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REVIEW

Advancing Therapeutic Strategies with Polymeric Drug Conjugates for Nucleic Acid Delivery and Treatment

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Abstract: The effective clinical translation of messenger RNA (mRNA), small interfering RNA (siRNA), and microRNA (miRNA) for therapeutic purposes hinges on the development of efficient delivery systems. Key challenges include their susceptibility to degradation, limited cellular uptake, and inefficient intracellular release. Polymeric drug conjugates (PDCs) offer a promising solution, combining the benefits of polymeric carriers and therapeutic agents for targeted delivery and treatment. This comprehensive review explores the clinical translation of nucleic acid therapeutics, focusing on polymeric drug conjugates. It investigates how these conjugates address delivery obstacles, enhance systemic circulation, reduce immunogenicity, and provide controlled release, improving safety profiles. The review delves into the conjugation strategies, preparation methods, and various classes of PDCs, as well as strategic design, highlighting their role in nucleic acid delivery. Applications of PDCs in treating diseases such as cancer, immune disorders, and fibrosis are also discussed. Despite significant advancements, challenges in clinical adoption persist. The review concludes with insights into future directions for this transformative technology, underscoring the potential of PDCs to advance nucleic acid-based therapies and combat infectious diseases significantly.

Keywords: polymer drug conjugates, polymeric therapeutics, nucleic acid

Introduction

The advancement of nucleic acid therapeutics marks a significant milestone in biomedicine, offering substantial potential in treating diverse genetic disorders.[1] Their main strength lies in their precise targeting and nucleic acid assisted genetic mutations and provide potential treatments for conditions once considered incurable.[2,3] In clinical settings, these combined therapies have been used for treating genetic abnormalities and cancer etc.[4] Advancements in this field are revolutionizing personalized healthcare by enabling the development of tailored treatment approaches that enhance therapeutic effectiveness while minimizing adverse effects.[5] The development of effective delivery mechanisms, the stabilization of nucleic acids, and the mitigation of potential adverse effects present substantial challenges in the field.[6] The integration of advanced nanotechnologies with a deeper comprehension of cellular mechanisms is essential for effectively tackling these challenges.[5]

Polymer drug conjugates (PDCs) have emerged as a new drug and nucleic acid delivery approach, aiming to augment therapeutic efficacy while minimizing toxicity.[7] PDCs are composed of pharmacologically active compounds, including small molecules, peptides, proteins, or aptamers, covalently bonded to a polymeric carrier. This carrier system

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significantly improves solubility, facilitates controlled release, enhances therapeutic efficacy, and optimizes the pharmacokinetic profile of the attached drug.[8] Numerous approaches, such as controlled polymerization, generated well-defined polymers with precise molecular weights and structures, improving the design and synthesis of efficient PDCs.[9,10] Among them, PEG conjugates such as PEG-doxorubicin demonstrated improved pharmacokinetics and reduced toxicity.[11] In recent research, the incorporation of targeting ligands, such as antibodies and peptides, into polymer-based drug delivery systems has gained prominence. This approach aims to facilitate site-specific drug delivery, thereby increasing drug accumulation at the intended site while minimizing off-target effects and enhancing therapeutic efficacy.[12,13]

PDCs consist of therapeutically active agents such as drugs, proteins, peptides, hormones, enzymes, or growth factors that are covalently linked to polymers. This conjugation serves to protect the therapeutic agents from degradation, enhance targeted delivery, and consequently improve overall therapeutic efficacy.[14,15] Previously, PDCs have been used as nucleic acid delivery carriers and therapeutic agents to treatvarious medical conditions, including liver fibrosis, angiogenesis, metastasis, and cancer.[16–21]

In 1975, Ringsdorf introduced the concept of a biocompatible polymer backbone, to which biologically active agents could be covalently linked. This model served as a foundation for subsequent advancements in polymer-drug conjugation. During the 1980s, Allan Hoffman and Robert Langer further explored this technique, focusing on the covalent attachment of drugs to polymers. Their research demonstrated the potential of PDCs to enhance the precision of therapeutic agent delivery and provide controlled drug release mechanisms.[21]

In 1990, the development of a polymer-protein conjugate, Adagen, marked a significant advancement in enzyme replacement therapy, specifically targeting adenosine deaminase deficiency in patients with severe immunodeficiency.[22] Later, in 2011, PEGylated interferon α-2b (Sylatron) received regulatory approval as an adjuvant therapy for individuals with high-risk melanoma. Furthermore, PEGylated interferon β-1a has been approved for its therapeutic potential in the management of multiple sclerosis.[23]

This review presents a comprehensive analysis of recent advancements in PDCs, with a focus on conjugation methodologies, the application of non-covalent interactions to enhance therapeutic efficacy, and strategic innovations that have propelled clinical progress. Various classes of PDCs are explored, including polymer-protein conjugates and small-molecule drug integrations, highlighting their potential in precision medicine.

The review further examines multifunctional polymeric carriers designed for combination therapies and targeted delivery approaches. In addition, the selection of suitable natural and synthetic polymers, alongside diverse techniques for PDC formulation, is discussed. Particular attention is given to the use of PDCs for nucleic acid delivery and their role in treating a wide range of diseases.

Advanced multifunctional systems capable of co-delivering multiple therapeutic agents, such as drugs and nucleic acids, are also reviewed. These systems support gene therapy and diagnostic imaging through a unified carrier (Figure 1). Despite the significant progress made, key challenges remain that hinder the widespread clinical adoption of PDCs. This review provides an overview of these limitations and offers insights into potential future directions for the field.

Overview of Nucleic Acid Therapeutics

Nucleic acid therapeutics have recently received significant attention as a promising tool for overcoming various diseases. These therapeutics utilize mRNA, siRNA, and miRNA to target specific genes to combat the devastating diseases.[16,24,25]

mRNA Therapeutics

mRNA therapeutics involve the delivery of synthetic mRNA that encodes therapeutic proteins into target cells (Figure 2). mRNA therapeutics offer rapid development, versatile applicability, and potential for personalized medicine.[26]

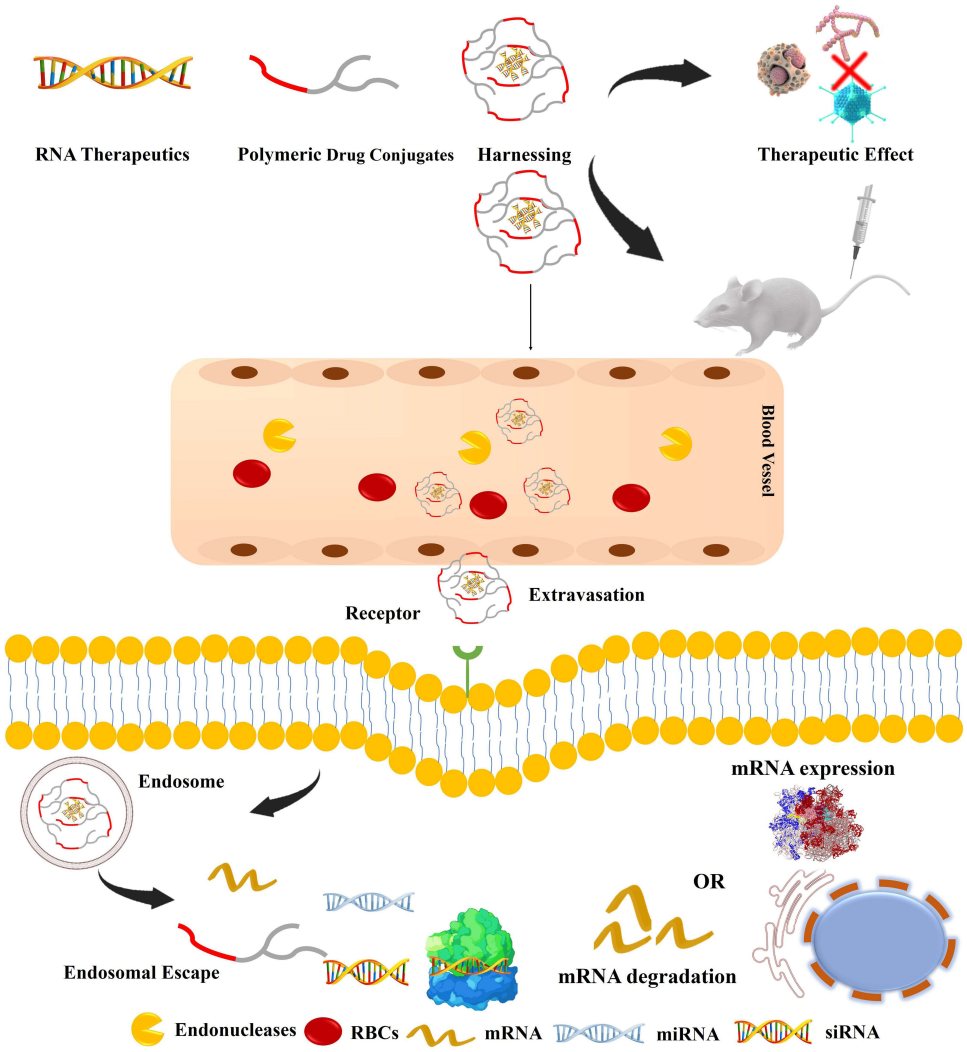

Figure 1 Schematic representation of the mechanism of polymeric drug conjugates in nucleic acid delivery and therapeutics.

siRNA Therapeutics

siRNA therapeutics function by silencing specific genes causing diseases by degrading their target mRNAs (Figure 3). They have shown promise in treating genetic disorders and viral infections.[27]

miRNA Therapeutics

miRNAs are non-coding RNA molecules modulating gene expression by binding to specific mRNAs, inhibiting mRNA translation.[28] miRNAs function primarily through the RNA-induced silencing complex (RISC). Within the RISC framework, the miRNA directs the complex to target mRNAs through base-pairing interactions, subsequently leading to mRNA cleavage or translation inhibition (Figure 3).[25]

Clinical Translation of mRNA Therapeutics

mRNA can be utilized in-vivo or ex-vivo for clinical applications. Patient-derived cells are isolated, subjected to mRNA modification ex-vivo, and reinfused. However, in vivo, mRNA is administered to the patient through the same routes as conventional medications.[29] mRNA therapeutics have profoundly altered the approach to treating numerous diseases with immunotherapy and protein replacement. Yet, most clinical trials that use mRNA are focused on gene editing, regenerative medicine, and immunotherapy.[30]

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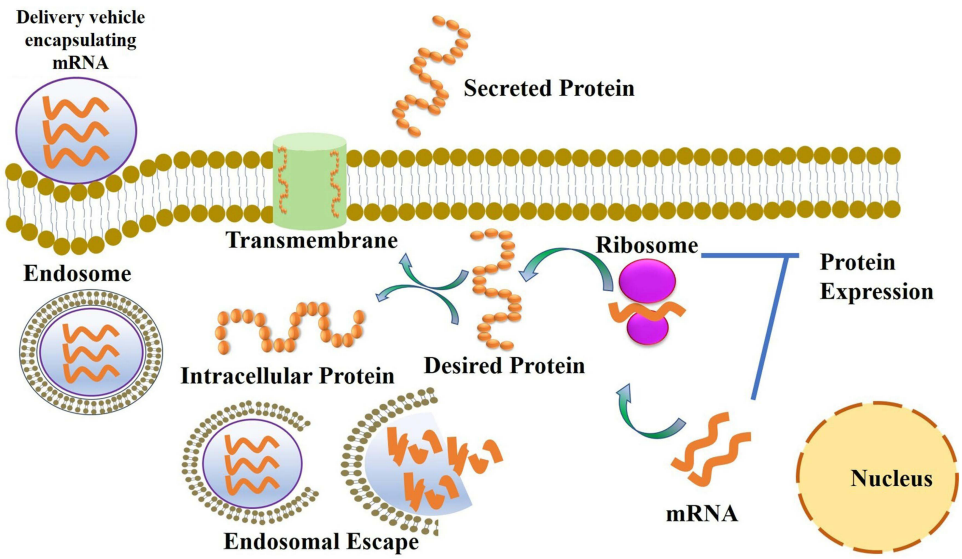

Figure 2 Mechanism of mRNA therapeutics.

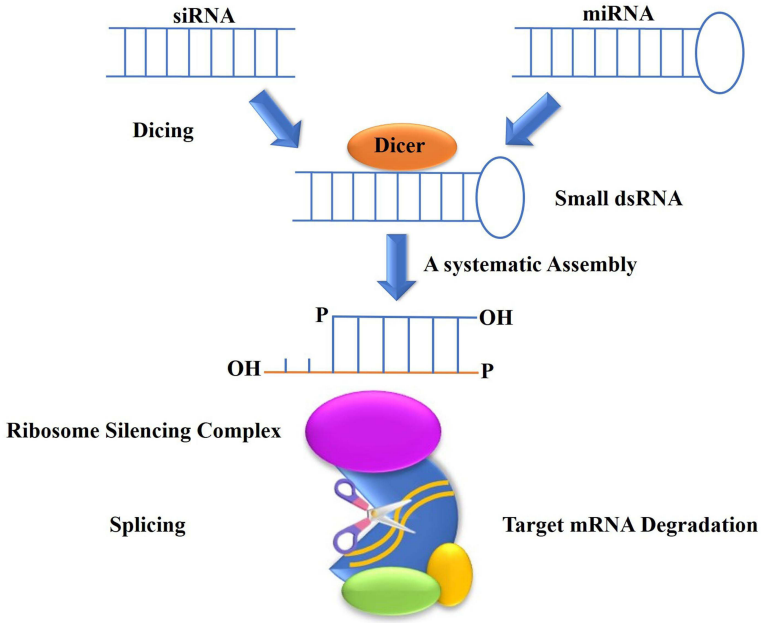

Figure 3 siRNA/miRNA mechanism of action in nucleic acid therapeutics.

Gene Editing

mRNA serves as a transient carrier of genetic information for protein synthesis. Synthetic mRNA is introduced into cells, directing them to produce specific proteins like gene editing enzymes.[30] CRISPR/Cas, Zinc Finger Nucleases (ZFNs), and Transcription Activator Like Effector Nucleases (TALENs) are employed for gene editing.[31,32] The CRISPR/Cas system traditionally uses DNA vectors to produce the Cas proteins and guide RNAs, and mRNA can be utilized to produce Cas proteins in target cells transiently. Within the context of in vivo CRISPR/Cas9-mRNA application, lipid NPs have been assessed for intravenous delivery, eg, a clinical trial involving corneal delivery of Cas9-mRNA.[32] ZFNs are focused on ex-vivo approaches that utilize ZFN mRNA, and TALENs can also be expressed using mRNA, allowing for the activity of these proteins in cells (Figure 4).[30,32]

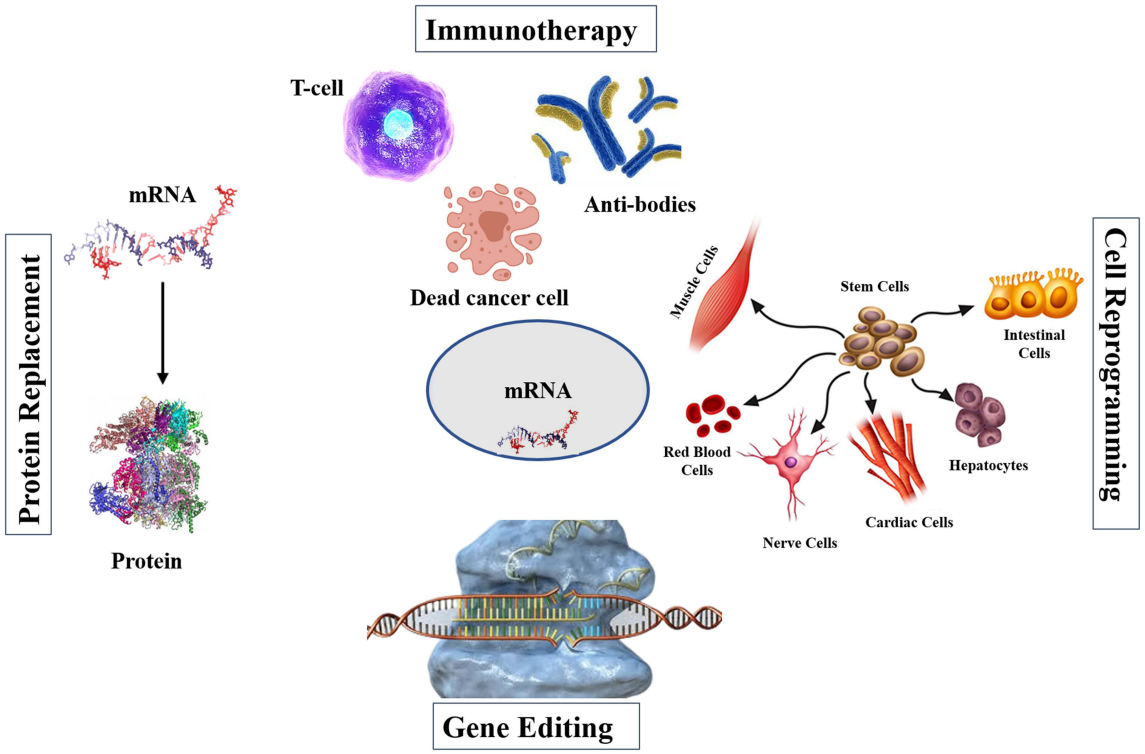

Figure 4 Clinical Translation of mRNA therapeutics.

Regenerative Medicine

The primary objective of regenerative medicine is to regenerate, repair, and replace defective cells, tissues, or organs.[33] Somatic cell transfection via mRNA-encoded transcription factors offers advantages over traditional reprogramming and trans-differentiation methods. Yet, its application remains primarily restricted to in vitro preclinical evaluations, with a single Phase I/II clinical trial underway (NCT02407470).[34] Engineered Mesenchymal Stem Cells (MSCs) represent a novel regenerative medicine approach. Transiently introducing mRNA can enhance MSCs' migratory attributes by expressing specific homing proteins. Once localized to the desired tissue, MSCs possess the capacity for self-renewal and can differentiate into diverse cellular lineages, including chondrocytes, adipocytes, and osteoblasts.[35] A clinical trial (NCT04524962) enrolled patients to assess the impacts of RNA-engineered allogeneic MSCs, termed "Descartes-30", on acute respiratory distress syndrome (ARDS) and COVID-19. Descartes-30 produces distinct DNases, targeting extracellular neutrophil networks pivotal to inflammation in ARDS (Figure 4).[36]

Immunotherapy

The principal aim of mRNA approaches is to elicit an immunological reaction, leading to a surge in clinical evaluations of mRNA-mediated immunotherapies.[37] The mRNA-transfected T-cell targeting against CD8+ T cells proposes an innovative therapeutic strategy for Type 1 diabetes.[38] Moderna reported Phase I (NCT03829384) findings for their systemic mRNA-1944, which encodes the CHKV-24 antibody targeting the chikungunya virus. Preliminary data showed the induction of CHKV-24 IgG neutralizing titers (Figure 4).[39]

Protein Replacement

Phenylketonuria (PKU) is caused by phenylalanine hydroxylase (PAH) gene mutations, leading to impaired phenylalanine metabolism and toxic accumulation, causing irreversible intellectual disabilities.[40,41] Previously, mRNA encoding PAH was encapsulated in LNPs, which increased levels of PAH expression in hepatocytes, demonstrating the therapeutic potential of mRNA-based protein replacement therapy (Figure 4).[42]

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Infectious Diseases

Around 24 mRNA vaccines are currently under clinical trial for various infectious diseases.[43] These mRNA vaccines are primarily designed to target viral infections such as SARS-CoV-2, rabies, influenza Zika, CMV, chikungunya, hMPV/PIV3, RSV, and HIV.[44–47] After the cell uptake, it leads to the production of antigens and subsequent presentation of T and B cells.[48] The efficacy and immunogenicity of the vaccine are substantially influenced by the mRNA structure and delivery method (Figure 5).[43]

SARS-CoV-2

CureVac's mRNA-based SARS-CoV-2 vaccine (CVnCoV) employs LNPs containing Acuitas Therapeutics' ionizable lipid (ALC-0315) encapsulating unmodified mRNA (spike protein) in a stabilized prefusion conformation.[49] Preliminary phase I trials (NCT04449276) indicated neutralizing antibodies at a 12 μg dose, comparable to post-COVID-19 recovery levels (Figure 5).[45–47]

Rabies

CureVac used LNPs for mRNA delivery in their novel rabies vaccine, CV7202. Phase I clinical trial (NCT03713086) indicates that dual intramuscular administrations at concentrations of 1 or 2 μg demonstrated good tolerability and elicited robust neutralizing antibody response (Figure 5).[50]

Zika Virus

Two phase I clinical trials (NCT03014089, NCT04064905) evaluated the safety and immune response of mRNA-LNP (mRNA-1325 and mRNA-1893). The mRNA-1325 induced intracellular expression of Zika structural proteins, facilitating Virus-Like Particles (VLPs) secretion. Contrarily, mRNA-1893 employed an analogous mechanism but incorporated a distinct sequence from mRNA-1325, enhancing immunogenicity and Zika VLP production. Preliminary data from the mRNA-1893 trial (NCT04064905) indicate promising tolerance and 94–100% seroconversion rates in seronegative

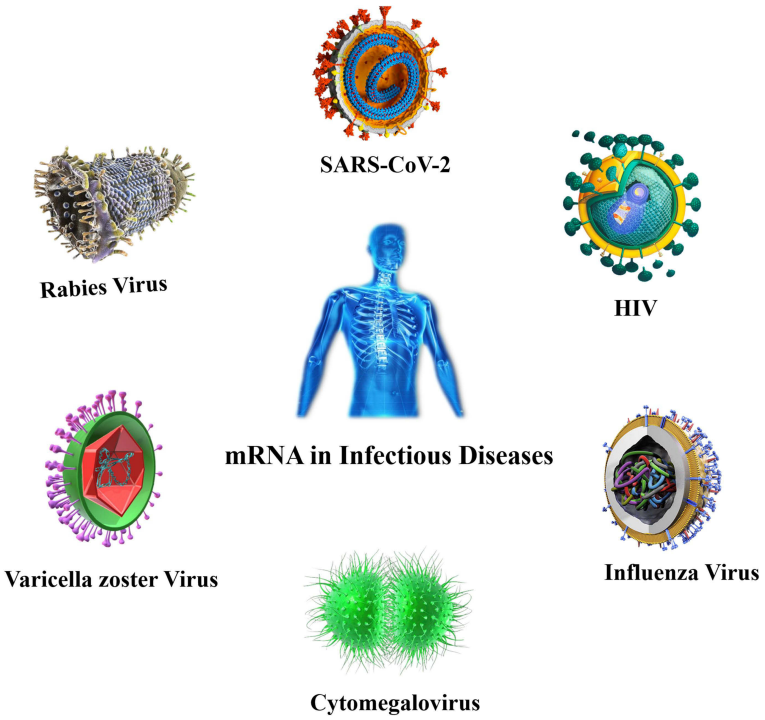

Figure 5 mRNA application in infectious diseases.

participants. A subsequent Phase II trial (NCT04917861) aims to measure the safety and immunogenicity of mRNA-1893 among individuals in both flavivirus-endemic and non-endemic regions (Figure 5).[51]

Clinical Translation of siRNA Therapeutics

The translation of siRNA therapeutics denotes a promising field in modern drug development. siRNA molecules can specifically silence target genes, providing a unique mode of action compared to traditional drugs. siRNAs are short, double-stranded RNA molecules that can degrade mRNA through RNAi, thereby inhibiting protein synthesis (Figure 6) [52]

Polyneuropathy

In 2018, Patisiran (Onpattro®; Alnylam) became the pioneer of siRNA therapeutic approved to treat hereditary transthyretin amyloidosis, a condition stemming from mutations in the TTR gene. The TTR protein, responsible for transporting thyroxine and retinol, can misfold due to these mutations, resulting in fibril deposition in various organs. Patisiran specifically targets and degrades TTR mRNA, reducing the production of the TTR protein. This, in turn, decreases the amount of amyloid deposits formed from the misfolded TTR protein, helping to alleviate symptoms of the disease. Patisiran's formulation incorporates a lipid nanoparticle containing DLin-MC3-DMA, cholesterol, DSPC, and PEG2000-C-DMG lipids that undergo a pH-sensitive fusion, optimizing their intravenous delivery (Figure 6).[53]

Acute Hepatic Porphyria

In 2019, the FDA approved the siRNA therapeutic Givosiran (Givlaari®; Alnylam), targeting δ-aminolevulinic acid synthase 1 (ALAS1).[54] ALAS1 is pivotal in acute hepatic porphyria (AHP), stemming from mutations in heme biosynthesis genes. Elevated hepatic ALAS1 causes an accumulation of heme intermediates, δ-aminolevulinic acid,

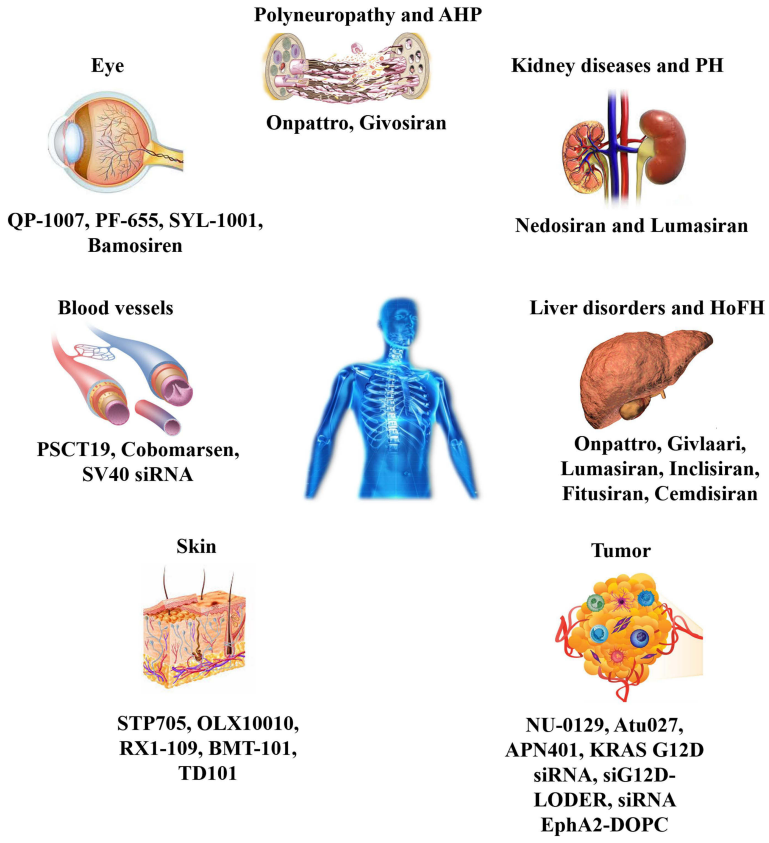

Figure 6 Current siRNA therapeutics at the clinical stage.

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and porphobilinogen, inducing neurotoxicity causing acute porphyria attacks.[55] Givosiran targets and reduces the levels of ALAS1 mRNA, thus lowering ALA and PBG levels and reducing the severity of AHP attacks.[56] Preclinical trials in mice, rats, and monkeys demonstrated a significant reduction in ALAS1, paving the way for clinical investigation (Figure 6).[57]

Homozygous Familial Hypercholesterolemia

Inclisiran (Novartis) is a GalNAc-siRNA conjugate designed for homozygous familial hypercholesterolemia (HoFH) and high LDL-C management. The inclisiran targets PCSK9, augmenting LDL receptor recycling on hepatocyte surfaces, elevating receptor expression in the hepatocyte membrane, thus amplifying LDL-C binding and diminishing circulating LDL-C levels (Figure 6).[58]

Primary Hyperoxaluria

Primary hyperoxaluria (PH) is a group of rare genetic disorders that result in the overproduction of oxalate, a waste product typically eliminated via the kidneys. Glyoxylate undergoes metabolism to oxalate via lactate dehydrogenase (LDH). Excess oxalate can combine with calcium to form calcium oxalate crystals, leading to recurrent kidney stones, nephrocalcinosis disease, and potentially end-stage renal disease. Nedosiran and lumasiran are two RNAi therapeutics developed for PH therapy. Nedosiran focuses on LDH knockdown to block the oxalate synthesis. Lumasiran targets glycolate oxidase, which converts glycolate to the primary oxalate precursor, glyoxylate.[57,59] Lumasiran (Alnylam) has gained FDA approval for PH type 1 (PH1) (Figure 6).[60]

Clinical Translation of miRNA Therapeutics

miRNAs have pivotal functions in controlling gene expression at the post-transcriptional level. Their dysregulation has been implicated in various diseases, making them promising targets for therapeutic intervention. The development and clinical translation of miRNA therapeutics has been an area of active research over the past decade.[61] Therapeutics can be designed to either restore or inhibit miRNA function. miRNA mimics are synthetic molecules that function like endogenous miRNAs and inhibit the target mRNAs, while miRNA inhibitors (or antimiRs) block the activity of endogenous miRNAs.[62] miRNAs serve as pivotal regulators across an array of pathological conditions such as immunological dysfunctions, Alzheimer's disease, cardiovascular diseases, rheumatoid arthritis, and cancer (Figure 7).[63]

Hepatitis C Virus Infection

Miravirsen is a unique therapeutic agent that targets the Hepatitis C virus (HCV). Miravirsen is an oligonucleotide that targets miR-122. miR-122 is a liver-specific miRNA that HCV relies upon for replication. By binding to and sequestering miR-122, Miravirsen prevents the miRNA from aiding the replication of the HCV RNA genome. In other words, rather than targeting the virus directly, Miravirsen targets a host factor (miR-122) that the virus requires to replicate.[64] Miravirsen (SPC3649), developed by Santaris Pharma, underwent Phase I and IIa clinical evaluations for HCV therapy. Results indicated a dose-dependent reduction in HCV RNA among patients with chronic HCV genotype 1 infection (Figure 7).[65]

Kidney Cysts

RGLS4326 is a novel oligonucleotide targeting miR-17, which plays a pivotal role in kidney cystogenesis. It is undergoing Phase I clinical evaluations to assess its safety profile under the Regulus Therapeutics (Figure 7).[66,67]

Cancer

The miR-34a (Mrx34) is recognized as a pivotal modulator in tumor suppression, downregulating 30 oncogenes. Therapeutics targeting miR-34a show potential in addressing NSCLC, ovarian, colon, cervical, and HCC. Mrx34 is undergoing a Phase 1 clinical evaluation for HCC (Figure 7).[68,69]

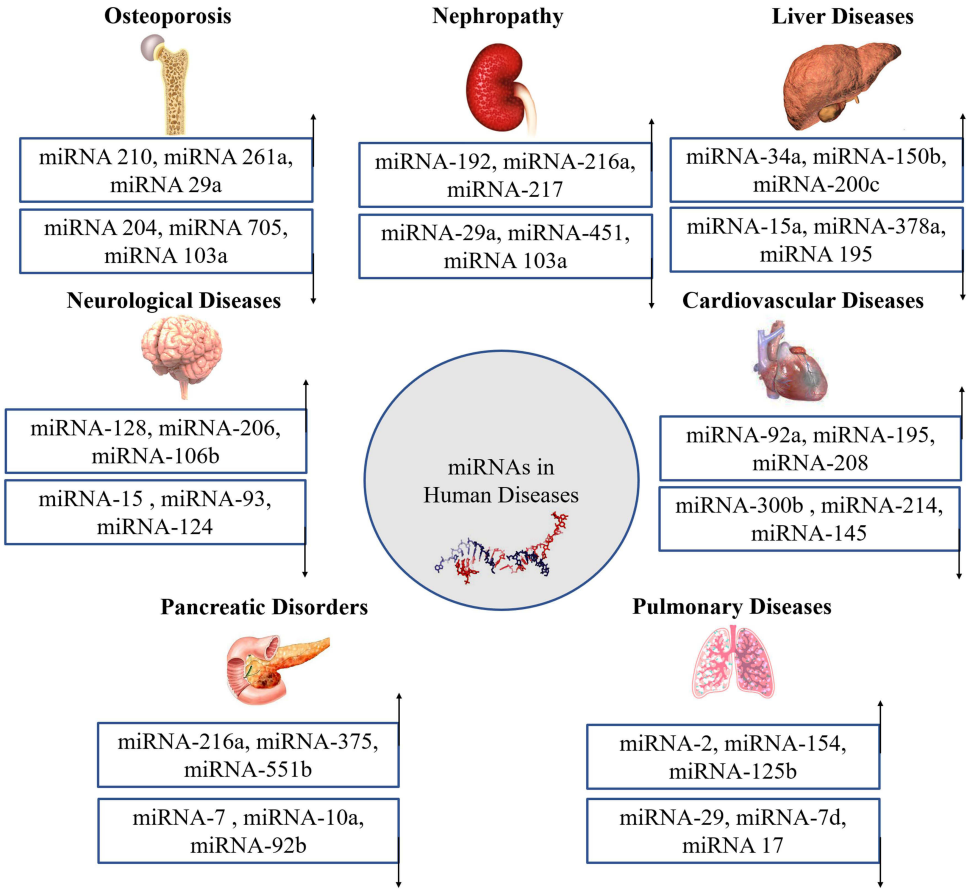

Figure 7 The potential of miRNAs in the treatment of human diseases.

Conjugation Strategies for Developing PDCs

Reversible Addition-Fragmentation Chain-Transfer (RAFT) Polymerization and Click Chemistry

RAFT polymerization tunes the molecular weight and distribution of polymer chains by utilizing a chain transfer agent (CTA), generating polymers with specific molecular weights and low polydispersity. Click chemistry facilitates the attachment of functional groups to polymers, which is effective for conjugating drugs to polymers and enhancing targeted delivery. Employing RAFT polymerization followed by click chemistry allows the development of sophisticated PDCs with enhanced delivery efficacies.[70,71]

RAFT polymerization facilitates the incorporation of functional end-groups that serve as effective sites for binding various biomacromolecules.[72,73] The Opaxio (Xyotax) exemplifies a sophisticated approach by combining PLGA with paclitaxel, leveraging the biodegradable nature of PLGA, allowing it to be metabolized normally and effectively bypassing renal clearance.[74]

Enzymatic Linkage

It involves using specific enzymes to conjugate drugs to polymers via amide, ester, or peptide bonds, which degrade under physiological conditions, releasing the drug in a controlled manner.[75] The enzymatic linkage of doxorubicin to a PEG via cleavable oligopeptide groups via reductive disulfide bonding.[76,77]

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Covalent Linking

Covalent linking involves the formation of the covalent bond between a polymer and a therapeutic agent, which facilitates controlled drug release.[78] This concept was introduced by Helmut Ringsdorf in 1975. It enhances drug solubility, targets, controls drug release, reduces toxicity, and has therapeutic effects.[79]

Non-Covalent Linking

Non-covalent linking involves the binding of molecules through hydrogen bonds, ionic bonds, van der Waals forces, and hydrophobic interactions, which are reversible and less robust than covalent bonds. It allows for the encapsulation of drugs within a polymer matrix without chemical alteration, preserving the functionality of chemically sensitive drugs. Additionally, the reversible nature of these interactions enables a controlled release of drugs, which can be triggered by specific environmental changes such as alterations in pH, temperature, or enzymatic activity, thus playing a pivotal role in improving drug solubility, stability and bioavailability, and targeted release.[80]

Strategic Design of PDCs

The design of PDCs draws from a model proposed by Helmut Ringsdorf in 1975.[81] Ideal polymers should be hydrophilic, biocompatible, and biodegradable, featuring functional groups for attaching drugs or spacers. The MW is kept below the renal threshold for non-biodegradable ones to ensure excretion and avoid accumulation in the body. Polymers with a MW under 50,000 Da are preferred.[82] Manipulating polymer properties like molecular weights, structures, and charges can tailor PDCs. For instance, adding solubilizing groups to the polymer chain enhances drug solubility and bioavailability. Drug release rates can be controlled by incorporating bio-responsive spacers, critical in maintaining enzymatic activity in polymer-enzyme conjugates.[83] Linkers between the polymer and drug, such as esters or amides, should be stable yet degrade upon pH changes, preventing premature release in the bloodstream.[84] Clinical evaluations of PDCs have shown varied success. Dextran-doxorubicin conjugate showed liver toxicity, while an HPMA copolymer-doxorubicin conjugate demonstrated better tolerability.[85] Overall, polymer-drug conjugation has been found to improve pharmacokinetic profiles, increase plasma half-life, reduce clearance, protect the drug from degradation, and potentially enhance therapeutic outcomes by targeting the drug to specific sites of action.

Selection of Polymers for the Development of PDCs

Natural Polymers

Chitosan

Chitosan (CT) is a unique cationic aminopolysaccharide notable for its non-toxicity, biocompatibility, and biodegradability.[86] The enhanced affinity between CT and ibuprofen forms a NPs complex (nanoplex) that significantly improves solubility and dissolution rates. This nanoplex transforms crystalline ibuprofen into spherical amorphous particles with substantially reduced sizes, optimizing drug delivery characteristics.[87–89] Copper citrate-chitosan nanoparticles (CuCC NPs) were engineered by combining copper citrate complexes with chitosan, effectively reducing copper ions' biotoxicity. In the acidic and glutathione-rich environment of tumors, these NPs degrade and release copper ions. The released copper ions convert excess hydrogen peroxide in the tumor into hydroxyl radicals, leading to tumor cell death. In vivo studies demonstrated that CuCC NPs are highly effective in cancer dynamic therapy (CDT).[90] Another study developed a triblock copolymer micelle from N-succinyl chitosan-poly-L-lysine-palmitic acid (NSC-PLL-PA) to co-deliver DOX and siRNA targeting P-gp. This micelle showed stability changes between acidic and neutral pH, leading to rapid release of the drug and siRNA in acidic conditions. In vitro findings revealed that this system significantly improved antitumor effects, particularly against HepG2/ADM cells, by downregulating P-gp. The micelles accumulated explicitly in the tumor, effectively inhibiting its growth and showing promise as a combined treatment approach for cancer therapy.[91]

Dextran

Dextrans can be chemically modified to develop prodrugs by attaching bioactive agents using direct linkage, spacer arm integration, or receptor-specific ligands. Direct linkage can improve drug release but may limit

enzymatic access, leading to pH-dependent hydrolysis. Using a spacer arm reduces steric hindrance, improves enzymatic access, and allows for precise control over drug release by modifying terminal functional groups with specific covalent bonds.[92] Dextran-5-aminosalicylic acid (5-ASA) azo-coupled prodrug has effectively countered the premature absorption of 5-ASA in the upper gastrointestinal tract, thus ensuring its targeted delivery to the colon. Additionally, a dextran-nalidixic acid ester has exhibited potential in colon-specific drug delivery, displaying both chemical stability and targeted release when exposed to cecal contents.[93–96] The Dextrin-MNP protects miRNAs against cellular nucleases and efficiently delivers them to cancer cells in vitro and in vivo.[97] Afshin Nikkhoo et al synthesized carboxymethyl dextran-conjugated trimethyl chitosan NPs loaded with NIK/STAT3-specific siRNA and BV6 for inducing apoptosis in breast, colorectal, and melanoma cancer cells.[98]

Cyclodextrins

Cyclodextrins (CDs) have a hydrophilic outer surface and a hydrophobic inner core, which allows CDs to encapsulate small, hydrophobic drug molecules.[99–101] A per-FOL-β-CD-ss-DOX attaches folic acid (FA) to the terminal primary hydroxyl groups of β-cyclodextrin (β-CD). A pH-sensitive spacer links DOX to the secondary hydroxyl groups of β-CD. The conjugate demonstrated significant cytotoxicity in EMT6/AR1 cells, resistant to free DOX. This indicates that per-FOL-β-CD-ss-DOX NPs could effectively target FR-expressing cells, even those resistant to standard chemotherapy treatments.[102]

An advanced amphiphilic cationic CD NPs, modified with PEGylated folic acid (FA), has been developed to deliver docetaxel (DTX) and siRNA targeting the RelA subunit of NF-κB. These CD.DTX.siRelA.PEG-FA NPs specifically bind to folate receptors on colorectal cancer (CRC) cells, leading to increased cellular uptake, enhancing DTX-induced apoptosis while simultaneously reducing RelA expression and substantially inhibiting CRC growth. These results highlight the potential of FA-targeted PEGylated CD NPs as an effective approach for combined DTX and siRNA delivery in CRC treatment.[103]

Hyaluronic Acid

Hyaluronic acid is a mucopolysaccharide formed from glucuronic acid and N-acetylglucosamine, renowned for its biocompatibility, biodegradability, and viscoelastic properties. It can attach CD44 highly expressed on tumor cells. Due to functional groups like hydroxyl and carboxyl, hyaluronic acid can be chemically modified, making it effective for targeted therapy.[104]

The HA-Hz-DOX conjugate successfully eradicated glioblastoma cells in invitro and demonstrated superior effectiveness in treating subcutaneous tumors in animal models compared to free DOX. These results highlight the promise of HA as a polymeric platform for targeted glioblastoma treatment.[105] siRNAs were effectively delivered via HA and PEI, complexed with AuNPs, and facilitated gene silencing, photothermal therapy, and chemotherapy. Upon radiation after treatment with the AuNP-integrated siRNA complexes in the MDA-MB-231 breast cancer cell line, a significant cytotoxic effect was observed. The enhanced gene silencing efficiency of the CXCR4 gene with the AuPEI-HA-DOX/siRNA complex was observed.[106]

Synthetic Polymers

Polyethyleneimine

PEI plays a crucial role in developing PDCs. The cationic nature of PEI allows for efficient conjugation with various drugs and nucleic acids.[16,107] The Herceptin-tagged PEI and poly(lactide) NP system (hPPD) was designed to deliver DOX to HER2-positive breast cancer cells, aiming to enhance the efficacy of chemotherapy. The findings indicate that hPPD markedly reduces the proliferation of cancer cells more effectively than DOX in vitro and in the xenograft tumor model.[108] Another study designed a modified hydroxyethyl cellulose conjugate with polyethyleneimine (HECP2k) to co-deliver DOX and Bcl-2 siRNA. This approach significantly enhanced anti-cancer effects by promoting apoptosis, indicating that HECP2k is a highly effective delivery system for DOX and Bcl-2 siRNA, with significant therapeutic potential.[109]

Polyethylene Glycol

PEG, known for its excellent solubility, non-toxicity, and non-immunogenicity, is synthesized via the polymerization of ethylene oxide.[110] PEG is highly valued for conjugation applications, is pivotal in developing polymer-protein conjugates, and enhances protein resistance to enzymatic degradation.[11,111,112] PEGylation has received FDA approval for modifying proteins, peptides, and small bioactive molecules by attaching PEG chains, which improves pharmacokinetic properties.[113–116] In the early 2000s, the FDA approved two PEGylated interferon conjugates (Pegasys® and PEG-Intron®) for chronic hepatitis C. Additionally, Neulasta®, a PEGylated version of recombinant granulocyte colony-stimulating factor (G-CSF), received FDA approval in 2002.[117] PEG-interferon α-2b (Sylatron) received approval in 2011 as an adjuvant therapy for high-risk melanoma. Additionally, other PEGylated therapeutics, such as PEG-asparaginase (Oncaspar®), PEG-adenosine deaminase (Adagen®), and PEG-growth hormone receptor antagonist (Somavert®), have been developed.[118–121]

Krishna Rao et al developed methoxy poly(ethylene glycol) (mPEG) and DOX linked by a pH-sensitive imine bond for cancer therapy. Among different, the smallest mPEG (1K) conjugates demonstrated superior release rates, cellular uptake, and cytotoxic effects compared to larger mPEG conjugates and free DOX.[122] Poly(ethylene glycol)-b-poly(d, l-lactide) NPs, loaded with siRNA and cationic lipids, were developed via a double emulsion-solvent evaporation technique. The NPs demonstrated effective cellular uptake and endosomal escape, leading to significant Plk1 gene silencing and substantial apoptosis in HepG2 and MDA-MB-435 cancer cells.[123]

N-(2-hydroxypropyl)methacrylamide

The drug delivery capabilities of hydrophilic N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers have been extensively studied.[124] HPMA is extensively employed in developing PDCs for targeted cancer therapy. HPMA allows for the incorporation of targeting moieties that direct the drug to specific cells, further optimizing treatment effectiveness.[125] The HPMA copolymer-DOX conjugate (PK1, FCE28068), developed in 1994, was the first synthetic PDC to enter clinical trials. PK1 links DOX to the HPMA copolymer through a degradable tetrapeptide linker, GFLG (glycylphenylalanylleucylglycine). This conjugate showed significant stability, improved DOX accumulation in melanoma tumors, and reduced cardiotoxicity and bone marrow toxicity compared to free DOX. In Phase II clinical trials, PK1 was well-tolerated, showing no typical DOX-induced alopecia or cardiotoxicity.[126,127] P-SS-AMD, a PDC, combines HPMA with the AMD3465 (CXCR4 antagonist) to enable controlled drug release and CXCR4 antagonism. P-SS-AMD features a methacrylamide monomer linked to AMD3465 via a disulfide bond, which is cleaved by glutathione inside cancer cells. This releases AMD3465 to block CXCR4 and inhibit cancer cell invasion. Additionally, P-SS-AMD's cationic charge allows it to bind with miRNA, forming complexes that deliver miR-200c mimics into cancer cells, suppressing the ZEB-1 gene and enhancing cancer cell migration inhibition.[128]

Dendrimers

Dendrimers are promising biomaterials for polymer-drug conjugation owing to their distinctive 3D hyperbranched, star-like structures.[129,130] Their nanoscale size promotes increased cellular uptake and prolonged circulation in the bloodstream, which augments drug delivery to tumors.[130–132]

Poly(amidoamine) (PAMAM), and dendrimer-trastuzumab conjugates were developed for targeting HER-2 over-expressing cancer cells with DOX. Previous research demonstrated that the PAMAM–DOX–trastuzumab conjugate was more effective than DOX alone or a basic PAMAM–trastuzumab conjugate. This indicates that trastuzumab improves targeting precision, making the PAMAM–DOX–trastuzumab conjugate a promising advancement for treating HER-2-positive tumors.[133] Positively charged dendrimers PAMAM and poly(propylene imine) (PPI) have shown potential in DNA complexation for gene delivery [147, 151, 152]. The solubility and bioavailability of curcumin were enhanced by incorporating it into a PAMAM dendrimer, which formed a polyplex with Bcl-2 siRNA. This combination improves cellular uptake and tumor inhibition via Bcl-2 silencing. The PAMAM-Cur/Bcl-2 siRNA NPs demonstrate superior effectiveness.[134]

Polymeric Micelles

Polymeric micelles are formed from amphiphilic block copolymers that self-assemble into nanostructures with a hydrophobic core to encapsulate poorly soluble drugs.[135] Poly(D, L-lactide), poly(L-lactic acid), poly(DL-lactic-co-glycolic acid), polycaprolactone, and poly(β-benzyl-L-aspartate) are commonly used hydrophobic blocks used in the core.[136] The "corona" or outer shell, composed of hydrophilic blocks such as PEG, protects the micelles from aggregation and facilitates evasion from the RES.[137]

A conjugate of methotrexate-polyethylene glycol (MTX-PEG) with CG/DMMA polymeric micelles was designed to enhance the targeted delivery of DOX to tumors. The findings showed that these micelles effectively improved cancer treatment.[138] Previously, chitosan-coated micellar polyplexes delivered siRNA and DOX to treat multidrug-resistant cancers simultaneously. The findings showed that these polyplexes were more effective at killing cancer cells than DOX alone, with reduced tumor size and extended survival times in both in vitro and in vivo models.[139]

Preparation Methods of PDCs

Solvent Evaporation Diffusion

The solvent evaporation diffusion (SED) technique primarily involves the formation of an oil-in-water emulsion. The process commences with blending an organic solvent, carrying both polymer and drug into an aqueous phase infused with a stabilizer like Polyvinyl Alcohol (PVA), which is then vigorously emulsified using a high-shear mixer to develop a primary emulsion.[140,141] Emulsification can be executed via single (oil-in-water) or double (water-in-oil-in-water) emulsion methods, employing high-speed homogenization or ultrasonication. The resulting emulsion is diluted in water and stirred magnetically at ambient conditions or under a vacuum to facilitate solvent evaporation, forming a nanoparticle suspension. These nanoparticles are isolated through ultracentrifugation and rinsing with distilled water and then freeze-dried to yield solid PDCs. Selecting the organic solvent is pivotal, with preferences for partially soluble solvents like ethyl acetate and polyethylene glycol that dissolve both the drug and polymer and are safely removable.[142]

Nanoprecipitation

Nanoprecipitation is a technique where a polymer precipitates as an organic solvent, which is partially water-soluble, is displaced from a lipophilic solution into an aqueous phase. This rapid diffusion lowers interfacial tension and increases surface area, forming fine droplets without mechanical agitation. Fessi et al demonstrated that adjusting pH and ionic strength can enhance the loading efficiency of procaine hydrochloride in PLGA NPs.[143]

Controlled precipitation techniques, such as NanoMorph® technology, is employed to prepare drug-loaded polymeric NPs. This process involves dissolving the drug in an organic solvent at high temperatures and rapidly mixing this solution with a cooled aqueous stabilizer. This rapid mixing induces nucleation, resulting in the formation of spherical amorphous NPs. Improvements in particle size and uniformity are often achieved through ultrasonic waves or adjustments to process parameters.[144]

Salting-Out

Salting out is a technique to develop a viscous gel by combining a hydrophilic polymer, like PVA, with an electrolyte solution (eg, sodium chloride, calcium chloride) without using high shear forces or surfactants. The drug and polymer are initially dissolved in an organic solvent, such as acetone, which helps solubilization and phase separation. The gel is mixed into this organic phase to form an oil-in-water emulsion. Adding water allows acetone to diffuse into the aqueous phase, triggering the salting-out process and forming nanospheres. These nanospheres are subsequently isolated through cross-flow filtration to remove any remaining electrolyte and solvent.[145] Diluting the emulsion with a large volume of water triggers a reverse salting-out effect, leading to the precipitation of the polymer and the formation of NPs. Previous studies have developed PTMC-dexamethasone nanoconjugates with particle sizes ranging from 183 to 251 nm. Other methods for NP production include supercritical fluid technology, dialysis, microencapsulation, nanoencapsulation, and various surface and diffusion-mediated drug loading techniques.[146,147]

Polymerization of Monomers

Different techniques for polymerizing monomers include emulsion, mini emulsion, microemulsion, surfactant-free emulsion, interfacial, and free radical polymerization. Choosing the right method depends on factors like safety, degradation rates, ease of preparation, drug encapsulation efficiency, release kinetics, targeted delivery, and overall therapeutic effectiveness.[148]

Classes of PDCs

Polymer Protein Conjugates

The introduction of recombinant insulin in 1982 represented a breakthrough in protein therapeutics. However, these biological drugs often face poor stability, rapid clearance, and possible immune reactions.[149] In 1977, Abuchowski et al pioneered PEGylation, a technique that involves attaching PEG to proteins, which reduces immune responses, improves solubility, and extends the protein's presence in the bloodstream.[115] In the 1980s, Matsumura and Maeda found that the polymeric conjugate of the anticancer protein neocarzinostatin, called SMANCS, mainly accumulates in tumor tissues via the EPR effect.[150] The mPEGylated peptide dendrimer-DOX conjugate (dendrimer-DOX) are NPs for targeted breast cancer therapy. It features a tetra-peptide linker that responds to specific enzymes, enabling controlled release of DOX at the cancer site.[151]

PEG masks parts of the protein that might trigger an immune reaction, protects it from being broken down or cleared too quickly, and ultimately leads to more effective and safer treatment regimens. The first PEGylated protein, Adagen, was approved in 1990 for treating severe combined immunodeficiency linked to adenosine deaminase deficiency.[114,152] PEGylated enzyme Pegaspargase (Oncaspar), approved in 1994 for acute lymphoblastic leukemia, significantly extends the drug's plasma half-life from 20 hours to 357 hours, reducing administration from 2–3 times per week to biweekly and lowers the enzyme's immunogenicity.[153,154] The technique of PEGylation has been successfully used with nucleic acids to improve the pharmacokinetics of treatments such as pegaptanib sodium for macular degeneration.[155,156]

Small Molecule PDCs

The first polymer-small-molecule drug conjugate was documented in 1955 by Jatzkewitz, who demonstrated that linking mescaline, a psychedelic alkaloid, to a copolymer of N-vinylpyrrolidone and acrylic acid increased its bioactivity's residence time in mice.[157]

PDCs for Combination Therapy

PDCs are being investigated for their potential in treating complex diseases like cancer, HIV/AIDS, and neurodegenerative disorders through various combination therapies such as chemotherapy with radiotherapy, etc. There are four types of PDC-based combination therapies: 1) using a PDC with additional free drugs, 2) combining different PDCs, 3) attaching multiple drugs to a single polymer carrier, and 4) integrating polymer-directed enzyme prodrug therapy (PDEPT) with polymer enzyme liposome therapy (PELT). In PDEPT, a polymer carrying an enzyme triggers drug release at the target site. PELT combines a liposome-based drug delivery system with a polymer that holds the enzyme for liposome breakdown and drug release.[9,158]

Vicent et al developed a novel PDC combination therapy incorporating HPMA copolymer, aminoglutethimide, and DOX to treat breast cancer by merging endocrine and chemotherapy. This approach led to a conjugate with markedly enhanced cytotoxicity against MCF-7 breast cancer cells compared to the free drugs, their combinations, or the drug conjugated with HPMA alone. The study highlights the potential for improved breast cancer treatments by integrating multiple therapeutic strategies within a single compound.[158,159]

Multifunctional Targeted Delivery

In drug delivery, traditional approaches often involve delivering a single bioactive agent. However, the increasing complexity of chronic diseases and the rise of multidrug resistance have revealed the limitations of this method, especially for conditions like cancer and antibiotic resistance. To overcome these challenges, research explores the co-

delivery of multiple bioactive agents to the targeted site to improve therapeutic effectiveness.[158] A single polymer capable of carrying multiple drugs offers the advantage of delivering various treatments simultaneously, which can enhance therapeutic effects. For instance, Z-DMC-CIS(N3) is a multifunctional PDC designed for chemotherapy and radiation therapy, especially for cancers resistant to cisplatin. This compound integrates three distinct drugs—platinum, azidyl radical, and demethylcantharidin (DMC). It features a Pt(IV)–azide complex that becomes active when exposed to UV light, enabling cancer cell destruction through dual mechanisms.[160]

PDCs for Nucleic Acid Delivery

In 1975, Ringsdorf introduced the concept of PDCs. This idea was later explored in more detail by Duncan et al, who investigated the biological principles and techniques involved.[81,161] Designing effective PDCs involves attaching pharmacologically active substances such as small molecules, proteins, peptides, and aptamers to a polymer scaffold using a biodegradable linker. This approach improves drug properties by enhancing solubility and bioavailability, extending the drug's presence in the bloodstream, increasing therapeutic effectiveness, enabling targeted drug delivery, and protecting the drug from enzymatic breakdown.[22] PDCs ensure structural stability, safeguard nucleic acid cargo from enzymatic breakdown, offer therapeutic benefits, and enhance efficient delivery to target cells.[162] PDCs integrate the advantages of polymeric vectors with therapeutic effects and the ability to deliver nucleic acids effectively (Figure 8).[16]

Considerations for the Design of PDCs to Optimize Nucleic Acid Delivery

The design of PDCs is pivotal in enhancing nucleic acid's delivery and stability via polymer selection, formulation method, surface chemistry, and targeting strategies.

Polymer Selection

Choosing a suitable polymer is vital for efficient nucleic acid delivery. Biocompatibility, biodegradability, stability, and nucleic acid encapsulation efficiency are significant. Natural polymers like chitosan and alginate exhibit superior biocompatibility and minimal toxicity. In contrast, synthetic polymers, eg, PEI and PLGA, allow precision in manipulating carrier characteristics.[162,163] The selection of polymers depends on the specific requirements of nucleic acid delivery and therapeutic targets. Utilizing a polymeric blend of polymer, drug conjugate, and nucleic acid can exploit their combined synergistic characteristics.[164–166]

Formulation techniques

Formulation techniques are crucial for enhancing the properties of PDCs. Nanoprecipitation, emulsion/solvent evaporation, and self-assembly facilitate the production of PDCs with controlled size, surface zeta potential, stability, and targeting.[167,168]

Surface Modification

Modifying the surface chemistry of PDCs can optimize their biocompatibility, circulation time, and targeting potential.[169] PEGylation of the PDCs reduces immunogenic responses and enhances biocompatibility, stability, targeting, and half-

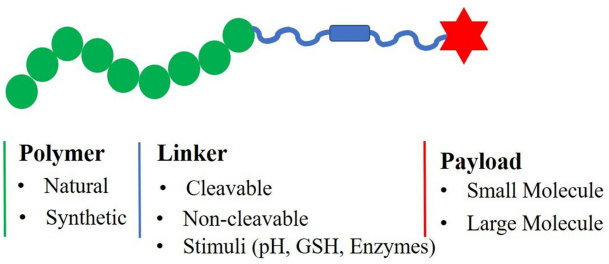

Figure 8 Schematic representation of a polymer-drug conjugate.

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life.[170] Incorporating targeting ligands, such as antibodies or peptides, on the surface facilitates precise cell recognition, promoting enhanced cellular internalization and improved delivery efficacy via active targeting.[171]

Stimuli-Responsive Systems

Stimuli-responsive PDCs facilitate site-specific controlled nucleic acid release, enhancing therapeutic effects in response to temperature, pH, and enzymatic activity.[172,173] By incorporating moieties that respond to stimuli, like linkers sensitive to pH that can be cleaved, to release nucleic acid molecules in response to the specific microenvironment of the target site in a controlled manner for on-demand delivery.[173,174]

Biocompatibility and Safety

When developing PDCs for nucleic acid delivery, biocompatibility and safety is crucial. The polymer should exhibit minimal cytotoxicity and immune response.[175] Biodegradable polymers facilitate clearance, diminishing potential adverse effects.[176]

Role of Polymeric Drug Conjugates in Nucleic Acid Delivery

Polymeric drug conjugates are a key approach to overcoming challenges in nucleic acid delivery. These conjugates provide a versatile platform for designing and synthesizing delivery systems that protect nucleic acids, improve cell uptake, facilitate endosomal escape, and enhance therapeutic outcomes. Efficient mRNA, siRNA, and miRNA delivery systems are crucial for maximizing their therapeutic potential. Nevertheless, stability, cellular uptake, endosomal escape, and intracellular release hinder the clinical translation of nucleic acid therapeutics.[112,166,177,178]

Polymeric Drug Conjugates Improve Nucleic Acid Stability

Nucleic acids are degraded by nucleases in the bloodstream and intracellular compartments. Protecting them from degradation is essential for maintaining their therapeutic efficacy.[179] A common approach to prevent nuclease degradation is encapsulating with NPs. Chemical and structural modifications have also improved siRNA serum stability.[180–182] PDCs protect nucleic acid cargo from degradation by nucleases. Conjugating nucleic acids with PDCs boosts their stability, extends circulation time, and improves delivery to target sites.[16,162,166]

Jing Li et al investigated the stability of reducible polymeric copper chelators (RPC) polyplexes with Cu(II). Without Cu(II), DNA release from RPC polyplexes began at 70 μg/mL heparin, with complete release at 80 μg/mL. However, in the presence of Cu(II), only partial DNA release occurred at 80 μg/mL heparin (approximately 80% for 50% Cu(II) and 30% for 100% Cu(II)). Cu(II) enhances the stability of RPC polyplexes against polyanion disruption and improves their transfection efficacy (Figure 9A and B). These polycationic Cu(II) chelates show promise as nucleic acid delivery vectors, suggesting potential for theragnostic applications.[183]

PDCs improve cellular uptake

The effectiveness of nucleic acid therapies relies on their efficient delivery into target cells. The cell membrane's anionic phospholipid bilayer and embedded proteins impede the passive diffusion of siRNAs because of its negative charge.[184] Zhou et al utilized a polymer-drug conjugate for VEGF siRNA delivery and observed no significant differences between PEI-Cyclam and PEI polyplexes. This suggests that PEI's delivery efficacy remained unaffected by cyclam modification, ensuring effective siRNA cellular uptake. Additionally, PEI-Cyclam demonstrated anticancer activity (Figure 9C).[17] Ullah et al demonstrated that PEI-cyclam significantly improves siRNA cell uptake, confirming its efficacy as a TGF-β siRNA delivery system and antifibrotic agent (Figure 9D).[16]

Polymeric Drug Conjugates Improve Immune Activation

Small molecules targeting CXCR4, such as AMD3100, elevate leukocyte counts in humans and animal models, including canines and rodents. CXCR4 is crucial for anchoring leukocytes and hematopoietic stem and progenitor cells in the bone marrow. Increased leukocyte levels following CXCR4 inhibition indicate the mobilization of these stem and progenitor cells.[185] Elevated PBL levels are generally observed within 1 hour after administering AMD3100 in humans and animals.[186]

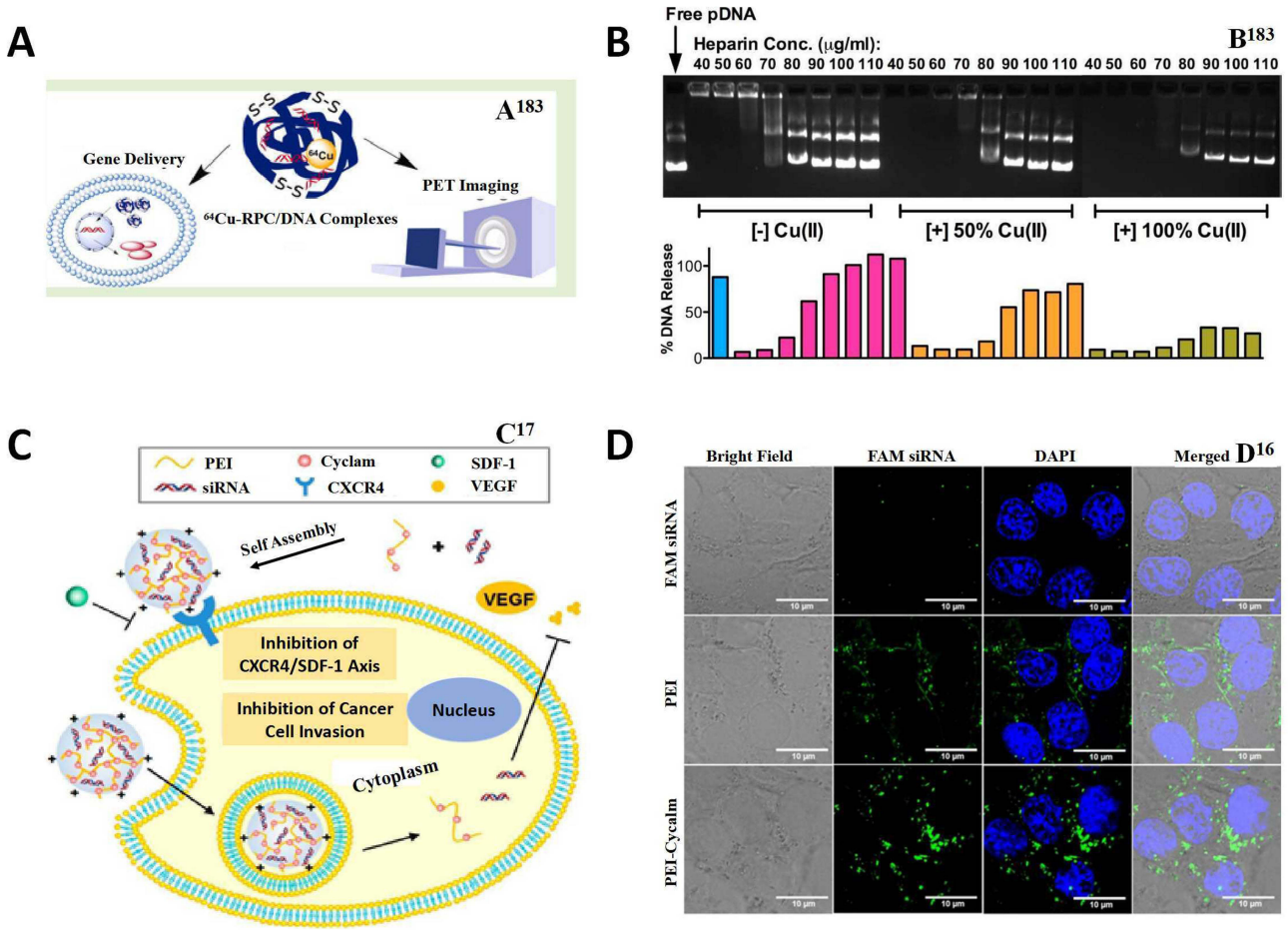

Figure 9 (**A**) Function and application of reducible RPC in enhanced gene delivery and PET imaging. Reprinted with permission from Li J, Zhu Y, Hazeldine ST, Firestine SM, Oupický D. Cyclam-based polymeric copper chelators for gene delivery and potential PET imaging. *Biomacromolecules*. 2012;13(10):3220–3227. Copyright © 2012, American Chemical Society.[183] (**B**) Stability of Cu(II) complexes of RPC/1.8 against heparin disassembly. Reprinted from permission from Li J, Zhu Y, Hazeldine ST, Firestine SM, Oupický D. Cyclam-based polymeric copper chelators for gene delivery and potential PET imaging. *Biomacromolecules*. 2012;13(10):3220–3227. Copyright © 2012, American Chemical Society.[183] (**C**) Mechanism of action of PEI-C/siVEGF complexes. Reprinted with permission from Zhou Y, Yu F, Zhang F, et al. Cyclam-modified PEI for combined VEGF siRNA silencing and CXCR4 inhibition to treat metastatic breast cancer. *Biomacromolecules*. 2018;19(2):392–401. Copyright © 2018, American Chemical Society.[17] (**D**) Cell uptake was evaluated by confocal laser scanning microscopy at 2 h post-incubation with polyplexes and free FAM siRNA (Green). Cell nuclei are stained with DAPI (blue). Reprinted with permission from Dove Medical Press. Ullah A, Chen G, Hussain A, et al. Cyclam-modified polyethyleneimine for simultaneous TGFβ siRNA delivery and CXCR4 inhibition for the treatment of CCl(4)-induced liver fibrosis. *Int j Nanomed*. 2021;16:4451–4470.[16]

The effects of polymeric CXCR4 antagonists (PCX) on leukocytosis and hematopoietic stem and progenitor cell mobilization were evaluated in vitro. PCX treatment resulted in a 1.6-fold increase in total PBL count, similar to PBS-treated controls. PCX-4's effects were comparable to AMD3100 ($p > 0.05$). This preliminary data suggests that polymeric CXCR4 antagonists may effectively induce leukocytosis and mobilize hematopoietic stem and progenitor cells, akin to AMD3100.[187] PCX provides CXCR4 inhibition and targeted siRNA delivery. Previously, PCX demonstrated more significant cytotoxicity than AMD3100 and effectively delivered siRNAs targeting the RUNX1 transcription factor in both mouse and human leukemia cells, highlighting the potential of PCX/siRNA NPs for advancing AML treatment.[188]

Polymeric Drug Conjugates Improve Specific Tissues Targeting

Targeted delivery to specific cells while reducing non-specific interactions is a pivotal concern.[189] Firefly luciferase (FLuc) and interleukin-10 (IL-10) mRNA were precisely delivered to Ly6c-expressing leukocytes by encapsulating the mRNA in LNPs, which were surface-modified with anti-Ly6c monoclonal antibodies to target Ly6c+ cells specifically.[190] A rapid decline in kidney function characterizes acute kidney injury (AKI). Tang et al developed a novel siRNA carrier by modifying chitosan with α-cyclam-toluic acid (C-CS), which targets injured kidneys. This modification specifically

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allows the C-CS polymer to bind and antagonize CXCR4-expressing cells in the kidneys. The C-CS/siRNA complexes selectively accumulate in damaged renal tissues, reducing inflammation and promoting kidney recovery, demonstrating their potential for effective AKI treatment.[191]

Polymeric Drug Conjugates Improve Endosomal Escape and Intracellular Release

Efficient endosomal escape is critical, enabling nucleic acids to reach their anticipated intracellular compartments and exert their therapeutic effects.[192] Effective nucleic acid delivery requires precise intracellular release from their vectors to ensure the anticipated therapeutic outcome.[193] PDCs facilitate enhanced endosomal escape, reducing the entrapment of nucleic acid in endosomes.[16,194] Ying Xie et al studied the combined effects of CXCR4 inhibition and miR-200c mimic treatment on cholangiocarcinoma cell invasion. Their results show that delivering the miR-200c mimic via PCX, in conjunction with CXCR4 inhibition, significantly improves anti-metastatic activity by targeting both the CXCR4 axis and epithelial-to-mesenchymal transition.

Furthermore, the minimal overlap between FITC-Oligo and lysosomal signals indicates effective endosomal escape of the PCX polyplexes (Figure 10A).[19] Zhou et al used PEI-C polyplexes carrying siVEGF, effectively reducing VEGF expression in vitro. In an in vivo breast cancer model, these polyplexes demonstrated significant antitumor and anti-metastatic effects, suggesting PEI-C's potential for combined siRNA delivery and CXCR4 inhibition. After 2 hours of exposure to PEI-C polyplexes, FAM-siRNA fluorescence (green) notably overlapped with LysoTracker (red), indicating lysosomal localization. By 6 hours, fluorescence patterns diverged, reflecting the endosomal escape of FAM-siRNA, likely due to PEI's "proton sponge effect" in the PEI-C formulation (Figure 10B).[17,195] Ullah et al demonstrated that PEI-Cyclam functions effectively as a siRNA delivery vector and antifibrotic agent. Their results show that PEI-Cyclam offers enhanced intracellular uptake and endosomal escape of siRNA, confirming its efficacy in nucleic acid delivery.[16]

Applications of PDCs in Disease State

PDCs have demonstrated efficacy in several nucleic acid-based therapeutics, such as siRNA-mediated gene silencing, mRNA vaccines, and miRNA-targeted interventions.[37,196–200] The ability of these polymeric vectors to protect and deliver nucleic acid to target cells or tissue sites holds significant importance for addressing genetic diseases, infections, tumors, and related pathologies.[37,201]

PDCs in the Treatment of Cancer and Metastasis

Zhou et al developed PEI-C for treating metastatic breast cancer. This system delivers VEGF siRNA and inhibits CXCR4/SDF-1. The results demonstrate PEI-C's potential as a dual-function carrier for VEGF silencing and CXCR4 inhibition, enhancing cancer treatment.[17] Ting et al created a lung-targeted delivery system utilizing perfluorocarbon (PFC) nanoemulsions to administer CXCR4 and STAT3 siRNA, which successfully inhibited CXCR4 and silenced

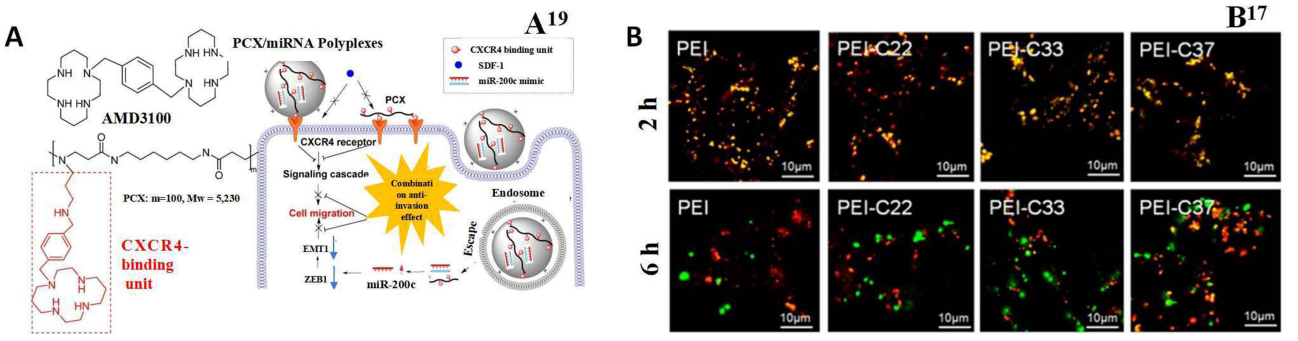

Figure 10 (**A**) Chemical Structure of AMD3100, PCX and mechanism of Action of PCX/miR-200c. Reprinted with permission from Xie Y, Wehrkamp CJ, Li J, et al. Delivery of miR-200c mimic with Poly(amido amine) CXCR4 antagonists for combined inhibition of cholangiocarcinoma cell invasiveness. *Mol Pharmaceut*. 2016;13(3):1073–1080. Copyright © 2016, American Chemical Society.[19] (**B**) Endosomal escape of PEI-C polyplexes (w/w 3) in 4T1 cells at 2 and 6 h post-incubation Lysosomes were stained with Lysotracker Red. Reprinted with permission from Zhou Y, Yu F, Zhang F, et al. Cyclam-modified PEI for combined VEGF siRNA silencing and CXCR4 inhibition to treat metastatic breast cancer. *Biomacromolecules*. 2018;19(2):392–401. Copyright © 2018, American Chemical Society.[17]

STAT3, demonstrating significant anticancer efficacy in lung metastasis models by diminishing cancer spread.[202] Wang et al developed polyplexes for delivering siNCOA3 siRNA to target and inhibit CXCR4 receptors in metastatic pancreatic carcinoma (PC) treatment.[203] Jing Li developed CXCR4 antagonists, including polymeric plerixafor (PAMD) and reducible PAMD (rPAMD), which effectively block CXCR4 and its interaction with SDF-1, thereby inhibiting CXCR4-mediated cancer cell invasion. Both biodegradable and non-biodegradable forms demonstrated efficacy in combating metastasis.[204] Ying Xie et al developed and evaluated PCX with a miR-200c mimic to target cholangiocarcinoma. PCX effectively inhibited cancer cell migration via CXCR4 antagonism. By complexing with the miR-200c mimic, PCX facilitated cellular delivery and decreased ZEB1 expression, indicating a promising approach for addressing cholangiocarcinoma and metastasis.[19]

PDCs Improve Immunotherapy

Zuk A. et al investigated a small-molecule CXCR4 antagonist (AMD3100), which caused a rise in leukocyte counts in both human and animal models. CXCR4 is crucial for retaining leukocytes and hematopoietic stem cells in the bone marrow. Increased leukocyte count following CXCR4 inhibition indicates mobilization of these stem and progenitor cells.[185]

Wang Y. et al observed that elevated PBL levels are typically noted within 1 hour post-AMD3100 administration in human and animal subjects.[186] Upon PCX administration, a 1.6-fold elevation in total PBL count was reported in vivo. The impact of PCX-4 was paralleled that of AMD3100, providing initial evidence that PCX promotes leukocytosis and mobilizes hematopoietic stem and progenitor cells, mirroring the clinical efficacy of AMD3100.[187] The findings indicated superior anticancer activity of PCX compared to AMD3100 and efficient delivery of siRNAs against the transcription factor RUNX1 in mice and human leukemia cells. They concluded that PCX offers simultaneous CXCR4 inhibition and siRNA delivery.[188]

PDCs Improve Fibrosis Therapy

Zhang and his team developed a cholesterol-modified CXCR4 inhibitor, Chol-PCX, to deliver anti-miR-155, which reduced fibrotic liver damage and significantly lowered liver enzymes and collagen in models of alcohol-induced liver injury, highlighting its therapeutic potential.[205] Ding et al developed PFOB nanoemulsions for the local delivery of therapeutic siRNA to treat lung fibrosis, enhancing STAT3 silencing and CXCR4 inhibition.[206] Ullah et al developed PEI-Cyclam-based polymeric CXCR4 antagonists that deliver TGFβ siRNA while inhibiting the CXCR4/SDF-1 axis, resulting in anti-fibrotic effects.[16]

PDCs in Diagnostic Imaging and Photodynamic Therapy

Jing Li et al developed a bioreducible cyclam-based polymer for gene delivery and potential PET imaging. The RPC polycations were synthesized via Michael addition, demonstrating low toxicity and improved in vitro transfection efficiency. The efficacy and safety of RPCs with Cu(II) depend on Cu(II) bonding. Due to their Cu(II) binding capability, RPCs are suitable for gene delivery and PET imaging applications.[183] Gang et al developed CXCR-targeted cholesterol-modified (PAMD-Ch) polyplexes with IR780. The dye's photothermal effects disrupt endosomal membranes, enhancing siRNA transfection. These micelleplexes impede CXCR4-mediated cancer cell invasion, offering a promising delivery system that integrates CXCR4 antagonism with NIR-triggered siRNA delivery for improved cancer treatment.[207]

Current Clinical Status of PDCs

PDCs represent a significant advancement in drug delivery systems, particularly in the treatment of complex diseases like cancer, autoimmune disorders, and infectious diseases. By attaching therapeutic drugs to a polymer carrier, PDCs enhance the drug's solubility, stability, and bioavailability, often leading to more efficient and targeted delivery to specific tissues or cells. PDCs represent a transformative approach to modern medicine, offering better control over drug delivery, enhancing therapeutic efficacy, and minimizing side effects, which is especially important for treating serious conditions like cancer and genetic disorders.[75]

Abraxane® is an innovative formulation of paclitaxel designed for injectable suspension. Manufactured by Celgene Corporation, it is clinically utilized in the treatment of various malignancies, including breast cancer, non-small cell lung cancer, and pancreatic cancer.[208] The paclitaxel is conjugated to human serum albumin nanoparticles, which serve as the delivery vehicle, enhancing the solubility and bioavailability of the drug while facilitating its targeted delivery to tumor sites. Paclitaxel functions as a potent microtubule inhibitor, disrupting cell division and thereby inducing apoptosis in proliferating cancer cells. This formulation aims to reduce the systemic toxicity typically associated with paclitaxel, providing a more effective and safer therapeutic option for patients.[209] OncoHist® is a novel polymer-drug conjugate currently under investigation for the treatment of hematologic cancers and solid tumors.[210] Manufactured by Pollex Life Sciences, this therapeutic utilizes a polyglutamic acid polymer to conjugate histone deacetylase inhibitors (HDAC inhibitors), enhancing the drug's stability, solubility, and circulation time. The polymeric carrier ensures that the HDAC inhibitors remain in the bloodstream for a longer duration, potentially improving their efficacy. HDAC inhibitors work by regulating gene expression, which can trigger cancer cell differentiation and induce apoptosis, making them a promising approach for targeting various types of cancers.[210,211] Kadcyla® (ado-trastuzumab emtansine) is a targeted therapy used for the treatment of HER2-positive breast cancer, particularly in patients who have previously been treated with trastuzumab and taxane chemotherapy.[212] Manufactured by Genentech, Inc., this polymer-drug conjugate combines trastuzumab, a monoclonal antibody that specifically targets the HER2 receptor on cancer cells, with DM1, a potent cytotoxic agent derived from maytansine. The conjugate is formed through a stable thioether linker, which enables the precise delivery of DM1 to HER2-positive cancer cells. DM1 is a powerful antimicrotubule agent that disrupts microtubule polymerization, effectively halting cell division and inducing apoptosis in rapidly proliferating cancer cells, thereby enhancing the therapeutic efficacy of the treatment while minimizing systemic toxicity.[213,214]

Looking forward, the future of PDCs is promising, with ongoing research aimed at expanding their applications beyond oncology to other diseases such as genetic disorders and infectious diseases. Advances in polymer design and drug conjugation techniques are expected to further enhance their precision and effectiveness, offering personalized treatment options with fewer side effects. Additionally, the incorporation of novel targeting strategies and combination therapies could revolutionize the treatment landscape for many complex diseases.

Challenges and Innovations in the Design of PDCs

Despite over 40 years of extensive research on PDCs, especially in oncology, they have not yet made significant market breakthroughs. This slow progress is often due to clinical failures related to non-specific drug release and suboptimal pharmacokinetic profiles resulting from inadequate conjugate design.[116,215,216]

Clinical setbacks may stem from variability in the composition, structure, and particle size of conjugates compared to conventional formulations, leading to inconsistent drug release profiles and potential trial failures. A rational design approach for PDCs with controlled size and predictable release profiles is essential to improve clinical outcomes. Developing polymers with low polydispersity and optimizing bioactive molecule conjugation or using protective moieties can reduce nanoparticle heterogeneity. The distinct physicochemical properties of PDC, such as high surface-to-volume ratios, adjustable size, and surface functionality, offer a valuable platform for creating more effective cancer therapies and diagnostics.[217]

Future Prospects of PDCs in Nucleic Acid Delivery

Advancements in Polymer Design

Designing effective polymer-drug conjugates necessitates a detailed analysis of process parameters and material properties and careful evaluation of the drug release rate in plasma and at the target site.[22] Advanced polymerization techniques are employed for polymer-drug conjugate design, including ring-opening polymerization (ROP) and RAFT polymerization. In this process, the therapeutic agent is initially conjugated to the monomeric unit, facilitating the formation of a covalently bonded polymer-drug complex.[218] Advances in polymer design and synthesis techniques will continue to enable the revelation of innovative PDC with improved properties, including enhanced stability, controlled release, and

targeted delivery. Tailoring the polymer-drug conjugate carriers to specific nucleic acid delivery requirements will improve the effectiveness and safety of nucleic acid-based treatments.[201]

Advancement in Manufacturing Processes

PDCs have shown promising results in pre-clinical settings, with limitations such as poor linker chemistry, reduced bioactivity, reduced drug conjugation, and polymeric toxicity, with pharmacoeconomic concerns that have restricted their clinical applications.[219] The development and manufacturing of PDCs can be significantly enhanced by employing reproducible and scalable manufacturing processes such as computational simulations, including molecular docking and molecular dynamics.[220,221] Computational approaches offer insights into molecular interplays and the physicochemical attributes of both carriers and drugs. Techniques such as docking protocols and molecular modelling are instrumental in prognosticating drug candidates' efficacy and binding affinity with specific protein targets.[220] This method would substantially reduce expenditures inherent in PDC research. Future endeavours will focus on developing cost-effective and scalable methods for producing these complex polymer-drug conjugates.

Biodegradability and Biocompatibility

Future designs will emphasize biocompatibility and biodegradability to minimize potential immunogenic responses. Using naturally derived or bio-inspired polymers might become more common, ensuring the degradation products are non-toxic and easily excretable.

Integration of Advanced Drug Delivery Strategies

Integrating PDCs with advanced drug delivery strategies, such as stimuli-responsive systems, extracellular vesicles, or exosomes, holds promise for overcoming existing limitations. These strategies can enhance the intracellular delivery, endosomal escape, and subcellular localization of nucleic acid cargo, improving therapeutic outcomes.[193,222]

Nucleic Acid Delivery

PDCs can be potential siRNA, miRNA, mRNA, and CRISPR/Cas systems carriers. The main challenge is ensuring these nucleic acids' stability, intracellular delivery, and efficient release. The evolution of PDCs will focus on overcoming these barriers to enable effective gene modulation therapies.

Combination with Gene Editing Strategies

Combining PDCs with emerging gene-editing technologies like CRISPR-Cas systems enables precision genome engineering. The polymeric entity will simultaneously act as a carrier to carry CRISPR components or mRNA, siRNA, and miRNA, allowing precise and efficient gene editing.[223,224]

Clinical Trials and Regulations

As PDCs advance, the need for rigorous clinical trials will grow. Establishing clear guidelines for evaluating the safety and efficacy of these conjugates will be crucial. Regulatory bodies worldwide must adapt to these new therapeutic modalities, establishing protocols and guidelines to ensure patient safety.

Conclusion

In a nutshell, PDCs are highly advantageous in nucleic acid delivery because they provide therapeutic and carrier potential and further enhance stability, targeted delivery, and combination therapy via combining small molecules and nucleic acids. Despite their limitations, ongoing research and advancements in polymer design, formulation, and delivery strategies show great promise for overcoming these obstacles. By revolutionizing the field of nucleic acid therapeutics, PDCs can pave the way for novel remedies for various diseases.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

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