspired by the structural and functional features of natural polyphenols such as EGCG in siRNA delivery, catechol-modified low molecular weight polymers were designed for siRNA delivery (Figure 3A).[80] The polycatechols efficiently condensed siRNA into stable nanoparticles and achieved high gene knockdown efficiency both in vitro and in vivo. ZnII-dipicolylamine complex (Zn-DPA) has high binding affinity with phosphate-containing molecules through specific interaction between the coordinated znic ion and the phosphate moiety.[81,82] Modification of polymers with Zn-DPA efficiently enhanced the association of polymers and siRNA. These polymers allow efficient siRNA binding and delivery even using a noncationic polymer scaffold.[82–84] Other functional ligands such as cholesterol and targeting ligands could be easily incorporated onto these coordinative polymers.[82,85] A catechol-cholesterol conjugate was decorated onto DPA-modified low molecular weight PEI via zinc-bridged coordination (Figure 3B).[85] The assembled polymer micelles efficiently bound siRNA through the combination effect of coordination, ionic interaction, and hydrophobic interaction.
Overall, various strategies have been successfully developed to enhance the ability of polymeric carriers to complex siRNA with either rational design of cationic polymers or preassembly of siRNAs themselves. When designing a polymer carrier, the introduction of second interaction force including hydrophobic, hydrogen bonding, and coordination interaction interactions besides electrostatic interactions between polymer chains and the siRNA molecules is usually essential. These carriers allows high stability in siRNA complexation and high siRNA delivery efficiency; however, the complicated structures for the materials may induce safety concerns in clinical application. On the other hand, preassembly of siRNA into structures allows the efficient complexation of siRNA using various carriers especially for some FDA-proved ones, thereby showing greater potential in clinical application of RNAi.

### 2.2 Serum stability

#### 2.2.1 PEGylated polymers

Cationic polymers usually have poor serum stability in siRNA delivery due to nonspecific interactions between polymers and serum proteins. To solve this problem, hydrophilic molecules such as PEG\[86\] and sugar\[87\] were decorated to polymers to shield the positive charges on polypelexes. The modification reduces the electrostatic interactions of polymers with serum proteins, and prevents their rapid clearance by RES. However, PEGylation on polymers may also reduce the internalization of polypelexes by target cells.\[88\] To address this problem, various PEG-detachable polymers were designed,\[89–91\] and this issue will be further discussed in the section of endocytosis.

#### 2.2.2 Zwitterionic polymers

Zwitterionic polymers also possess excellent serum stability due to their excellent hydrophilicity.\[92\] Poly(carboxybetaine) (PCB), a zwitterionic polymer, was complexed with siRNA under acidic conditions due to the protonation of PCB.\[93,94\] The prepared complexes were reverted to a neutral state in physiological conditions, and thus, could resist nonspecific protein adsorption. When accumulated in tumor tissues, the charge property of PCB polypelexes turned back to a positive state, which facilitated the cellular uptake and subsequent endosomal escape.

#### 2.2.3 Fluorinated polymers

Perfluorocarbons (PFCs) are both hydrophobic and lipophobic, and thus fluorinated materials usually exhibit
FIGURE 4  Fluorinated polymers in siRNA delivery. (A) Fluorinated polymers improve the serum stability of siRNA complexes in gene delivery. Fluorinated oligoethylenimine nanoassemblies for efficient siRNA delivery in serum-containing medium. Reproduced with permission from Ref. 115. Copyright 2018, ACS. (B) Transmucus and transmembrane siTNF-α delivery mediated by fluorinated and guanidinated bifunctional helical polypeptides. Reproduced with permission from Ref. 144. Copyright 2020, ACS

antifouling properties. Conjugation with fluoroalkyl chains may improve the serum stability of cationic polymers in gene delivery. Unlike PEGylation and zwitterionic polymer modification, fluorination on polymers does not sacrifice membrane binding and cellular uptake of the polymers. Therefore, fluorinated polymers can achieve both high transfection efficiency and excellent serum stability. To date, fluorination has successfully improved the siRNA delivery efficiency of various cationic polymers including branched PEI and PAMAM dendrimers. For example, a series of fluorinated oligoethylenimines (fOEIs) were developed for siRNA-mediated gene therapy (Figure 4A). The fOEIs could assemble into vesicle-like nanostructures with the fluorous segments densely embedded in the bilayers. The assembled fOEIs exhibited high gene knockdown efficiency in the presence of fetal bovine serum. Mechanism studies revealed that fOEIs/siRNA complexes displayed good colloidal stability and serum protein adsorption resistance in protein solutions, while the complexes by alkylated OEIs and siRNA formed large aggregates with serum proteins. The superior serum stability of fluorinated polyplexes is attributed to the antifouling behaviors of fluorous tags with extremely low surface energy. The physiological stability of fluoropolymer polyplexes against serum could be further improved by PEGylation. The combination of fluorination and PEGylation not only conferred resistance against serum-induced disassembly but also decreased protein binding to the siRNA polyplexes.

The fluorination strategy could be incorporated into reversible cross-linking polymers to construct highly efficient and low cytotoxic carriers. For example, bioreducible disulfide bond was introduced to the backbone of a cationic polymer or as linker to bridge cationic polymers. The combination of reversible cross-linking and fluorination provided a highly compact and stable structure to minimize nonspecific interactions with serum proteins. The combinational of fluorination and reduction sensitivity also improved siRNA delivery efficiency by promoting cell uptake, endosomal escape, and cytoplasmic siRNA release. Besides, the fluorinated polymers could also act as amphiphilic surfactants to stabilize PFC nanoemulsions via a fluorophilic effect. Fluorophilic interactions increased the polyplex stability as well as siRNA transfection efficiency. Moreover, the fluoropolymer-stabilized PFC nanoemulsions allowed ultrasound-triggered siRNA release.

2.3 Targeting

The selective delivery of siRNA to target tissues or cells while avoiding nonspecific uptake by normal cells is crucial for clinical RNAi therapy. Many studies have demonstrated that nanoparticles can accumulate in solid tumors through the leaky tumor vasculature and immature lymphatic drainage, termed enhanced permeability and retention (EPR) effect. However, this passive strategy has been proved with a very poor efficiency that only
an average of 0.7% of injected nanoparticles reached tumor.\textsuperscript{[119]} The EPR effect is now a controversial issue for nanomedicines.\textsuperscript{[120,121]} Therefore, active targeting strategies that selectively binding to biomarkers present on target cells are required for cancer therapy, as well as other disease treatment.

### 2.3.1 Proteins

Introducing targeted proteins such as transferrin (Tf) and monoclonal antibodies onto a polymeric carrier can improve the \textit{in vivo} siRNA delivery efficiency.\textsuperscript{[122–123]} Davis et al reported an example of successful RNAi in humans by systemically administration of a targeted siRNA nanoparticle, CALAA-01.\textsuperscript{[5]} The nanoparticle is composed of a cyclodextrin-containing polycation (CDP), an adamantane-conjugated polyethylene glycol (AD-PEG), a human Tf (hTf)-decorated AD-PEG (AD-PEG-hTf), and a therapeutic siRNA (Figure 5A).\textsuperscript{[124–126]} The hTf receptor is overexpressed on the surface of tumor cells, and the targeting nanoparticle successfully delivered therapeutic siRNA to tumors and downregulated the target gene.\textsuperscript{[5]} Moreover, the targeted nanoparticles exhibited deep tissue penetration due to transferrin-activated endocytosis/exocytosis events.\textsuperscript{[127]} A study on 3D cancer spheroids demonstrated that transferrin-appended nanocaplet delivered siRNA into spheroids with a depth up to 70 µm (Figure 5B).\textsuperscript{[127]} Similarly, $\beta_7$ integrin is highly expressed on gut mononuclear leukocytes. Nanoparticles coated with $\beta_7$ antibody exhibited high efficiency in the delivery of siRNA to silence cyclin D1 (CyD1) gene in the leukocytes.\textsuperscript{[122]} However, proteins often have a large molecular size, and the conjugation of protein on polymeric carriers will greatly change the nanocarrier size and cause steric hindrance on the polymers to further load cargo siRNAs.

### 2.3.2 Peptides

Numerous targeting peptides have been used to design polymeric carriers for targeted RNAi. RGD peptide with high binding affinity with integrins ($\alpha_v$,$\beta_3$ and $\alpha_v$,$\beta_6$) overexpressed on many cancer cells is the most widely used
targeting peptide in these studies. Polymers decorated with RGD are capable of carrying siRNA to cross tumor vessels and reach the extravascular tumor parenchyma after intravenous administration. Polymeric delivery systems based on HA/siRNA complexes were reported. HA modified with targeting peptides also allowed to address the barriers such as blood-brain barrier (BBB) during drug administration. The treatment of brain diseases such as glioblastoma (GBM) by gene therapy is challenging due to the existence of BBB. Angiopep-2, a peptide composed of 19 amino acids, was used to facilitate the delivery of siRNA therapeutics into GBM. The peptide could initiate endocytosis into GBM through the receptor-related protein 1 (LRP-1) expressed on both BBB endothelial cells and GBM cells. A polymeric nanogel containing therapeutic siRNAs was further decorated with angiopep-2 for targeted delivery (Figure 5C). The angiopep-2 functionalized nanogel exhibited 2.2-fold higher BBB penetration and 1.9-fold higher internalization by U87MG GBM cells compared to nontargeted nanogel. In vivo results demonstrated that the targeted nanogel efficiently silenced the expression of polo-like kinase 1 (plk-1) and inhibited the growth of orthotopic U87MG xenografts via a single intravenous administration. Besides, peptide-targeted polymers also showed promising results in the delivery of siRNA to treat other diseases such as obesity and osteoporosis.

2.3.3 | Polysaccharides and small-molecule ligands

Hyaluronic acid (HA) is a natural biomacromolecule with high binding affinity toward cell surface receptor CD44 overexpressed on many tumor cells. The negatively charged HA often served as a coating material to shield the cationic charges on polymer/siRNA polypelexes to reduce the polymer toxicity, prolong the blood circulation, and enhance tumor accumulation via HA-mediated active targeting. Recently, an anionic polymeric delivery system based on HA/siRNA complexes was reported. HA with a molecular weight of 200 kDa could complex with siRNA to form nanoparticles around 85 nm via hydrogen bonding and hydrophobic interactions. The HA-based nanoparticles successfully delivered therapeutic siRNAs into CD44-positive cancer cells and primary stem cells with 60% gene knockdown efficiency. Although high molecular weight polymer was used in the delivery system, the nanoparticles exhibited minimal toxicity on the cells due to the negative charge characteristic of HA. Many small-molecule ligands also showed high binding affinity to the receptors on cell surface, and hence, were employed to design targeted polymers for siRNA delivery. For example, sugars such as galactose were decorated to polymers for targeted delivery of siRNA into hepatocytes or hepatoma cells. Folic acid (FA) was conjugated to polymers for targeted delivery of siRNA into tumor cells overexpressing folate receptors.

2.4 | Tissue penetration

After entering target tissues, the polypelexes should penetrate deeply in the tissues for efficient RNAi. Generally, the carriers with a small size and a cationic surface are responsive for high tissue penetration. In addition, decoration of tumor targeting ligands such as transferrin and RGD peptide may improve the tissue penetration ability of nanocarriers. Recently, it is reported that fluoropolymers possess high tissue penetration capability due to the antifouling properties of fluorous ligands. For example, a series of fluorinated α-helical polypeptides were designed for pulmonary siRNA delivery to treat acute lung injury. Pulmonary drug delivery is often hindered by the mucus barriers on pulmonary epithelia. The high density of negatively charged mucin glycoproteins in the mucus layer will interact with positively charged polypeptides and prevent their penetration. Benefited from the fluorous tags, fluorinated polypeptides exhibited much higher gene silencing efficiency in RAW 264.7 cells, and showed 239-fold higher mucus permeation than a nonfluorinated polymer. As a result, the fluorinated polymer-based siRNA nanoformulation efficiently silenced tumor necrosis factor α (TNF-α) gene in the inflammation tissues and showed a promising therapeutic performance in an acute lung injury model.

2.5 | Endocytosis

Efficient internalization of polypelexes by target cells is the prerequisite for efficient gene silencing. Cationic polymers are beneficial for efficient endocytosis, and these polymers could be decorated with cell penetrating peptides (CPPs) to further promote cell internalization. However, positively charged materials usually show rapid clearance by RES and poor serum stability during siRNA delivery. Conjugation of polymers with PEG is often required for prolonged blood circulation, but may lead to less efficient cellular uptake. This dilemma could be addressed by the design of PEGylated polymers with pH-sensitivity. For example, a triblock polymer consisting of PEG, 2-mercaptoethylamine (MEA)-grafted poly(L-aspartic acid) (PAsp(MEA)), and PEI (PEG-PAsp(MEA)-PEI) was designed for in vivo siRNA delivery (Figure 6A). At pH 5.0, the amines on the polymer were protonated, which enabled efficient siRNA condensation into nanoparticles at a relatively low nitrogen to phosphate (N/P) ratio of
FIGURE 6  (A) The amine-protonation degree-mediated charge-reversible polyplex for long circulation, tumor-specific cell uptake, and facilitated intracellular siRNA release. Reproduced with permission from Ref. 150. Copyright 2014, Wiley. (B) pH-responsive linkage breakage of acid labile 2-propionic-3-methylmaleic anhydride linker induces PEG detachment in tumor tissues. Reproduced with permission from Ref. 90. Copyright 2015, ACS. (C) The composition of the pPTPs/CPDs polymer.  

3:1. The nanoparticles were then cross-linked via disulfide linkages to improve their stability. The prepared formulations were negatively charged at pH 7.2-7.4 in the bloodstream, and turned to a positively charged state when localized in tumor (pH 6.8), which promotes efficient endocytosis by tumor cells. When localized in endosomes (pH 5-6.5), the amines on polymers were further protonated, and the increased positive charges on the nanoparticles further facilitated the endosomal escape process. Besides, PEG-detachable polymers were designed to address the PEGylation dilemma. The PEG segments could be cleaved from cationic polymers by tumor extracellular acidity,[89,90,151] hypoxia,[152] or matrix metalloproteinase[153] to convert the polyplexes from a neutrally charged state to a positively charged one. For example, PEG-conjugated poly(ε-caprolactone)-nona-arginine (PCL-R9) via an acid labile 2-propionic-3-methylmaleic anhydride linker was synthesized for in vivo siRNA delivery (Figure 6B).[190] PEGylation on PCL-R9 provided a “stealthy effect” during blood circulation, and maintained the serum stability of polyplexes. After entering tumor tissues, the acidic microenvironment triggered the detachment of PEG shells and promoted the internalization of polyplexes by tumor cells. In a separate study, PEG-decorated thermosensitive polymers bearing phosphate residues (pPTPs) and RGD-decorated poly(disulfide)s bearing guanidinium residues (rCPDs) were grafted onto mesoporous silico-coated gold nanorods (Figure 6C).[154] The phosphate/guanidinium paired motifs allowed the binding of PEG shells with rCPDs during blood circulation. After accumulated in tumor tissues, the acidic microenvironment will protonate the phosphates on pPTPs and detach the PEG shell bound on rCPDs. As a result, RGD will be exposed on the nanoparticle surface, which further facilitates the selective uptake of siRNA-loaded nanoparticles by tumor cells.

2.6 Endosomal escape

After endocytosis, the polyplexes were usually transported to acidic cell compartments that steadily progress from early (pH 6-6.5) to late (pH 5-5.5) endosomes, and finally to lysosomes (pH 4.5-5). The cargo siRNA should escape from the endosomal/lysosomal pathway before being degraded by the enzymes to achieve efficient RNAi. Several strategies have been developed to enhance the endosomal escape of polymeric carriers via endosomal membrane fusion or disruption.

2.6.1 pH-buffering polymers

High pH-buffering capability was reported to be an advantageous factor for endosomal escape.[155] It was
reported that pKa values of the carriers did dramatically affect gene knockdown efficiency that lipid nanoparticles with pKa of 6.2–6.5 showed the best silencing effects, while this value for tri-block copolymers was 5.8–6.2. Polymers with the appropriate pKa values underwent acid triggered hydrophobic to hydrophilic transition and “proton-sponge” effect in endosome, which might significantly change gene silencing efficiency. After endocytosis, the polyplexes are trapped within the early endosomes. These pH-buffering polymers could act as “proton-sponges” to prevent endosomal acidification. Proton pumps on the endosome membrane continue to transport protons inside endosomes, and chlorides and waters were also transported into endosomes to balance the charges. Finally, the vesicle swells and ruptures to release the entrapped polyplexes into the cytosol. The “proton-sponge” hypothesis was supported by cationic polymers such as PEI and PAMAM dendrimers, the most widely used cationic polymers in cytosolic biomacromolecule delivery. Besides the “proton-sponge” effect, high pH-buffering polymers may facilitate endosomal escape by increased membrane disruption. The pH-buffering groups such as tertiary amine and imidazole with a pKa around physiological pH will be protonated when the pH drops in endosome, which will enhance the interactions of polymers with endosomal membranes. An endosome-disrupting polymer consisting of poly(dimethylaminoethyl methacrylate-co-dioisopropylethy methacrylate) (PDPA) and guanidinated poly(aminioethy methacrylate) (PG) was designed for siRNA delivery. The DPA segment with a pKa around 6.0 is hydrophobic and noncharged at physiological pH, and the amphiphilic copolymer tends to self-assemble into nanomicelles at pH 7.4. When entrapped in endosomes, PDPA converts to a hydrophilic polymer due to protonation of DPA moieties, which results in the disassembly of the nanomicelles and the enhanced endosomal disruption by the cationic PDPA. Therefore, the endosomal escape of pH-buffering polymers could be attributed to a synergistic effect of the “proton-sponge” effect induced endosomal swelling and increased endosomal membrane disruption by the polymers after protonation. Besides polycations, hydrophobic ligands such as lipids and fluoroalkyl chains were also reported to facilitate endosomal escape via membrane disruption.

2.6.2 Small-molecule adjuvants

Chloroquine (CQ) has widely been used to promote endosomal escape by reducing the activity of lysosomal enzymes. The molecule is lipophilic at physiological pH and can easily penetrate across cellular membranes. The presence of CQ could improve the endosomal escape of polyplexes and as well as their transfection efficiency. Besides CQ, several cationic and amphiphilic drugs could serve as endosomal escape enhancers to promote siRNA delivery. These drugs tend to accumulate in acidified lysosomal compartment and increase the permeabilization of lysosomal membranes, which facilitates the diffusion of siRNA from the lysosomal lumen into cytosol. These amphiphilic adjuvants promoted cytosolic delivery of siRNA by various nanocarriers. Amphoterin B (AmB) is known to transiently increase membrane permeability at sublethal concentrations by forming transmembrane pores. The hydrophobic molecule was encapsulated in polymer nanomicelles consisting of poly(2-(dimethylamino)ethyl methacrylate)-block-poly(2-(disopropylamino)ethyl methacrylate) (PDMA-b-PDPA). The nanomicelles achieved efficient endosomal escape during siRNA delivery due to the effect of released AmB and the protonation of PDPA during acidification.

2.7 Intracellular siRNA release

Cationic polymers need to form stable polyplexes with siRNA to protect it from enzymatic degradation and favor cellular uptake, while the enhanced siRNA binding is always accompanied by insufficient siRNA release. To overcome this problem, various stimuli-responsive polymers that could trigger intercellular siRNA release by external or internal stimulus such as light, temperature, glutathione, reactive oxygen species (ROS), and pH were designed. These polymers could undergo a rapid degradation in response to these stimuli, and release the bound siRNA into cytosol. Hammond et al reported a series of degradable poly(β-amino ester) (PBAEs) via Michael addition of bifunctional amines to diacrylates. The polymers enable efficient siRNA release after endocytosis due to hydrolytic degradation of PBAEs. These PBAEs could be incorporated with other responsiveness by using diacrylates bearing disulfide or ketal moieties. A novel cationic polymer based on bicyclo[3.3.1]nonane (BCN) were synthesized for siRNA delivery. Polymerization of 9-thiabicyclo[3.3.1]-dichloride with tris(pyridine) trinucleophiles generated a class of positively charged and branched polymers (Figure 7A). These polymers exhibited high siRNA binding, and could be degraded into small molecule diols and pyridines under hydrolysis at 37 °C. The degradation of polymer scaffold efficiently promoted siRNA release and gene silencing. The responsive polymers can be fabricated by crosslinking of cationic polymers using dynamic covalent linkages. The cross-linked
polymers with stimuli-responsiveness also allow efficient siRNA release by intracellular stimulus-triggered polymer degradation.\cite{150,194} A class of light-degradable polymers was synthesized by copolymerization of photocleavable diacrylate (based on \(\alpha\)-nitrobenzyl chemistry) with disulfide bond containing bisacrylamides and amines.\cite{182} The release of siRNA could be triggered by ultraviolet (UV) light exposure and intracellular glutathione. Besides, self-immolative polymers consisting of a pH-cleavable PEG head, a programmable hydrophobic body, and a positively charged PEI tail were designed for site-specific cargo release (Figure 7B).\cite{195} The polymers could self-assemble into programmable nanoparticles with logic gates and hierarchical structures. The acidic tumor microenvironment removes the PEG shell and triggers endocytosis by tumor cells, while the following self-immolative depolymerization responding to external stimulus such as light, \(\mathrm{H}_2\mathrm{O}_2\), glutathione, esterase, phosphatase, and acidic pH promotes the release of cargoes in the cytosol.

Cationic polymers with degradable side chains were also designed to facilitate intracellular siRNA release. siRNA binding moieties were grafted onto polymer scaffolds via responsive linkages, which could be cleaved by intracellular stimulus.\cite{196} For example, poly(acryloyl hydrazide) was reacted with guanidinium aldehyde to prepare pH-responsive guanidinium polymers (Figure 7C).\cite{197} The guanidinium groups on the polymer enable efficient siRNA binding. Once internalized into cells, the hydrazone linkages between guanidium and polymer were cleaved by the endosomal acidity to release the bound siRNA. Nishiyama et al recently proposed a pyruvate-triggered siRNA release mechanism for hydrazide-bearing polymers (Figure 7D).\cite{198} Pyruvate is a highly abundant molecule in the cytoplasm (3.0 mM). It could react the hydrazide moieties modified on polyglutamidetogenerate negatively charged \(\alpha\)-oxohydrazone, which facilitates the siRNA release by converting the positively charged polymer to a negatively charged one. A triblock copolymer consisting of \(\text{PEG-}b\text{-poly(2-dimethylaminoethyl methacrylate)-}b\text{-poly(pyrenylmethyl methacrylate)}\) (PEG-\(b\text{-PDMAEMA-}b\text{-PPy)}\) was designed for light-responsive siRNA release.\cite{199} PPy is a hydrophobic moiety that stabilizes the polyplex by hydrophobic interactions. UV light irradiation on the polymer cleaves the ester bond to release pyrene moieties, converting the hydrophobic PPy segment to hydrophilic poly(methacrylic acid) (PMAA). The increased hydrophilicity and number of negative charges on the polymer lead to fast disassembly of the polyplexes.

**FIGURE 7** Degradable polymer backbones (A, B) and polymers with degradable side chains for intracellular siRNA release. (A) Schematic representation of condensation polymerization and depolymerization of BCN-based hyperbranched polycations. Reproduced with permission.\cite{193} Copyright 2018, ACS. (B) A programmable polymer with PEG-detachable shell and self-immolative backbone.\cite{195} (C) Postfunctionalization of poly(acryloyl hydrazide) scaffold with cationic and hydrophobic aldehydes. Reproduced with permission from Ref. 197. Copyright 2016, Wiley. (D) Pyruvate-triggered siRNA release for hydrazide-bearing polymers. Reproduced with permission from Ref. 198. Copyright 2019, ACS
and siRNA release. A similar approach was designed based on ROS-responsive phenylboronic ester. The phenylboronic esters grafted on PMAA were degraded into small molecules, yielding a negatively charged PMAA to promote siRNA release via charge repulsion.

2.8 | Reduced cytotoxicity

The clinical translation of polymers for siRNA delivery requires a very rigorous safety evaluation. For cationic polymers, high charge density is usually required to achieve efficient gene delivery, but leads to increased cytotoxicity. Numerous efforts have been made to address this issue. For example, a noncharged biomimetic platform based on ribonucleoprotein (RNP) has been designed for targeted siRNA delivery. The carrier system contains a dsRNA-binding domain (dsRBD), a histidine-rich polypeptide (H$_2$E)$_{30}$, and a PEG chain (Figure 8A). The dsRBD domain allows the efficient siRNA binding via a sequence-independent binding mechanism, while the polypeptide facilitates endosomal escape via histidine-mediated pH-buffering. Moreover, the cargo siRNA is modified with FA via a disulfide linker for targeted delivery. The designed polymer can efficiently bind siRNA and form compact RNP nanoparticles around 12.3 nm. Such RNP nanoparticles are stable in buffers and serum solutions. In vivo results demonstrated that RNP nanoparticles have high tumor accumulation and caused tumor inhibition by efficient downregulation of plk-1. In another study, a series of base pairing-inspired bifacial polymer nucleic acids was developed for siRNA delivery (Figure 8B). The triazine-derivatized polymers exhibited high binding affinity with T/U-rich DNA and RNA due to the formation of melamine-thymine triplex via multiple hydrogen bonding. These noncharged polymers showed potent applications in siRNA delivery.

Guanidination has been proved to be an efficient way to improve the cellular uptake and gene transfection efficiency of a polymer. Although guanidinium has high binding affinity with cell membranes, polymers grafted with guanidinium were usually less toxic than those modified with the primary or secondary amines. A recent study reported that polymeric metformin (PolyMet) generated by direct reaction of PEI with dicyandiamide has relatively low toxicity but high transfection efficiency (Figure 8C). PolyMet-based siRNA nanoformulations efficiently downregulated target genes in vivo and successfully inhibited tumor growth via a combination of gene therapy and the inherent anticancer activity of PolyMet. Besides nitrogen-based cationic moieties, Frechet et al reported a class of phosphonium-based polymers for efficient siRNA delivery (Figure 8D). These phosphonium polymers possess much lower toxicity but higher transfection efficiency than their ammonium analogs. The phosphonium polymers even showed potent siRNA delivery efficiency in the presence of serum. The exact reasons behind the low toxicity but high RNAi efficiency of metformin- and phosphonium-based polymers require further investigations.
3 DESIGN OF POLYMER NANOSTRUCTURES FOR siRNA DELIVERY

Besides polymer synthesis and chemical modification, the polymers were fabricated into various nanostructures such as nanogels and layer-by-layer (LBL) nanocapsules for efficient siRNA delivery. Alternatively, the polymers were used in combination with lipids, cell membranes, virus nanoparticles, and inorganic nanoparticles to prepare hybrid nanocomposites for improved RNAi. These strategies were discussed in details as follows.

3.1 Polymeric nanogels

Nanogels with excellent biocompatibility and stability allow the controlled release of encapsulated siRNA. The nanogels are usually prepared by in situ polymerization of cationic monomers and biodegradable cross-linkers on siRNA molecules. For example, nanogels consisting of spermine-acrylate, glycerol dimethacrylate and N-[Tris(hydroxymethyl)methyl]acrylamide, and siRNA were prepared by radical polymerization (Figure 9A). The spermine unit ensures siRNA binding and cell internalization and endosomal escape, while the acid-labile cross-linker glycerol dimethacrylate allows serum stability of the siRNA nanoformulation and intracellular siRNA release after internalization. In a separate study, siRNA with two single-stranded overhangs at both sides were introduced to DNA-grafted polycaprolactone (DNA-g-PCL) via complementary base pairing (Figure 9B). The direct mixing of the siRNA with the DNA-g-PCL brushes resulted in the formation of cross-linked nanogels. The nanogels exhibited good serum stability in physiological condition and allows the rapid endocytosis without any polycations. Moreover, the stability of nanogels could be further improved by modification with polydopamine (PDA) and PEG.
3.2 | LBL nanocapsules

LBL assembly is based on sequentially deposition of oppositely charged polyelectrolytes onto a nanosized core to obtain multilayered polyelectrolyte films or capsules. When siRNA was used as an anionic polyelectrolyte, it could be loaded within the LBL layers with high serum and RNase stability. Numerous studies have demonstrated that the loading of siRNA into LBL assemblies can achieve high and sustained gene silencing both in vitro and in vivo.[211–218] This approach allows the simultaneous incorporation of multiple functional polymers into a discrete nanoparticle through the polymer design or postmodification.[219] For example, positively charged poly-L-arginine (PLR) and negatively charged siRNA were sequentially adsorbed on an anionic liposome nanoparticle, and the outmost layer was coated with propargyl-modified poly-L-aspartate (pPLA) (Figure 9C).[220] The anionic pPLA provides excellent colloidal stability against serum proteins, and enables the further conjugation with azide-functionalized species such as iRGD and PEG. The fabricated LBL nanoparticles allow efficient siRNA delivery. Besides liposomes, organelles in cells were used as the substrate to prepare LBL capsules. A mitochondria-based LBL complex was fabricated to overcome multidrug resistance in cancer cells (Figure 9D).[221] The siRNA loaded in the complex efficiently downregulated pump resistance-related proteins, while delivered mitochondria reactivated the drug-induced apoptosis pathway. As a result, both pump and nonpump mediated drug-resistance could be addressed by this LBL complex. Similarly, LBL-coated on gold nanostar exhibited efficient photothermal therapy via the delivery of siRNAs.[222]

3.3 | Polymer/lipid hybrid nanoparticles

Polymer/lipid hybrid nanoparticles combine the advantages of both polymers and liposomes in siRNA delivery. These polymer/lipid hybrid nanoparticles can be divided into two types: coassembly of amphiphilic polymers with lipids and coating polymer/siRNA polyplexes with a lipid shell. For example, formulating nanoparticles with 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), DSPE-PEG, and cholesterol is a general tool to fabricate long blood circulating materials.[149,153,223–225] Coformulation of cationic polymers with lipids not only improves the polyplex stability, but also shields the positive charge on the surface of polyplexes.[129,140,176,226–229] For noncationic amphiphilic polymers, lipid incorporation allows the formation of polymer/lipid chimeric nanoparticles with high siRNA loading efficiency and stability. For example, nanoparticles composed of PEG-b-PLA or PEG-b-PLGA have gained great attentions in drug and gene delivery. However, the direct encapsulation of siRNA by PEG-b-PLA or PEG-b-PLGA by double emulsion method is always limited by low encapsulation efficiency due to the weak interactions between siRNA and the polymer. To solve this problem, a cationic lipid-assisted nanoparticle system named “CLAN” with high siRNA encapsulation efficiency was developed.[230–232] The cationic lipid can efficiently bind siRNA at the water-oil interface during the primary emulsification step, and this prevents the diffusion of siRNA into external water phase in later emulsion processes (Figure 9E).[231] With the help of a cationic lipid, N,N-bis(2-hydroxyethyl)-N-methyl-N-(2-cholesteryloxy carbonyl aminooethyl) ammonium bromide (BHEM-Chol), the encapsulation of siRNA by PEG-b-PLA was dramatically increased, resulting in increased gene silencing efficiency and RNAi-mediated therapy.[233,234] This “CLAN” strategy could be extended to other amphiphilic copolymers and cationic lipids,[235,236] and allowed the codelivery of small-molecule drugs and siRNAs for combination therapy.[235] Polymer/lipid nanoparticles were also prepared by mixing polynplexes with liposomes to form hybrid complexes where the lipids are coated on the surface of polyplexes. A microfluidic chip was recently used to fabricate uniform polymer/lipid nanoparticles.[237] These hybrid nanocomposites allow efficient encapsulation of siRNA within the polymer core, and the lipid shell could facilitate the cellular uptake of nanoparticles.[238,239]

3.4 | Cell-membrane-coated polymer/siRNA polyplexes

Cell membrane coating has been developed as an efficient strategy to improve the in vivo performance of nanoparticles. Membrane coating on nanoparticles not only enhances their colloidal stability and blood circulation, but also allows targeted nanoparticle delivery. For example, cancer cell membrane-coated biomimetic nanoparticles could specifically delivery siRNA to homologous tumors in vivo (Figure 10A).[240,241] One of the challenges for cell membrane-decorated polyplexes is how to release the cargo siRNAs from coated membranes after endocytosis. To solve this issue, charge-reversal polymers with negative surface charges at physiological pH were proposed to disrupt the lipid bilayers by converting the polymers to positively charged ones under acidic conditions.[242,243] PEI/siRNA polyplexes were coated with citraconic anhydride-grafted poly-L-lysine (PLL-CA) to generate negatively charged polyplexes, which was further coated with angiopoept-2 peptide decorated red blood cell membrane (Ang-RBCm) (Figure 10B).[242]
The decoration of RBC membrane enables the long-term blood circulation, and the targeting peptide angiopep-2 improves the BBB permeability and tumor accumulation of the nanoparticles. Once entrapped in acidic endosomes, the acid-labile linkage between CA and PLL was degraded, resulting in the switching of polymer charges from negative to positive. As a result, the decorated RBC membrane was destroyed and the PEI/siRNA polyplexes were released from the membranes for gene silencing. Such cell-membrane-coated charge conversional hybrid nanocomplexes efficiently suppressed the growth of GBM by downregulation of target genes via siRNA delivery.

### 3.5 Polymer/virus chimeric nanoparticles

Virus/polymer chimeric nanoparticles enable simultaneous expression and silencing of multiple genes (Figure 10C).\[^{244,245}\] The nanoparticles containing a Bcl-2-interacting mediator of cell death (BIM)-encoding adeno-associated virus (AAV) core and an acid-degradable polyketal (PK) shell. The polymer shell was synthesized via photopolymerization of acid-cleavable ketal-based amino monomers and cross-linkers in the presence of siRNA. Benefiting from the polymer coating, the AAV
was shielded from capture by immune cells during blood circulation. After endocytosis, the PK shell was degraded into small molecules in acidic endosomes, resulting in the release of siRNA targeting myeloid cell leukemia-1 (MCL-1). The BIM-encoding AAV was subsequently transported into cell nucleus for BIM expression. BIM promotes the apoptosis of leukemia cells, while MCL-1 serves as a prosurvival Bcl-2 member. The polymer/virus chimeric nanoparticles simultaneously upregulated BIM expression and silenced MCL-1 expression, and synergistically suppressed the proliferation of leukemia in vivo.

3.6 Polymer/inorganic nanoparticle hybrid nanocomposites

Inorganic nanomaterials have exhibited various promising features in biomedical applications. Up to now, various inorganic nanoparticles such as gold nanostructures, superparamagnetic iron oxide nanoparticles (SPIONs), mesoporous silica nanoparticles (MSNs), carbon nanotubes, graphene, and upconversion nanoparticles (UCNPs) were coated with polymers to fabricate hybrid nanocomposites for siRNA delivery. The polymers were usually engineered on nanoparticles via covalent conjugation, LBL assembly, and in situ synthesis. Gold nanoparticles and SPIONs are the most popular inorganic nanoparticles used to construct hybrid nanocomposites for siRNA delivery. These materials have reported with low cytotoxicity, inherent optical or magnetic properties, and facile surface modification. Gold-polymer hybrid nanoparticles were usually developed for enhanced photothermal therapy via downregulation of heat-resistance genes or photoactivation of siRNA release from the nanoparticles. SPIONs-based nanomaterials allow efficient magnetofection and magnetic resonance imaging (MRI)-based in vitro and in vivo tracking. For example, encapsulation of SPIONs within cationic polymersomes consisting of poly(etherimide)-b-poly(D,L-lactide) (PEI-PDLLA) yielded an MRI-visible nanocomplex. The nanocarriers efficiently delivered an siRNA targeting Nogo-66 receptor (NgR) into neural stem cells (NSCs) and significantly promoted the neuronal differentiation of NSCs without inducing adverse effect on the secretary profile of NSCs. After transplantation of the transfected cells into a rat model of acute ischemic stroke, MRI-visible cell migration and homing were observed. Moreover, the sustained neuronal differentiation of NSCs was detected after cell transplantation, resulting in improved recovery of neural function in the model. Besides metallic nanoparticles, nonmetallic materials such as MSNs were always used as the scaffold to fabricate hybrid nanocomposites. The porous nanostructure of these materials enables high polyplex loading, and protects the polyplexes against serum and RES. Moreover, the nanochannels in MSNs can be loaded with therapeutic drugs for codelivery of multiple cargoes. For example, MSNs were loaded with an osteogenic peptide osteostatin, and further coated with PEI for siRNA delivery. The siRNA nanoformulation efficiently downregulated sclerostin expression, which inhibits Wnt/β-catenin pathway and increases osteoblast differentiation. The codelivered osteostatin by MSNs served as an osteoclast inhibitor and caused a synergistic effect in bone regeneration.

4 CONCLUSIONS AND PERSPECTIVES

With a growing number of approved siRNA therapeutics in clinics, RNAi-mediated gene therapy for various diseases has gained great interest in recent years. Polymeric carriers hold great promise for efficient siRNA delivery into various target cells both in vitro and in vivo. Various functional polymers were designed to overcome the multiple extracellular and intracellular barriers during siRNA delivery. Furthermore, polymer-based hybrid nanostructures were reported for improved siRNA delivery by combining the features of polymers and other carrier systems. It is worth mentioning that gene delivery is a complicated biological process requiring the carriers to overcome substantial extracellular and intracellular barriers to RNAi delivery. However, there are often some contradictions need to be resolved when design materials, such as the paradox between siRNA complexation and release as well as difference between extracellular barriers and intracellular barriers. Cationic polymers need to form stable polyplexes with siRNA to protect it from enzymatic degradation and favor cellular uptake, while the enhanced affinity between polymeric carriers and siRNA is always accompanied by the insufficient siRNA release in the cytoplasm. On the other hand, the strategies to overcome extracellular barriers and intracellular barriers also need to be balanced. PEGylation is a commonly used strategy to facilitate the in vivo application of cationic polymers-based siRNA delivery for it shielding the positive charges on polyplexes, reducing the electrostatic interactions of polymers with serum proteins, and preventing their rapid clearance by RES, while modification of such neutral hydrophilic compounds may also reduce the internalization of polyplexes by target cells. These problems could be solved with the rational design of responsive systems mimicking the responsiveness of living organisms.

Despite the continuous advances of polymeric siRNA delivery systems, considerable challenges remain to be overcome. Most of the polymer designs were focused on
a few siRNA delivery processes, and the designed materials cannot address all the barriers for in vivo gene therapy. Understanding the mechanism for each process is essential to guide the design of efficient siRNA carriers. However, the mechanisms of polymers in addressing the multiple barriers during siRNA delivery still require in-depth investigations. For example, the “proton-sponge” effect is the most widely proposed concept to design polymeric carriers with fast endosomal escape in the literatures. Although this hypothesis has successfully explained the behaviors of some classic cationic polymers such as PEI and PAMAM dendrimers, it has been challenged by several experimental observations that some pH-buffering polymers failed to cause endosomal lysis and escape. Moreover, it is believed that the rupture of late endosomes/lysosomes may cause significant toxicity to the cells. Furthermore, it is believed that the rupture of late endosomes/lysosomes may cause significant toxicity to the cells. Another mechanism under debate is the EPR effect for nanoparticles to accumulate in tumor tissues. A recent study proposed an alternative transport mechanism that most of the nanoparticles enter tumors via an active process through endothelial cells rather than a passive process through the gaps between endothelial cells. Refining and manipulating the specific transport mechanisms will help the researchers to rationally design nanoparticles to overcome the poor tumor accumulation problem. For clinical applications, the polymeric carriers should be reproducible, degradable, and biocompatible. Considering the existence of multiple extracellular and intracellular barriers during siRNA delivery, various polymer-based nanostructures such as nanogels and nanocapsules and hybrid nanocomposites of polymers with lipids, cell membranes, virus, and inorganic nanoparticles were designed to overcome these barriers. However, the complicated structures for these materials may induce safety concerns and hinder their clinical translation. It is highly required to develop simple formulations with high siRNA delivery efficiency and safety. Besides, the polymers should be used in combination with chemically modified siRNAs with improved enzymatic stability and bioactivity to promote their in vivo RNAi performance. We believe that polymer-based siRNA delivery systems will benefit to human beings in a near future.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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