Molecular Therapy
Methods & Clinical Development
Review

Engineering Nanoparticles for Targeted Delivery of Nucleic Acid Therapeutics in Tumor

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In the past 10 years, with the increase of investment in clinical nano-gene therapy, there are many trials that have been discontinued due to poor efficacy and serious side effects. Therefore, it is particularly important to design a suitable gene delivery system. In this paper, we introduce the application of liposomes, polymers, and inorganics in gene delivery; also, different modifications with some stimuli-responsive systems can effectively improve the efficiency of gene delivery and reduce cytotoxicity and other side effects. Besides, the co-delivery of chemotherapy drugs with a drug tolerance-related gene or oncogene provides a better theoretical basis for clinical cancer gene therapy.

Gene therapy is a promising therapeutic strategy aimed at altering or modifying defective and/or missing gene sequences to cure acquired or inherited diseases, including genetic disorders, cancer, cardiovascular diseases, and autoimmune disease, through introducing foreign genetic materials to cells, tissues, or organs.[1] RNAi, as a posttranslational gene regulation technology of gene therapy, can specifically inhibit the gene expression of interest triggered by small interfering RNA (siRNA), genome origin microRNA, and double-stranded small hairpin RNA (shRNA).[2] In addition, RNA-programmed CRISPR/Cas9 mRNA has proven to be a versatile tool for therapeutic genome editing in mice, as recently reported.[3–6] In just the past 5 years, more than 100 antisense oligonucleotide-based therapies have been tested in phase I clinical trials, and a quarter of them have reached phase II/III. Nusinersen, a modified antisense oligonucleotide to cure spinal muscular atrophy, following Formivisen and Mipomersen treating cytomegalovirus retinitis and high blood cholesterol, respectively, has been approved by the U.S. Food and Drug Administration (FDA).[7–10] The continued improvement of innovative DNA/RNA modifications and delivery carriers, such as nanoparticles (NPs), will aid to solve the challenges and barriers of DNA/RNA-based therapeutics.

It is known that naked DNA/RNA molecules are rapidly degraded by nuclease, with a high clearance rate of kidney, poor efficiency of cell uptake by intravenous injection, and severe unintended side effects when off target.[11] Therefore, it is necessary to select a safe and stable transport carrier to deliver it to the target cells and tissues in clinical research. The transport carrier should meet these conditions: (1) it must protect DNA/RNA molecules from degrading and release with control; (2) it must be designed to increase the transfection efficiency, with the ability to penetrate deep into the tumor and reach tumor cells remote from the blood vessels for cellular internalization;[12–14] and (3) it should be stable and safe during blood circulation (no interaction with biomolecules resulting in an immune response) and accumulate in the tumor site, however, it must be able to stick to the cells for cellular uptake.[15]

Generally, transporters are divided into viral and non-viral vectors. Viral vectors, including retroviruses, adenoviruses, and lentiviruses,[16] are more efficient than non-viral vectors in numerous cell lines due to their controlled nucleic acid packing and unpacking in or from the capsid, as well as the ability of the virus to overcome various extracellular and intracellular delivery barriers or defense mechanisms of the targeted cells; however, there are safety concerns about severe off-target immunogenicity, inflammatory response, and toxicity.[16–19] In contrast, non-viral vectors, such as cationic lipid, polymers, and peptides using chemical entities that are able to mimic the main features of viral vectors, are able to compact and deliver nucleic acids in a similar manner. Also, these are much safer compared to viral vectors because the artificial design is usually not recognized (immediately) by the immune system. In addition, the chemical structure is controllable and easier to scale up and synthesize commercially.[20–22] However, there are still some problems with non-viral carriers to confront.

The first is how to improve the efficiency of passive targeting, namely, the enhanced permeability and retention (EPR) effect. The EPR effect is the unique and most crucial phenomenon occurring in tumor tissue, with excessive production of vascular mediators and extravasation of macromolecules from blood vessels into the tumor tissue interstitium.[23] Maeda et al.[24] summarized the characteristics of the EPR effect of nanomedicines and macromolecular drugs in most, if not all, solid tumors, including biocompatibility, property size (over 40 kDa), weakly negative to near neutral, maintain at least 30 min to achieve tumor site and cleared rapidly in cells, then drug release. The second one is choosing a highly specific molecular target to even a single epitopic antigen, receptor, or kinase, which can modify NPs to target tissue and enhance the therapeutic efficacy,[25,26] in

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DNA/RNA delivery caused by positively charged materials, the property of electrostatic interaction with the negatively charged DNA/RNA molecules.[27]

In this review, we introduce the current clinical applications of gene delivery, and we summarize the barriers and challenges to systemic delivery of nucleic acids. In addition, we highlight the exploitation and discovery of different NPs and their modification in DNA/RNA delivery to discover more efficiency and safer NPs in gene delivery.

Clinical Application of NPs-Based RNAi Therapy

In the last decade, several biotechnology companies have invested massive manpower, physical resources, and funds in NPs-based gene therapeutics. The NPs-RNA complex is intended to enhance its circulation, promoting safe delivery to the desired location and silencing of the target mRNAs.[28] Most of the NPs-based siRNA/microRNA delivery systems currently are approved for clinical trials in cancer therapy, virus infection, and other diseases (Table 1).

CALAA-01 is a polymer-based NPs siRNA delivery system containing a linear, cationic cyclodextrin-based polymer and a siRNA that targets the M2 subunit of ribonucleotide reductase (RRM2) (Figure 1A). Adamantane polyethylene glycol (PEG) was used as a surface modifier on the NPs to provide steric stabilization and a targeting ligand (human protein transferrin) on its surface as a positive target. The cationic polymer interacts electrostatically with anionic siRNA to assemble into nanocomplexes below approximately 100 nm in diameter that protect the siRNA from nuclease degradation in serum. The siRNA-containing nanocomplexes are targeted to cells that overexpress the transferrin receptor (TfR), and then anti-R2 suppresses RRM2 expression, resulting in cell-cycle arrest and cell death.[29]The trial has been terminated due to 21% of the patients having an adverse event because of drug instability (specifically the transferrin-targeting ligand).[30] Tolerable dose, siRNA plasma concentration, and elevated plasma levels of cytokines have no association with adverse events. In addition, a dose-dependent accumulation of CALAA-01 within tumors, but not adjacent tissue, was found in CALAA-01-specific staining, and the expression of RRM2 in protein and mRNA level were inhibited effectively.[31] Thus, how not only to engineer nanomedicines to make them effectively target tumor tissues but also to choose an appropriate delivery system are the keys to developing new-generation nanomedicines of high therapeutic efficacy.

siG12D LODER is composed of Local Drug EluteR (LODER), a novel biodegradable polymeric matrix that shields drugs against enzymatic degradation and siRNA against G12D-mutated KRAS (siG12D), which is released by LODER slowly (Figure 1B).[32,33] siG12D LODER was implanted regionally as miniature cylindrical pills into pancreatic tumors by endoscope ultrasound (EUS) biopsy for a long term, showing good anticancer effects. It has successfully passed a phase 1/2a clinical trial. Most importantly, LODER is a miniature (millimetric) biodegradable polymeric matrix that is composed of a copolymer of poly (lactic-co-glycolic) acid (PLGA) with good safety, biodegradability, and biocompatibility in this tissue for a long period.[34–36] Furthermore, the knockdown of KRAS activated the alternative pathway of the complement system[37] and stimulated immunity under certain conditions.[31]

TKM-080301, targeting polo-like kinase 1 (PLK1), is a siRNA for intravenous (i.v.) administration formulated in stable nucleic acid lipid particles (SNALPs), and it was evaluated in patients with gastrointestinal neuroendocrine tumors and adrenocortical carcinoma.[38] In this delivery system, PLK1 is a protein overexpressed in cancer cells promoting an uncontrolled cell proliferation,[39] and researchers used siRNA-silencing PLK1 molecules incorporating into the aqueous inner part with the help of cationic lipids (1,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane,DlinDMA). Helper lipids with DSPC (1,2-distear-oyl-sn-glycero-3-phosphocholine) promote the release of siRNAs from SNALPs in the cell cytoplasm. Polyethylene glycosylated lipids (PEG-C-DMA) offer an external surface that is more hydrophilic and neutral, for stability and crossing barriers. Cholesterol helps to stabilize the lipid particle formulation.[40]

Nowadays, some of these trials are in phase III and the results will hopefully be approved. Engineering NPs carriers has received wide attention in gene delivery due to their outstanding characteristics, including diverse modifications and high stability biocompatibility to adapt desirable properties in particle forms. Among the nanopaticles used in gene delivery carriers, the cationic polymer and lipid were the most widely used polymer for the delivery of DNA and RNA molecules.

The Challenges and Barriers of Polymer-Mediated Gene Therapy

Cellular Uptake and Endosomal Escape

Naked siRNA/microRNA permeating the cell membrane is blocked by its size and negative charge. Thus, using the electropositivity of cationic polymer leads to the formation of complexes containing siRNA/microRNA molecules promoting cell uptake and preventing degradation by RNase. Most complexes interact with the cell membrane electrostatically, because of the anionic cell surface proteoglycans, and enter into cells. For cell uptake, clathrin- and caveolae-mediated endocytosis, which are energy-dependent and controlled by the cell internalized molecules, are the common processes (Figure 2); the other occurs in phagocytes (macrophages, dendritic cells, and neutrophils), and the sizes of engulfing particles are larger than 0.5 μm, driven by actin.[41–44] Additionally, the efficiency of NPs is affected by the physicochemical properties, such as size, shape, and surface chemistry, as well as cell type. Some researchers said that uptake of PLGA copolymer-DNA complexes in Caco-2 cells was size dependent, with the highest uptake seen for particles with a mean diameter of 100 nm;[45] but, in COS-7 and HEK293 cell lines, it was higher for particles with mean diameters of 70 and 200 nm, respectively.[46] Furthermore, the highest transfection efficiency was seen for polyetherimide (PEI) nanogels with 75- and 87-nm mean diameters, when transfected in complexes with different diameters but with similar surface charge in different cancer cell lines.[47] These results

Table 1. The siRNA/MicroRNA-Based Drugs Targeting Different Diseases in Clinical Trials

Disease	Target	Vehicle	Drug Name	Sponsor	ClinicalTrials.gov Identifier (Phase)
Cancer					
HC, ST, ACC, GNT	siRNA target PLK1	lipid nanoparticle	TKM 080301	Tekmira Pharmaceuticals	NCT02191878 (I/II) NCT01262235 (I/II) NCT01437007 (I)
ST, MM, NHL	siRNA target MYC	lipid nanoparticle	DCR-MYC	Dicerna Pharmaceuticals	NCT02110563 (I) NCT02314052 (I/II)
ST	siRNA target RRM2	polymer nanoparticle	CALAA-01	Calando Pharmaceuticals	NCT00689065 (I)
ST	siRNA target EphA2	liposome	siRNA-EphA2-DOPC	M.D.Anderson Cancer Center	NCT01591356 (I)
Leukemia	antisense target GRB-2	neutral liposomes	BP1001	Bio-Path Holdings	NCT01159028 (I)
ASC, PC	siRNA target PKN3	lipid nanoparticle	Atu027	Silence Therapeutics	NCT01808638 (I/II) NCT00938574 (I)
PDA, PC	siRNA target K-RAS	biodegradable polymer matrix	siG12D LODER	Silenseed	NCT01188785 (I) NCT01676259 (II)
Glioblastoma	siRNA target p53	nanoparticle (NPs)	Temozolomide/SGT-53	SynerGene Therapeutics	NCT02340156
Lung cancer	siRNA target Fus1	DOTAP-Chol	Fus1/Erlotinib	Genprex	NCT01455389 (I/II)
HC	siRNA target CEBPA	liposomal nanoparticle	MTL-CEBPA	Mina Alpha	NCT02716012 (I)
Glioblastoma	siRNA target Bcl2L12	spherical gold nanoparticle	NU-0129	Northwestern University	NCT03020017 (early I)
IMG	siRNA target UGT1A1*28	nanoliposomal	CPT-11	University of California, San Francisco	NCT00734682 (I)
Advanced, metastatic cancer, ST	shRNA STMN1	BIV-lipoplex	pbi-shRNA STMN1 LP	Strike Bio	NCT01505153 (I)
Ewing's sarcoma	shRNA EWS/FLI1 type 1	BIV-lipoplex	pbi-shRNA EWS/FLI1 Type 1 LPX	Strike Bio	NCT02736565 (I)
NR	eIF5AK^{50R} plasmid eIF5A siRNA	polyethylenimine	SNS01-T	Senesco Technologies	NCT01435720 (II)
MPM, NSCLC	microRNA -16 mimic target EGFR	EDV	TargomiRs	Asbestos Diseases Research Foundation	NCT02369198 (I)
Virus infection					
EVD	siRNA target VP24, and VP35 regions, EBOV polymerase inhibitor	lipid nanoparticle	Favipiravir	INSERM, France	NCT02329054 (II)
Other Disease					
Hepatic fibrosis	siRNA target HSP47	lipid nanoparticle	ND-L02 s0201 injection	Bristol-Myers Squibb	NCT02227459 (Ib/II)
Hypercholesterolemia	siRNA target APOB	lipid nanoparticle	PRO-040201	Tekmira Pharmaceuticals	NCT00927459 (I), terminated

Source: https://clinicaltrials.gov. ACC, adrenocortical carcinoma; ASC, advanced solid cancer; GNT, gastrointestinal neuroendocrine tumors; HC, hepatocellular carcinoma; MM, multiple myeloma; MPM, malignant pleural mesothelioma; NHL, non-Hodgkins lymphoma; NSCLC, non-small-cell lung cancer; PC, pancreatic cancer; PDA, pancreatic ductal adenocarcinoma; ST, solid tumor; ALC, advanced liver cancer; SCLC, squamous cell lung cancer; IMG, intracranial malignant glioma; NR, not recorded; EGFR, epidermal growth factor receptor; GRB-2, Growth Factor Receptor Bound Protein-2; RRM2, Ribonucleotide Reductase Regulatory Subunit M2; PLK1,Polo-Like Kinase 1; HSP47, Heat Shock Protein 47; EphA2, Ephrin type-A receptor 2; eIf5A, Eukaryotic translation initiation factor 5A-1; EDV, EnGeneIC Delivery Vehicle; CEBPA,CCAAT/enhancer-binding protein alpha; BIV-lipoplex, bilamellar invaginated vesicle lipoplex; EVD, Ebola virus disease; PKN3, protein kinase N3; K-Ras oncogene, Kirsten rat sarcoma viral oncogene; APOB, apolipoprotein B; VP24, virus protein 24; VP35, virus protein 3.

suggest that the optimal size for gene transfer of non-targeting cationic vector-DNA complexes is between 70 and 90 nm. As the cellular uptake pathway of receptor targeting vector-DNA complex is different from non-targeting NPs, the effective size needs to be smaller. Several temperature- and pH-sensitive polymers or other smart polymers rapidly used in gene delivery to enhance cell uptake and lysosomal escape are introduced in Different Stimuli-Responsive Gene Delivery Systems.

Nuclear Entry for Plasmid shRNA

For shRNA, the carrier vector must carry it into the nucleus of the host cell, and nuclear localization sequences (NLSs) have been

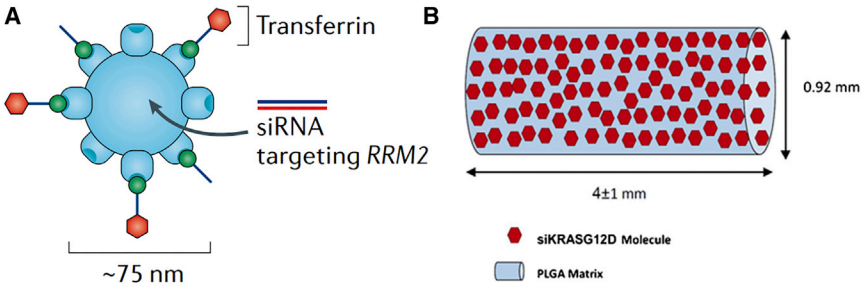


Figure 1. Schematic Illustrations of the siRNA-Based Therapeutics of CALAA-01 and siG12D-LODER

(A) CALAA-01 is a polymer-based nanoparticle containing a targeting ligand on its surface (the human protein transferrin) and a small interfering RNA (siRNA) that targets the M2 subunit of ribonucleotide reductase (*RRM2*). Reproduced with permission from Zuckerman and Davis.[30] Copyright © 2015 Springer Nature. (B) siG12D-LODER is a polymeric matrix of poly(lactic-co-glycolic) acid (PLGA) in a shape of a small cylindrical rod of 0.8 mm in diameter and 5.5 ± 1 mm in length. Reproduced with permission from Titze-de-Almeida et al.[33] Copyright © 2017 Springer Nature.

utilized. There are reports of the transfection efficiencies of cationic DNA nanocarriers or polycations coupled with NLS derived from the SV40 large T antigen, which can be recognized and go through the nuclear pore complex (NPC).[48–50] A peptide or protein such as Histon H1 can also improve the efficiency of entering into the nucleus.[51] Besides, sugar residue can also transport cargoes into the nucleus, and the pathway is used by neoglycoproteins to enter the nucleus. Lactosylated polylysine (PLL)/cDNA complex induced nuclear localization by binding to a potential lectin-like shuttling protein with galactose/lactose specificity, and this binding interaction was suggested to trigger the nuclear internalization of the complex.[52,53]

Some cationic polymers like PEI and T443, a poly-(glycoamidoamine), are capable of inducing nuclear membrane permeability, but they also have the highest level of cytotoxicity.[53]

Nanotoxicity

Despite the increasing biocompatibility of the material and the decreasing side effects, NPs-mediated toxicity still exists. The physicochemical properties of NPs, such as small size, large surface area, and flexible chemical compositions or structures that facilitate their use in nanomedicine, have also been found to contribute to their enhanced toxicological side effects. Reports have said that smaller sized NPs exhibit higher toxic effects due to the increased surface area,[54] and the structure and shape of NPs also contribute to nanotoxicity.[55] Besides, the ability of NPs adsorbing with ions and biomolecules influences the cellular responses, resulting in toxicity.[56]

As Baeg and colleagues[57] reviewed, polymeric NPs are safer than inorganic NPs and metallic NPs. Thus, a better understanding of cationic polymer NPs and the mechanisms of cationic polymer-based drug and gene delivery involved in nanotoxicity may help to further develop and utilize NPs in the field of nanomedicine.

The Smart NPs Engineered for Gene Delivery

Now, the researchers are focused on the design of more sophisticated NPs, aimed at addressing multiple challenges at the same time, such as controlled delivery, nucleic acid separation, controlled cell patterning, and gene release. Here we introduce three types of commonly used gene carriers that are lipid based, polymeric, and inorganic. In the subsequent section, different stimuli response modifications of NPs are addressed. Lastly, we discuss how to improve nanomedicine tumor accumulation and penetration (EPR).

Gene Delivery Systems

Lipid-Based Systems

Lipid-based NPs have been developed for systemic delivery of RNA or DNA into tumors, including liposomes, solid lipid NPs (SLNs), and reconstituted high-density lipoprotein (rHDL) NPs. Cationic liposomes have been extensively applied because of their high encapsulation efficiency, effective transfection, and easy-to-surface modification. Zhang et al.[58] used a liposome-protamine-IL-22-binding protein mRNA complex to inhibit the growth of C26 tumor cells exhibiting a high mRNA transport and expression efficiency because of the protamine, which can package DNA denser than histones. However, the potential clinical use of cationic liposomes is limited by their instability, rapid systemic clearance, toxicity, and induction of immunostimulatory responses. Therefore, many cationic lipid-containing liposomes are being developed that efficiently capture nucleic acids internally, but there are some NPs with neutral or anionic surface charge, such as lipidic NPs (LNPs).[59] Researchers found that 3β-[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol/dioleoyl phosphatidylethanolamine (DC-Chol/DOPE) cationic liposomes were easy to aggregate with negatively charged blood components and thus were allowed only by direct injection into local targets, but PEGylated DC-Chol/DOPE-siRNA lipoplexes can effectively reduce the excretion by kidneys and scavenging in liver, prolong the circulation time *in vivo*, and ultimately increase their preferential tumor accumulation; in addition, the systemic administration of PEGylated lipoplexes won't lead to any activation of innate immune responses in the immunocompetent mice.[60] Zou et al.[61] used low-molecular-weight PEI conjugating to lipid and prepared poly(D,L-lactide-co-glycolide) NPs; as a potential effective gene delivery system, it showed low toxicity and higher transduction efficacy when compared to high-molecular-weight PEI *in vitro*.

There are some other lipid-based NPs systems like cationic SLN[62] and rHDL.[63,64] SLNs bounded with p5α-red were developed as a

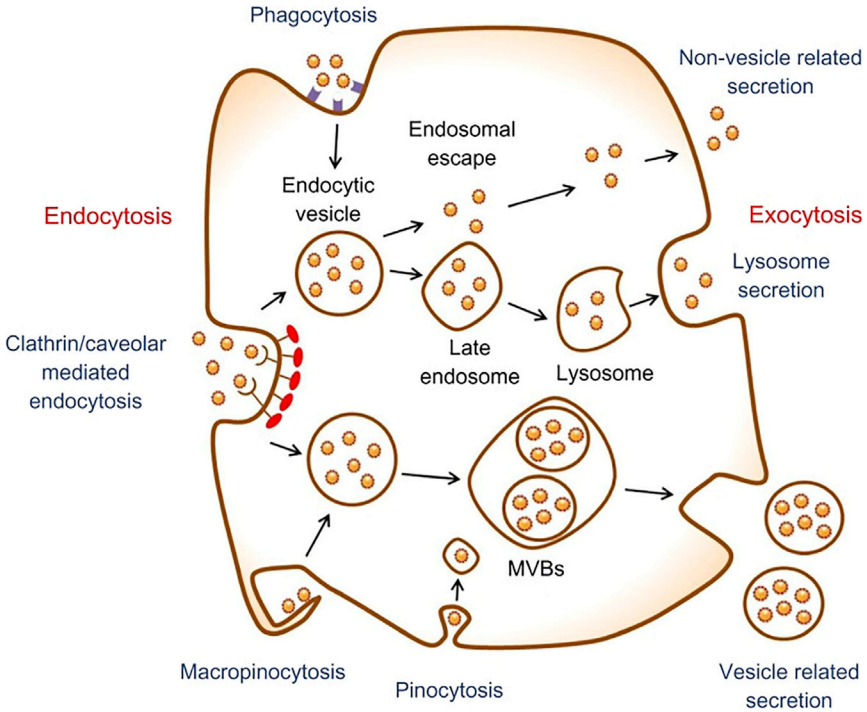


Figure 2. Schematic of Endocytosis and Exocytosis Patterns of Nanoparticles

Nanoparticles enter the cell via four types of pathways: clathrin-/caveolar-mediated endocytosis, phagocytosis, macropinocytosis, and pinocytosis. Nanoparticles exit the cell via three types of pathways: lysosome secretion, vesicle-related secretion, and non-vesicle-related secretion. MVBs, multivesicular bodies. Reproduced with permission from Oh and Park.[42] Copyright © 2014 Dove Press Ltd.

gene vector, and no significant cytotoxicity was observed with high gene-silencing efficiency *in vitro*.[65] However, a main drawback of SLNs is the stability of NPs, which may hinder their implementation for therapeutic purpose. Thus, researchers focus on overcoming this problem and developing effective NPs systems for delivering nucleic acids.

Polymeric Gene Delivery Systems

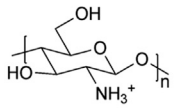
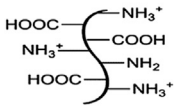
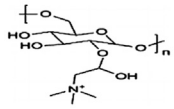
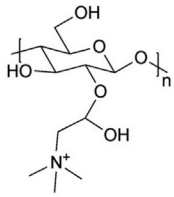
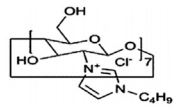
Despite of lipid, natural polymers like proteins and oligopeptides and synthetic cationic polymers, including cyclodextrin derivatives, PEI, and polyamidoamine (PAMAM), have been explored, owing to their unique physicochemical properties and the ability to form electrostatic complexes with anionic biomolecules, nucleic acids, and proteins used in clinical trials along with their inherent bioactive properties, such as being antimicrobial, antioxidant, stimuli responsive, anti-inflammatory, and antitumor. Also, the delivery efficiency was affected by the molecular weight, charge density, and chemical structure of the polymer.[66]

Natural Polymers. Cationic chitosan is a deacetylated derivative of chitin, which is the second most abundant natural polymer. The uses of chitosan include with proteins,[67] peptides,[68] DNA,[69] and siRNA delivery.[70,71] Given the cytotoxicity and stability of chitosan in the siRNA delivery process, further developments of chitosan-based delivery systems occurred. Grafting small molecules or polymer chains onto the chitosan backbone or alkalifying the amino groups to modify chitosan were most investigated. Mad2 siRNA-loaded epidermal growth factor receptor (EGFR)-targeted chitosan NPs can effectively inhibit cell growth in a cisplatin-sensitive and -resistant lung cancer model.[72] Poly(ethylene glycol)-modified chitosan (PEG-CS) could improve chitosan solubility, by forming stable siRNA-loaded NPs with smaller particle size, and enhance transfection efficiency in cancer cell lines.[73,74] Using a carboxymethyl dextran (CMD)-chitosan NPs platform to encapsulate HMGA2 siRNA and doxorubicin (DOX), it had a high efficiency for siRNA and drug encapsulation (about 78% and 75%, respectively), and it was stable against serum and heparin.[75] When CD73-siRNA was encapsulated into chitosan-lactate NPs, it also exhibited low cytotoxicity and high transfection.[76] The applications of other natural polymers have been listed in Table 2.

Synthetic Polymers. Compared with natural cationic polymers, synthetic cationic polymers are easy to improve the control properties and modify the structure. The bioactive moieties and functional groups can be readily incorporated into synthetic polymer systems to produce therapeutic potential and degradation characteristics of gene delivery. The research most focuses on PEI, PLL, polyacrylic acid (PAA), poly(aliphatic ester) (PAE), and poly(N,N-dimethylaminoethyl methacrylate) (PDMAEMA) and some required modification on them (Table 3).

PEI is one of the most prominent and extensively cationic polymers capable of gene transfection. The high charge density of PEI and composition of primary (25%), secondary (50%), and tertiary (25%) amines make it have high gene transfection activity (Figure 3A).[77] Primary amines are protonated at physiological pH 7.4, and they enable PEI to bind negatively charged genes effectively via electrostatic interactions. The secondary and tertiary amines are able to sequester protons in the endosomes, namely, the proton-sponge effect.[78] Furthermore, the efficacy and toxicity of PEI are strongly correlated with its molecular weight and structure (branched or linear). The higher the molar mass with higher cationic charge densities and better cellular uptake, generally the higher cytotoxicity is.[79–82] Thus, researchers modified PEI structure to improve the transfection efficiency and decrease the cytotoxicity in PEI and gene delivery systems. One strategy is modifying PEI with PEGylation to create a hydrophilic exterior that reduces interactions of the polyplex with plasma proteins and

Table 2. Overview of the Most Widely Used Natural Cationic Polymers in Gene Delivery

Cationic polymer	Component	Structure	Advantages	Disadvantages	Modification in gene delivery
Cationic Chitosan	N-acetyl glucosamine; D-glucosamine		biocompatibility,non-toxicity,biodegradability,antioxidant,mucoadhsive	Low transfection efficiency due to poor endosomal escape, Poor solubility in aqueous solutions	Trimethyl chitosan (TMC) PEGylated chitosan Guanidinylated chitosan
Cationic Gelatin	18 non-uniformly distributed amino acid		biodegradability, biocompatibility, without pH dependency		aminated gelatin, PEGylated gelatin, thiolated gelatin
Cationic Dextran	Glucose units linked by α-1,6-linkages		biodegradability, wide availability, easy to modification ,solubility		PEGylated dextran, aminated dextran, Dextran modification with histidine, Dextran–glycidyltrimethylammonium chloride
Cationic Cellulose	Linear glucose units linked by β-1,4-D-linkage		its derivatives are hydrophilicit, biodegradability, antibacterial	insolubility of itself in water and most organic solvents	HPDs, Quaternized celluloses
Cationic Cyclodextrin	six to eight cyclic glucose units linked by α-1,4-linkage		monodisperse saccharide structure, low immunogenicity, easy to modification, favorable toxicology	Difficulty in processing	PEGylated cyclodextrin, PEI-cyclodextrin, PLL-cyclodxtrin

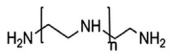
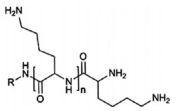
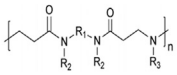
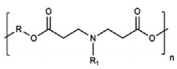
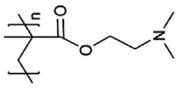
erythrocytes. The other important strategy is modifying PEI with hydrophobic moieties, like lipids, stearic acid, cholesterol, or palmitic acid, to increase cellular uptake and stability with systemic administration.[83,84] For branched PEI (bPEI) (1.8 kDa), conjugation with cholesterol can increase the transfection efficiency and reduce the toxicity.[78] In addition, PEI as a functional group to modify carbonaceous quantum dots or gold NPs (AuNPs) can effectively deliver and release a siRNA in the tumor site (Figure 3B).[85,86] Folate-rPEI-CDs with high biocompatibility and gene delivery efficiency can be accumulated in lung cancer cells selectively through receptor-mediated endocytosis, resulting in better gene silencing and anti-cancer effects.

PLL, as one of the most widely studied gene carriers, is a synthetic polypeptide composed of a large number of primary amines, which can interact with negatively charged biomolecules through electrostatic interaction. Due to the proximity group effect in the polymer chain, only a portion of the primary amine groups of the PLL are protonated at physiological pH. Therefore, the PLL exhibits a relatively low buffering capacity (pH 5.7–7.7), whereas PEI does not effectively escape the endosome without the endosomal solubilizing agent and strongly impairs its transfection ability.[87–89] In addition, it has been indicated that high-molecular-weight PLL has shown a tendency to aggregate and precipitate, which may cause cytotoxicity depending on the ionic strength.[90] Thus, conjugation to hydrophilic or amphiphilic macromolecules, such as PEG, has been widely investigated.[91] However, grafting PEG to the side chains of PLL indeed prolonged its lifetime in blood circulation and tumoral accumulation with loss of the ability to associate with siRNA,[92] but it also reduced cell uptake and binding at the target site. Thus, choosing specific targeting moieties, like antibodies, peptides, sugars, and folate, to conjugate to PEG-PLL can improve cellular uptake via receptor-mediated endocytosis and conferred tissue specificity.[93] Another method to promote the transfection efficiency is stimuli-responsive polymer, such as pH-sensitive, reduction-sensitive, or enzyme-responsive linkages. We introduce the smart polymer in detail in the next section.

PAA is a bisacrylamide by the addition of a primary or tertiary secondary amine to a Michael-type polyamine used for gene transfer due to their biocompatibility, water solubility, biodegradability, and lower toxicity, including linear and branched PAA. Therein, bioreducible PAAs or poly(disulfide amines) are a unique family of PAAs with lower cytotoxicity and improved gene transfer compared to branched PEI-25 kDa (Figure 4A).[94,95] Synthesized reducible poly(amino ethylenimine)s (SS-PAEIs) exhibited a 2-fold higher efficiency compared with PEI-25 kDa. Moreover, Lin et al.[96] found that the branching degree had a significant effect on transfection efficiency. They indicated that low-branched PAA could more effectively compact plasmid DNA (pDNA) into positively charged NPs than both high-branched PAA and linear PAA.

PDMAEMA is one of the most important pH-responsive cationic polymers, with combined transfection efficiency and acceptable cytotoxicity.[97] There were various modifications that have been investigated in an attempt to improve the transfection of PDMAEMA.

Table 3. The Overview of the Most Widely Used Synthetic Cationic Polymers in Gene Delivery

Cationic polymer	Component	Structure	Advantages	Disadvantages	Modification in gene delivery
PEI	Polyethylenimine LPEI and BPEI		High transfection efficiency	Non-degradability, cytotoxicity, low hemocompatiblity	PEGylation PEI, PCL-PEI, mercaptan-modified PEI, amine-modified PEI, Polysaccharide-modified PEI
PLL	Homopolymer of amino acid L-Lysine		using for polyplex formation	low transfection efficiency high MW PLL is cytotoxic	PEGylation PLL, stimuli-responsive PEG-PLL
PAA	tert-amino and amino groups		biodegradability, biocompatibility, water solubility, a lower toxicity		cholesterol-PAA
PAE	Amine containing polyesters		hydrolytically degradable, no negative effect on cell viability		PEG-PAE,RGD-PAE, sugars-PAE,PDA-PAE
PDMAEMA	Poly(2-N,N-dimethyla-minoethylmethacrylate)		pH- and temperature- responsive, water-soluble, low cytotoxicity	Poor biodegradation	PEGylated PDMAEMA, chitosan-PDMAEMA, ester linkages of PDMAEMA

PEGylated PDMAEMA not only induced cytokine production by murine macrophages but also it could effectively deliver a DNA vaccine, which can enhance adaptive immune responses by activating innate immunity (Figure 4B).[98,99]

Endogenous proteins and synthetic oligopeptides have been used for siRNA or DNA delivery like HAS, transferrin, atelocollagen, poly (Pro-Hyp-Gly), cholestery oligoarginine, and MPG-8, a 21-residue amphipathic peptide. Wang et al.[100] developed PEGylated NPs based on the cationic α-helical polypeptide poly(γ-4-((2-(piperidin-1-yl) ethyl)aminomethyl)benzyl-l-glutamate) for the delivery of Cas9 expression plasmid and small guide RNA (sgRNA) to various cell types and gene-editing scenarios. The results showed that the colloidally stable P-HNPs achieved a Cas9 transfection efficiency up to 60% and sgRNA uptake efficiency of 67.4%, representing a versatile gene-editing platform for biological research and therapeutic applications.[100]

Inorganic Gene Delivery Systems

Inorganic materials are frequently used for drug delivery and imaging, including gold, calcium phosphate, cadinum (quantum dots), and iron oxide. Actually, inorganic substances are biologically inert and afford excellent controls for gene delivery.

AuNPs can be suitably engineered for applications in gene delivery and as carriers of peptides and proteins. AuNPs can be stabilized by a large variety of stabilizers like polymers, ligands, biomolecules, denrimers, surfactants, etc.;[84,101–104] the stabilizers affect not only the relative instability and aggregation but also cell uptake and toxicity, which points to the necessity of strategic engineering of NPs surface chemistry.[105–107] Gu and colleagues[108] developed a novel AuNP beacon by optimizing the sequence amount, and they modified PEG and cell-penetrating peptide (CPP) on the gold core (Figure 5). When the molecular beacon got into tumor cells through membrane recognition by a scavenger receptor, the detecting DNA identified target mRNA like Akt, mTOR, and HIF-1 mRNA, resulting in the expression of Akt, mTOR, and HIF-1 being blocked and the fluorescence signal amplified.

Cationic polymer (chitosan and poly-L-lysis) and cysteine-coated AuNPs also showed good stability and resistance to aggregation in blood circulation, with significant transgene activities in all cell lines.[104] Son et al.[109] developed an exquisite RNAi-AuNP nanoconstruct with various geometries, which exhibited a precise conjugation and separation of a designated number of therapeutic siRNAs onto AuNP to create various geometries of RNAi-AuNP nanoassemblies, based on the hybridization between complementary nucleic acid base-pairing. Besides, using the property of mesenchymal stem cell (MSC) high affinity to bind arginine-glycine-aspartic (RGD) peptide, modifying RGD to dendrimer-entrapped AuNPs exhibited a highly efficient and specific gene delivery to stem cells. It revealed that the coexistence of RGD and AuNPs allows the design of a dendritic vector with specific stem cell-binding ability to bind to the cell surface through integrin receptors and improve the three-dimensional

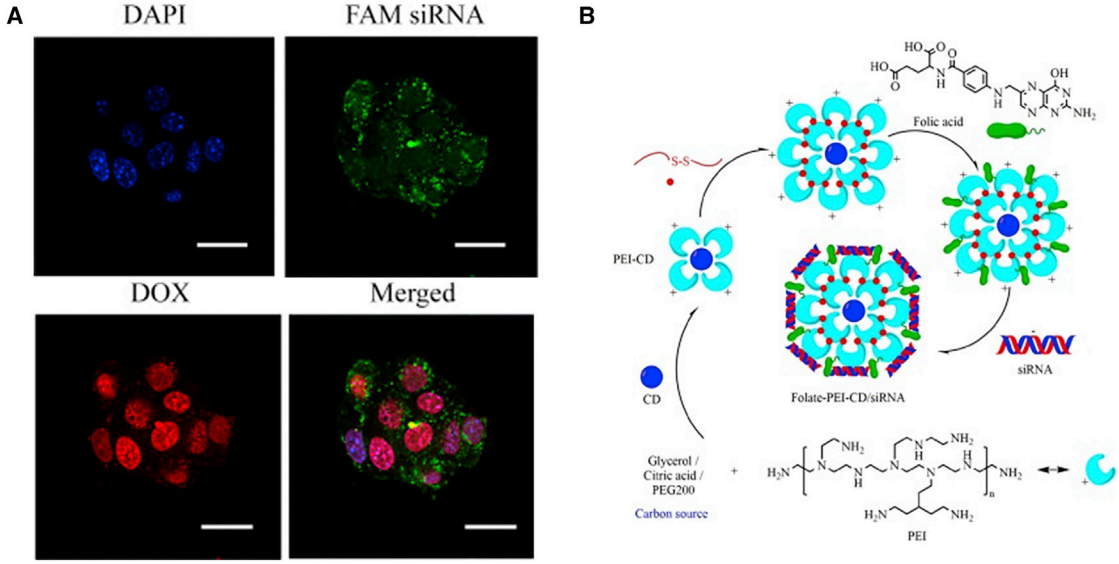


Figure 3. The Application of PEI for Co-delivery of siRNA and DOX and the Schematic Synthesis of Folate-PEI-CDs/siRNA Nanoparticles

(A) Confocal laser scanning microscope (CLSM) images of B16F10 cells incubated with PEI/siRNA/DOX for 24 hr. Scale bars, 20 μm. Reproduced with permission from Xu et al.[77] Copyright © 2017 Elsevier. (B) Schematic diagram of synthesis route of folate-PEI-CDs/siRNA nanoassemblies. Reproduced with permission from He et al.[85] Copyright © 2017 Elsevier.

conformation of dendrimers, which facilitates efficient and specific stem cell gene delivery applications.[110] Further, poly(thymine)-functionalized AuNP (AuNP-p(T)-DNA) is also another strategy for gene delivery. The gene of interest was inserted separately into pcDNA6 plasmid vector containing BGH polyadenylation(P(A)) signal; the produced poly(A) tail can hybridize with poly(T) oligonucleotide on AuNPs, facilitating the increased production of the respective proteins (Figure 6A).[111]

The application of calcium-based biomaterials in gene delivery causes calcium ion to form ionic complexes with the helical phosphates of DNA, and these complexes have easy transportability across the cell membrane via ion channel-mediated endocytosis, including calcium phosphates, calcium carbonates, calcium silicate, and calcium fluoride. Taking calcium phosphates as an example, there was a large number of -OH groups with Ca^{2+} cations in their surface, which can effectively adsorb organic molecules with acid groups such as carboxylic(-COO^{-}) and phosphoric groups. In addition, the solubility of calcium phosphates (CaPs) increased with the decrease of the solution pH value and dissolved into ions at a low pH value, revealing themselves to be pH-responsive nanocarriers for gene and drug delivery. A report said that low-to-moderate elevation of Ca^{2+} concentration (0.2–0.4 μM) triggers apoptosis and higher concentrations of Ca^{2+} (>1 μM) are associated with necrosis; the normal extracellular Ca^{2+} concentration is ~1.2 mM and the cytosolic concentration is ~0.1 μM.[112] However, in the application of CaPs, researchers performed one more layer of CaPs covering DNA-coated CaPs, including PEI,[113] liposome,[114–117] hyaluronan (HA),[118] or PEG-biophosphonates,[119] which can enable the prepared CaP NPs to remain physically stable over a long time and effectively protect siRNAs from enzymatic degradation under physiological conditions, although the elevated Ca^{2+} concentration was only a transient event and not toxic to the cells.[120]

Chen et al.[121] constructed a PEG/lipid/calcium phosphate-$Onco^{Ad}$ (PLC-$Onco^{Ad}$) delivery system for ZD55-IL-24 carried by oncolytic adenovirus (Figure 6B). It exhibited a highly efficient targeted gene delivery to the tumor site without the innate immune response and specific sequestration and toxicity in liver through systemic administration. Besides, using alendronated-hyaluronan graft polymer (AHA) instead of PEG-bp as the outer shell to carry the siRNA exhibited a stronger interaction between calcium ions and negatively charged phosphate, and it enabled CD44-mediated targeting of tumor cells in the study performed by Qiu et al.[118] In this delivery system, the negatively charged CaP-AHA/siRNA NPs (−12 mV) could effectively deliver EGFR-targeted siRNA and downregulate EGFR expression through CD44-mediated endocytosis; and, the internalized CaP-AHA/siRNA NPs exhibited a pH-responsive release of siRNA benefitting from CaP.

Nano-sized quantum dots (QDs) exhibit uniquely optical properties that are tunable with different sizes and shapes as attractive vectors for imaging, guided by the properties of emitting narrow symmetric bands under a wide excitation range, having antiphotobleaching stability, and being bio-functionalized on the large surface area. In addition, QDs can potentially promote the efficient delivery of siRNA into target cells and track the distribution of siRNA in cells *in vitro* or *in vivo*.[85,86,122]

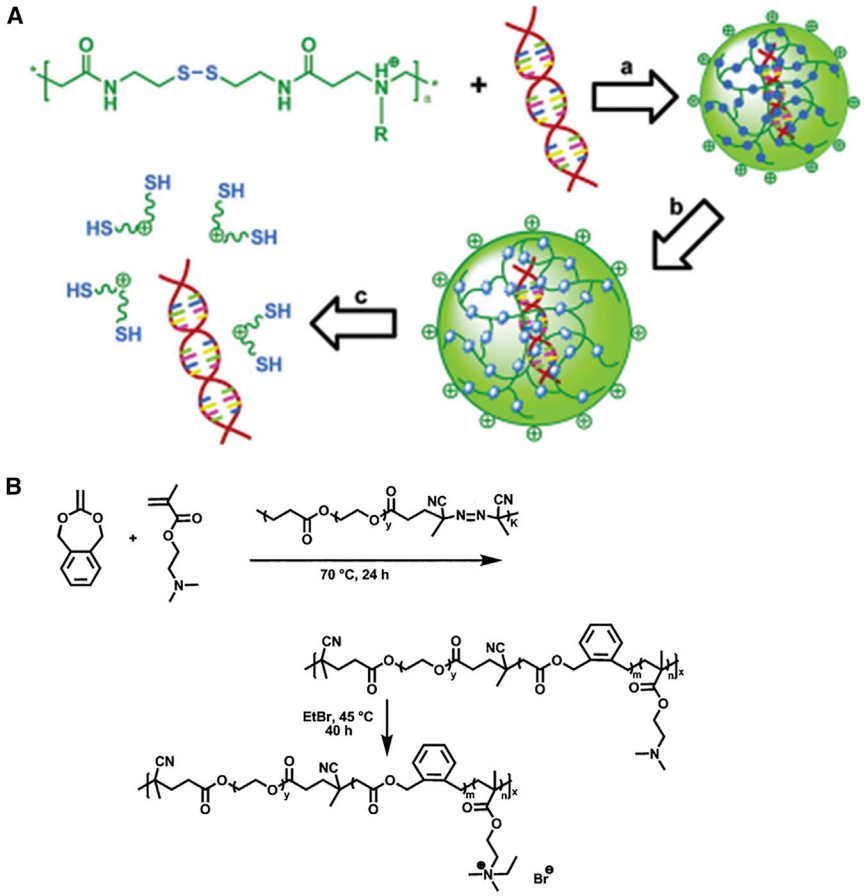


Figure 4. The Schematic Process of SS-PAA/DNA *In Vitro* and the Formation of Poly(PEG-co-(BMDO-co-DMAEMA)) with EtBr

(A) The concept of DNA condensation and subsequent intracellular release. (a) Formation of SS-PAAs/DNA polyplexes that are stable in the extracellular environment, (b) intracellular reduction of the disulfide linkages in the polymer of the polyplex, and (c) dissociation of DNA from the degraded polymer are shown. Reproduced with permission from Lin et al.[94] Copyright © 2007 American Chemical Society. (B) Synthesis route for the formation of the poly(PEG-co-(BMDO-co-DMAEMA)) and poly (PEG-co-(BMDO-co-DMAEMA))·EtBr. Reproduced with permission Zhang et al.[98] Copyright © 2012 American Chemical Society.

Different Stimuli-Responsive Gene Delivery Systems

pH-Responsive Systems

The pH gradient is one of the most exploited stimuli to design stimuli-sensitive NPs for DNA/RNA delivery in tumors, because pH exists differently among healthy (pH 7.2–7.4) and tumor (pH 6.5–6.9) sites, cytosol (pH 7.4) and lysosomes (pH 4.5–5) and endosomes (pH 5.5–6).[123–125] These pH-responsive components can be used to design pH-sensitive nanocarriers with the properties of being protonizable, acid labile, and destabilizing.[126] At a pH above the acid dissociation constant (pKa), a specific pH that when altered can affect the structure, the side chain amine groups remain non-ionized, allowing the polymer chain to contract while capturing siRNA or DNA. As the pH decreases below the amine pKa, for example, in a lysosome or endosome, the amine group becomes protonated and the polymeric chain swells due to electrostatic repulsion, resulting in the siRNA or DNA being encapsulated by cationic polymer released into the surrounding medium.[87]

PEI, chitosan, and PAA can be used as pH-responsive carriers because of their primary, secondary, and tertiary amine groups. When polyhistidine and poly-arginine coupled to PEI, the gene transfection abilities were substantially improved with low levels of cytotoxicity.[127] In addition, conjugating PEI to biodegradable lipids, such as cholesterol[128] or poly-glutamic acid derivates,[129] can increase the stability of the siRNA and PEI complexes and reduce the toxicity. Meanwhile, CaP NPs coupled with PEG-grafted carboxymethyl chitosan (CMCS), which have the stabilization of the CaP NPs and the high releasing efficiency of CMCS, showed a highly efficient delivery ability.[130] Other calcium-based biomaterials have been introduced in the part of inorganic gene delivery systems. Besides, a carboxybetaine ester functional (CBE) group can also mediate the interaction between DNA and AuNPs via pH.[131] The negatively charged phosphate backbone of the DNA interacts with and adsorbs on the positively charged CBE on the self-assembled monolayer (SAM). DNA release can be carried out by hydrolyzing CBE to a zwitterionic carboxybetaine state, and the adsorption of the SAM-modified common gold surface can be controlled by pH control of the negatively charged citrate-terminated AuNPs.

Thermo-responsive Systems

Temperature is among the most often investigated stimuli to control drug release, because the structural properties of some NPs are changed in response to temperature.[87] The hyperthermic nature of most inflamed pathological sites and tumors can act as an internal stimulus. Poly(N-isopropylacrylamide) (PNIPAAm)[132,133] or pluronic F-127 as temperature-responsive moieties can incorporate or graft to NPs to achieve the thermo-stimuli; and, PEG attachment can solve the toxicity, immunogenicity, and circulation time concomitant with thermo-sensitive moieties. PNIPAAm-co-2-(dimethylamino)ethyl methacrylate (DMAEMA)-co-butylmethacrylate (BMA) contained 8 mol% DMAEMA and 11 mol% BMA with a lower critical solution temperature (LCST) at 21°C; therefore, the copolymer was insoluble above 21°C and soluble below 21°C. At a temperature above the LCST, the complexes between the polymer and the pCMV-lacZ plasmid encoding for β-galactosidase become more densely bound

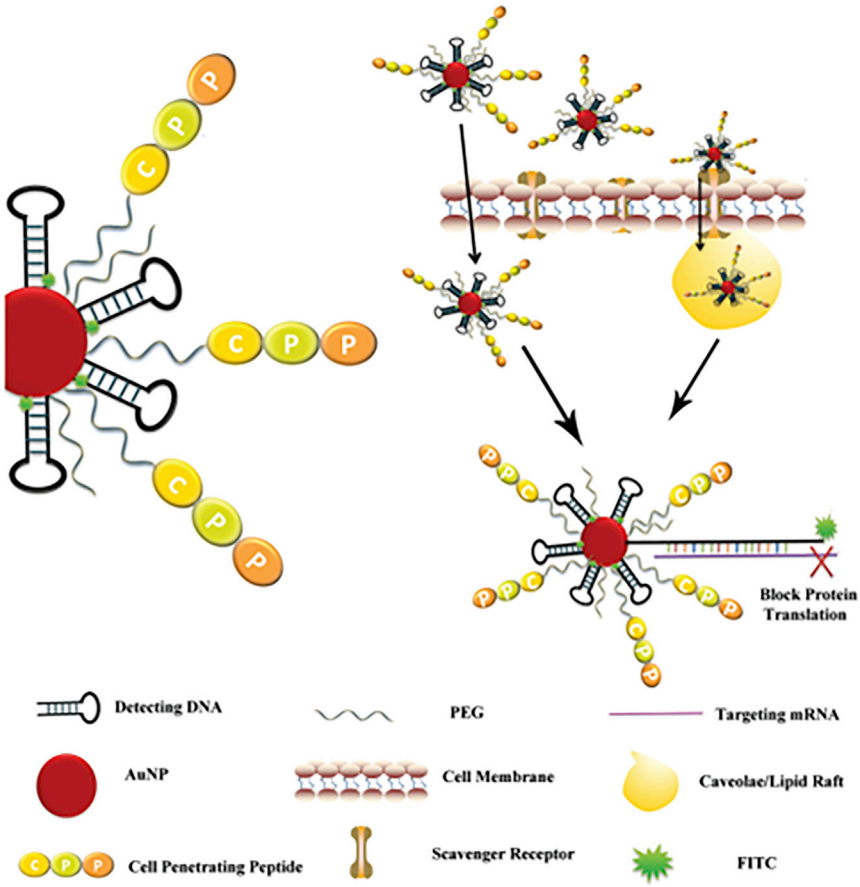


Figure 5. Schematic Illustration of the Modification of AuNP Beacon and the Mechanism of Cell Uptake
Reproduced with permission from Li et al.[108] Copyright © 2018 John Wiley & Sons, Inc.

and better protect the plasmid from enzymatic degradation. When the temperature is lower than the LCST, hydration occurs and the water solubility of PNIPAAm increases, resulting in the complexes being less compact and DNA being released. The transfection efficiency incubated at 37°C for 48 hr was greater than that incubated at 20°C for 3 hr and 37°C for 45 hr.[134]

Feng et al.[135] prepared an efficient nonviral cationic block copolymer gene delivery system containing poly(ethylene glycol)-block-poly {N-[N-(2-aminoethyl)-2-aminoehtyl]aspartamide} [PEG-b-PAsp (DET)] and poly(N-isopropylacrylamide)-blockPAsp(DET) [PNIPAM-b-PAsp(DET)] with high tolerability against nuclease and strong resistance toward protein adsorption. From the results *in vitro* and *in vivo*, it showed a higher gene transfection efficiency than that of regular polyplex micelles prepared form solo block copolymer of PEG-b-PAsp (DET)(SPM), with low cytotoxicity and improved colloidal stability.[135] In addition, thermo-sensitive PEI-plurinic nanocapsules prepared by an interfacial crosslinking reaction between preactivated Pluronic F-127 and low-molecular-weight PEI conjugated with PEG showed a controlled delivery manner.[136] The introduction of isobutyramide groups attached to the hyperbranched PEI side chain also indicated that the nanomedicine has thermal response characteristics.[137]

Redox-Responsive Systems

The redox-stimuli system is one of the most efficient systems for stimulus-sensitive cancer and gene therapy.[138–140] Glutathione (GSH) is an ubiquitous small molecule involved in important cellular pathways, such as in the maintenance of intracellular redox state because of the different redox conditions between the intracellular (2–10 mM) and extracellular (2–20 μM) compartments.[141,142] Furthermore, tumors can be regarded as a reducing environment because the concentration of GSH in tumor tissues and the cytoplasm of tumor cells is at least four times higher than that in normal tissues.[143] The rational design of reduction-sensitive delivery systems containing disulfide linkages has prepared for tumor targeting and intracellular delivery of siRNA.[144,145] The disulfide linkage in the complex can increase siRNA transfection efficiency and decrease toxicity in cells with high levels of GSH, like tumor cells. Hu et al.[146] constructed a redox-sensitive, oligopeptide-guided, self-assembling, and efficiency-enhanced (ROSE) system by mixing PEI-hβCD with Ad-SS-PEG and Ad-PEG-SP94 for miR-34a delivery, representing a significant effect improvement over conventional gene delivery strategies. In addition, the semblable strategy of MC11 peptide targeting EGFRs conjugated to PEI-hβCD could efficiently condense pDNA into NPs after mixing with Ad-SS-PEG, and it exhibited high transfection efficiency.[147] It is said that, compared to non-reducible polyplexes such as PAA, redox-sensitive polyplexes show higher gene transfection activity in response to intracellular GSH levels.[148] Meanwhile, the degradable behaviors of silica nanocomposite with disulfide bonds or embedded drugs revealed the redox-triggered degradable silica NPs for drug and gene delivery.[149]

Enzyme-Responsive Systems

Different from normal tissue, the levels of certain local enzymes, such as matrix metallo proteinases (MMPs), β-glucuronidase, hyaluronidase, urokinase plasminogen activator (uPA), human leukocyte elastase (HLE), and cancer-associated proteases (CAPs), are out of control, and they have been designed to develop enzyme-sensitive NPs for siRNA/microRNA delivery.[150–152] For example, MMPs involved in cancer metastasis, progression, and invasion are highly expressed in tumor cells, and they have become one of the hotspots in antitumor therapy research.[153] Zhu et al.[154] used PEG-pp-PEI-PE to co-deliver drug and tumor-targeted siRNA by MMP2-sensitive response, which not only has the ability to co-incorporate different drugs, excellent physical

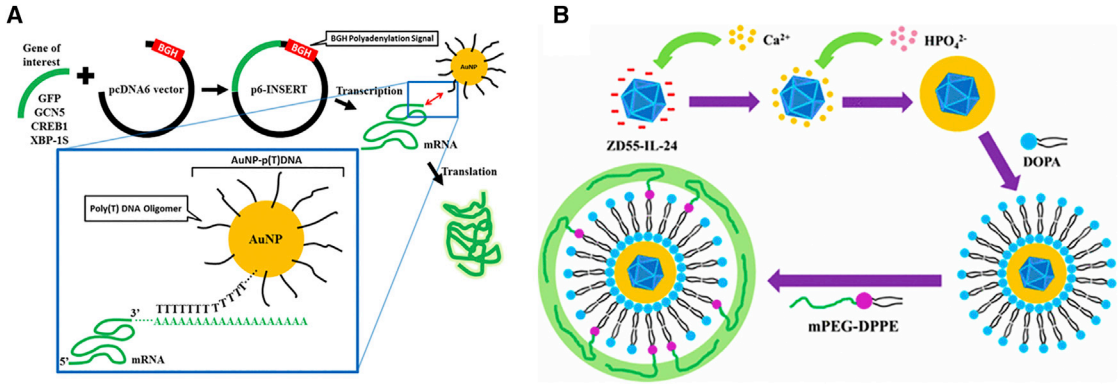


Figure 6. The Schematic Process of AuNP-p(T)DNA and PLC-ZD55-IL-24

(A) Schematic showing the use of AuNP-p(T)DNA in enhancing the translation of different mRNA templates. Genes of interest were inserted separately into pcDNA6 plasmid vector containing BGH polyadenylation (P(A)) signal and transcribed to produce the respective mRNA templates with a poly(A) tail. AuNP-p(T)DNA was added to mRNA templates to allow hybridization between the poly(T) oligonucleotide on AuNP and the poly(A) tail on the mRNA, which facilitated the increased production of the respective proteins. Reproduced with permission from Chan et al.[111] Copyright © 2018 American Chemical Society. (B) Synthetic route used to prepare PLC-ZD55-IL-24. Reproduced with permission from Chen et al.[121] Copyright © 2016 American Chemical Society.

characteristics and passive tumor targeting via the EPR effect, and, most importantly, the ability to release the siRNA preferentially in tumors where upregulated tumor levels of MMP2 triggered the de-shield of PEG and the exposure of the previously hidden PEI.[154,155] An enzyme-responsive system acts as a positive target delivery system that requires two components: the first one is the substrate or a substrate mimic as an enzyme-sensitive moiety, and the second component can lead to macroscopic transitions of nanomaterial and drug and gene release after interacting with the first component.[156]

Light-Responsive Systems

Light is a clean energy that is easy to apply, is controllable spatially and temporally, and has become another appealing stimulus for gene delivery. There are three regions of light used for triggering drug and gene delivery from appropriately designed nanocarriers: UV (10–400 nm), visible, and near-infrared (NIR) regions (650–900 nm). However, UV irradiation can't be used in a light-responsive system because it is much more cytotoxic than the other regions of the light spectrum and because of its inability to penetrate deeply into the tissue due to the absorption by endogenous chromophores. For NIR, it is hardly absorbed by water, hemoglobin, and lipid, and it causes less damage to cells than visible light, which gives NIR the characteristics of lower absorption and scattering in tissue; thus, researchers have focused on NIR to induce drug or gene release.[157] One strategy is attaching a carrier to the gold surface and then loading the gene onto the carrier, such as PLL. The PLL is attached to the surface of the Au nanoshell as a nucleic acid acceptor via Au-thiol binding and electrostatic binding, and then RNA or DNA was electrostatically attached to the cationic PLL with controllable releasing and no cytotoxicity.[158] The other strategy is to covalently attach the gene to the gold surface. Wijaya et al.[159] loaded two different DNA onto nanorods through thiol conjugation; the two different DNA were released selectively by the different wavelengths of NIR (λ = 800 or 1,100 nm) irradiation, and they did not exhibit any side effect from NIR or gold nanorods.[159,160]

Multi-responsive System

Because of the complex environment of the human body, multi-responsive nanocarriers, as a new development in the field of environmentally responsive gene delivery, are designed to be two or more stimuli responsive, including pH and redox, pH and temperature, pH and light, light and redox, and other stimuli triggers. An et al.[161] exploited a pH- and redox-responsive carrier; it not only rapidly released DNA under the presence of 10 mM GSH but also, when the pH reduced to the range of 7.4–6.3, the surface charge markedly increased. It benefited from the disulfide histamine composing the 4-arm PEG-b-poly(disulfide histamine) copolymer.[161] Another similar system is a mPEG-SS-PLL_{15}-glutaraldehyde star cationic polymer by Cai et al.[162] Yang et al.[163] developed polymer NPs with light- and pH-responsive polypeptides (PPPs), namely DSPE-PEG2000-PPP. Under NIR illumination and low pH at the tumor site after intravenous injection, DSPE-PEG2000-PPP could accumulate at tumor sites selectively and internalize into tumor cells.[163] Besides, a redox- and light-responsive system like GNR-DSPEI-PEG-RGD could release DNA from the complex at 808-nm irradiation, and it exhibited degradability of disulfide crosslinked short PEI.[164] The pH- and temperature-responsive carrier poly(ethylethylene phosphate)-block-poly[2-(dimethylamino)ethyl methacrylate] (PEEP-b-PDMAEMA) can effectively condense DNA because of the property of double-hydrophilic diblock copolymer. From the results of TEM and UV-vis measurement, PEEP-b-PDMAEMA was able to self-assemble into aggregates with different particle sizes and morphologies in aqueous solution in various pH media, and it exhibited a reproducible temperature-responsive behavior with a LCST.[165]

Design for Tumor Accumulation and Penetration

As an effective penetration system mentioned by Shen and colleagues,[14] the NPs must be small, less than 30 nm, which is the opposite of blood circulation and tumor accumulation that favor larger

Table 4. Multifunctional Cationic Polymer for Preclinical Gene Delivery Systems

Functionalization	Advantage
PEGylation	stabilization enhanced, circulation time improved, prevention of protein absorption
Targeting	gene target efficacy *in vivo* enhanced
Stimulus response	gene target efficacy *in vivo* enhanced
Cell penetrating	cellular uptake enhanced, cross cell membrane
Endosome escaping	endosomal escaping enhanced, cross cell membrane
Nuclear localization	nuclear localization

sizes.[166] Cationic nanomaterials because of their cationic charges can improve tumor penetration, but a neutral or a slightly negatively charged one can have a long blood circulation.[167] They synthesized polycaprolactone (PCL)-block–PEI/amide-folate acid (FA) with high cellular uptake, which can reverse its charge triggered by pH change, because amides with β-carboxylic acids can hydrolyze in acidic conditions to regenerate the amines, giving rise to a negative-to-positive charge reversal.[168] Thus, as we design an effective gene delivery system, the NPs should be neutral or negatively charged in blood circulation; once in the tumor's acidic extracellular medium, they become positively charged for cellular uptake, lysosomal escape, and nuclear localization. Zhang and colleagues[169,170] also synthesized a similar delivery system, poly(l-lysine)-block-poly(l-leucine) with a nuclear target Tat peptide to achieve this purpose[169] and FK/p53/PEG-PLLcomplexes composed of FK/p53 with PEG-PLL coated by folate.[170] Also a polyion complex coating using micelles was constructed, which was composed of poly (lysine)-b-polycaprolactone (PLys-b-PCL) as the cationic core and poly (glutamic acid)-g-methoxyl poly (ethylene glycol) (PGlu-g-mPEG) as the anionic coating material. The charge reversal profile was manipulated by controlling the polymer chain entanglement and electrostatic interaction in the polyion complex layer through glutaraldehyde-induced shell crosslinking that facilitated the therapeutic effect via tumor pentration.[171] In addition, quaternary amines carrying N-propionic 4-acetoxybenzyl ester substituents to make esterase-presponsive polymer were used as a gene carrier because it can undergo a quick intracellular esterase-catalyzed hydrolysis and then triggers the polymer's charge reversal from cationic to zwitterionic. This esterase-catalyzed hydrolysis can be tracked by monitoring the release of 4-hydroxybenzyl alcohol by high-performance liquid chromatography (HPLC).[172]

To conjugate with tumor-homing and -penetrating cyclic peptide iRGD, it was shown that the tumor accumulation and penetration enhanced significantly, but also the C-terminally exposed cryptic (R/K)XX(R/K) CendR element can trigger neuropilin-1 (NRP-1) binding, resulting in cellular internalization and malignant tissue penetration.[173–176]

Recently, Ji et al. developed peptide-based nanomaterials targeting and depleting cancer-associated fibroblasts (CAFs) to overcome the poor penetration and even low perfusion of molecular drugs[177] through remodeling tumor microenvironments to enhance nanomedicine penetration and, thus, efficacy. Like inhibiting platelet-derived growth factor receptor-β expression by imatinib mesylate,[178] reducing tumor extracellular matrix by collagenase,[179] inhibiting the transforming growth factor β (TGF-β)-signaling pathway by LY364947,[180] inhibiting the expression of collagenI and TGF-β by pirfenidone,[178] depleting tumor collagen by losartan,[181] and disrupting tumor extracellular fibronectins by cyclopamine[181] have been shown to significantly improve tumor perfusion, accumulation, and intratumoral distribution.

Future Direction of Cationic Polymer Design in Gene Delivery

As mentioned above, engineering NPs was always designed via conjugation with some functional group to decrease the cytotoxicity and increase the efficiency of gene transfection, especially in preclinical trials with application prospects in both drug and gene delivery. The different modifications have been explored (Tables 2, 3, and 4) in the literature, as detailed above. We have found the advantages of modified NPs in RNAi therapy to be as follows: (1) biologically degradable with minimal cytotoxicity, (2) easy-to-modify physicochemical characteristics and simple purification procedures, (3) functional ligand conjugation with improved targeted delivery of siRNA, (4) most can deliver siRNA and chemotherapeutics for an additive or synergistic effect, and (5) siRNA delivery can be monitored by visualizing themselves or through proper labeling.

All the modifications are based on maximally decreasing the potential adverse human health effects. This requires that NPs do not impair mitochondrial function, form apoptotic bodies, produce reactive oxygen species under phasilogical conditions *in vivo*, and maintain their ideal properties. Furthermore, minimizing the problem of off-targeting, which causes an off-target immune response, is the most important consideration. Nevertheless, the different sizes affect the nanomaterials' chemical block lengths and ratios, as well as stability, mechanical properties, charge, PEG-chain length or density, and even hydrophilic shell thickness. Besides, detailed analysis about tumor accumulation data for various nanomedicines found that only about 0.7% of injected doses actually accumulated in the tumor, because of the tumor's inherent pathological characteristics.[182] The large size of the NPs and tumor's crosslinked matrix components also hinder the penetration of NPs.

Choosing an appropriate oncogene for gene therapy is also considered in our study. Oncogenes and oncoproteins are involved in the regulation of tumorigenic cell growth,[183,184] and some of them are accepted as tumor markers, such as RAS, WNT, MYC, ERK, and TRK. They include the following: (1) growth factors or mitogens, which can induce cell proliferation like c-sis;[185] (2) receptor tyrosine kinases, which transduce signals for cell growth and differentiation, such as EGFR, vascular EGFR (VEGFR), and HER2/neu;[186–191] (3) cytoplasmic tyrosine kinases, which mediate the responses and the activation receptors of cell proliferatioin, migration, differentiation, and survival;[84] (4) cytoplasmic serine/threonine kinases and their

regulatory subunits involved in organism development; cell survival, differentiation, and apoptosis; cell cycle regulation; and cell proliferation;[192–194] (5) regulatory GTPases such as ras protein, which are involved in signaling pathways of cell proliferation;[195,196] and (6) transcription factors like myc gene, which regulate the transcription of genes that induce cell proliferation.[197] Furthermore, DNAzyme/RNAzyme[198] and microRNA also are involved in cell growth and gene transcription, especially of oncogenes, and they act as tumor suppressors, such as miR-21, miR-34a, miR-132, and miR-155.[199–203]

Another application of NPs is drug delivery; thus, the number of studies on the co-delivery of siRNAs and drugs has been increasing, especially studies for drug-resistant cancer. For multidrug resistance (MDR), siRNA and anti-cancer drugs are delivered into cancer cells simultaneously; the siRNA is chosen to silence the genes related to drug resistance, decreasing the drug efflux pumps and activating apoptosis pathways. Following the release of siRNA, the accumulation of co-delivered anti-cancer drug inside of the cancer cells will increase, resulting in promoted chemotherapeutic effects.[204] Co-delivery of siRNAs and anti-cancer drugs has been the most potential therapy to cure cancer.

AUTHOR CONTRIBUTIONS

Y.X. conducted the writing and literature review of the entire article; K.S. conducted the modification and proofreading of "The smart nanoparticles engineeried for gene delivery"; Y.Q. and B.C. conducted "Clinical application of nanoparticle-based RNAi therapy" and "The challenges and barriers of polymers-mediated gene therapy"; and Z.Q. conducted the conception of the whole review and the final proofreading.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

ACKNOWLEDGMENTS

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