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Advanced drug delivery systems for enhancing the efficacy of RNA-based therapeutics

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***Abstract*---**Background: RNA-based therapeutics, including antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), and messenger RNAs (mRNAs), offer significant promise in treating genetic and acquired diseases by targeting specific RNA sequences, encoding therapeutic proteins, or facilitating genome editing. However, the effective delivery of these RNA therapeutics remains a major challenge due to their large size, negative charge, and susceptibility to degradation. Aim: This review aims to explore advanced drug delivery systems developed to enhance the efficacy of RNA-based therapeutics, focusing on both viral and non-viral methods, and to evaluate the progress and limitations of these systems in clinical applications. Methods: The review synthesizes recent advancements in RNA delivery technologies, including viral vectors, lipid nanoparticles (LNPs), polymer-based nanoparticles, and hybrid systems. It also examines

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various targeting strategies such as passive and active targeting to improve the specificity and efficiency of RNA delivery. Results: Significant progress has been made with both viral and non-viral delivery systems. Viral vectors, though effective, face challenges related to immunogenicity and production costs. Non-viral systems, particularly lipid nanoparticles and polymer-based carriers, have shown promising results, with several FDA-approved products demonstrating clinical efficacy. Advances in targeting strategies, including ligand-based and antibody-based methods, have improved the precision of RNA delivery. Conclusion: The development of effective RNA delivery systems is crucial for advancing RNA-based therapeutics. Innovations in delivery vehicles and targeting strategies have led to significant clinical advancements, though challenges remain in optimizing delivery efficiency and minimizing off-target effects. Future research should focus on refining these delivery systems and addressing remaining hurdles to fully realize the potential of RNA-based therapies.

***Keywords*---**RNA therapeutics, delivery systems, lipid nanoparticles, polymer nanoparticles, viral vectors, targeted delivery, mRNA vaccines.

Introduction

RNA therapies have the potential to alter gene expression or produce therapeutic proteins, making them applicable to diseases with well-characterized genetic targets, such as infectious diseases, cancers, immune disorders, and Mendelian conditions (including neurological disorders). Additionally, advancements in genome sequencing, single-cell gene expression analysis, and programmable nucleases are driving the identification of new targets for gene therapies. However, the challenge of manipulating these targets, particularly non-coding DNA and the 85% of the genome that might be undruggable by small molecules [1], is compounded by the need for effective delivery of therapeutic RNA to diseased cells. This Review addresses therapeutic RNA, including antisense oligonucleotides (ASOs) like gapmers, which have DNA nucleotides flanked by RNA [2], small interfering RNAs (siRNAs), and larger RNAs such as messenger RNA (mRNA) (Fig. 1). These RNA therapies work by targeting RNA or proteins, encoding missing or defective proteins, or facilitating DNA or RNA editing. Despite their therapeutic mechanisms, the large size of some RNA therapies, such as mRNAs, their anionic charge, and their susceptibility to RNases present in the bloodstream and tissues complicate efficient cellular entry and function. To overcome these barriers, researchers have developed both viral and non-viral delivery systems designed to protect RNA from degradation, enhance delivery to target cells, and minimize off-target exposure. While viral gene therapies [3] have shown successful clinical outcomes [4,5,6,7,8,9], their effectiveness can be limited by pre-existing immunity [10], viral-induced immunogenicity [11], unintended genomic integration [12], payload size constraints [13], re-dosing challenges, upscaling issues [14], and high production costs. Although some of these limitations are being addressed [15], they have spurred interest in alternative

delivery methods. Advances in synthetic materials for RNA encapsulation, such as polymers, lipids, and lipid nanoparticles (LNPs), have invigorated research into non-viral delivery systems, leading to FDA approvals for subcutaneously administered N-acetylgalactosamine (GalNAc)–siRNA conjugates targeting hepatocytes [16,17,18], intravenously administered LNP-based siRNA drugs targeting hepatocytes [19], and emergency use authorization (EUA) and FDA approval [20] for intramuscularly administered LNP-based mRNA COVID-19 vaccines [21,22]. These approvals suggest that improvements in delivery to non-hepatic tissues, including the central nervous system, eye, and ear, may lead to new therapeutic options. Furthermore, nanoparticle-based delivery systems may offer potential for non-viral DNA delivery, which has been reviewed elsewhere [23].

Therapeutic RNA Payloads

- **Classification and Mechanisms of RNA Drugs:**
  - RNA-based therapeutics are classified by their biochemical mechanisms of action, which dictate the requirements for effective drug delivery (Fig. 1). Oligonucleotide drugs, such as antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs), use endogenous cellular enzymes—RNAse H1 and the RNA-induced silencing complex (RISC), respectively—to facilitate delivery. This approach avoids the need for introducing large enzymes into the system [24].
  - Significant advances have been made in delivering small molecules and macromolecules [24], yet many therapeutic oligonucleotides still require maintenance at high concentrations to achieve gene manipulation [25]. For instance:
    - **Givosiran** is administered subcutaneously at 2.5 mg/kg monthly.
    - **Lumasiran** is given subcutaneously at 3.0 mg/kg monthly for three months, followed by 3 mg/kg every three months.
    - **Inclisiran** involves a single subcutaneous dose of 284 mg on days 1 and 90, and every six months thereafter, with potential for annual dosing with further improvements [25].
- **Advantages and Limitations of Delivery Systems:**
  - DNA nucleases, including CRISPR-based systems, can induce long-term cellular effects even with transient expression [27]. MicroRNAs (miRNAs) recruit RISC to complementary mRNA sequences, facilitating targeted RNA interference. This has led to the development and clinical testing of miRNA mimics and anti-miRNAs. For example:
    - **MRX34**, a double-stranded miRNA-34a mimic delivered via liposomes, was tested in advanced solid tumors [29].
    - **Miravirsen**, an anti-miRNA-122 drug, was evaluated for hepatitis C treatment [30].
    - **RG-101**, an anti-miRNA-122 drug, initially reduced viral load in hepatitis C patients but was discontinued due to hyperbilirubinemia [31,32].

- mRNA drugs represent a versatile therapeutic option for a range of diseases, including vaccines (e.g., COVID-19), protein replacement therapies, and genome editing.

- **siRNA Therapeutics:**
  - siRNA-based gene silencing uses double-stranded RNAs approximately 13 kDa in size to suppress protein translation. This is achieved by recruiting RISC to mRNA through Watson–Crick base pairing, with Ago2 protein cleaving the target mRNA. Other Ago proteins (Ago1, Ago3, Ago4) may facilitate nonspecific mRNA degradation by localizing mRNA to processing (P)-bodies [33,34].
  - siRNAs have been approved by the FDA and EMA for several indications:
    - **Patisiran**: Treats hereditary transthyretin-mediated amyloidosis (hATTR) [19].
    - **Givosiran**: For acute hepatic porphyria [16].
    - **Lumasiran**: For primary hyperoxaluria type 1 [18].
    - **Inclisiran**: For hypercholesterolemia [17].
  - The FDA has also accepted a new drug application for **Vutrisiran**, an investigational RNA interference (RNAi) therapeutic for hATTR amyloidosis with polyneuropathy, following successful Phase III trials [36].
  - The rapid clinical implementation of siRNA is attributed to:
    - The small size of siRNA allowing for solid-phase synthesis with site-specific chemical modifications.
    - Use of RISC, which is endogenous to eukaryotic cells, avoiding the need for large enzyme delivery.
    - siRNA's requirement for only cytoplasmic delivery, which is simpler than nuclear delivery.
- **Antisense Oligonucleotides (ASOs):**
  - ASOs are oligonucleotides with a molecular weight of 6–9 kDa and share manufacturing advantages with siRNAs. They have been FDA-approved for several conditions, including familial hypercholesterolemia [41], hATTR amyloidosis with polyneuropathy [42], certain subtypes of Duchenne muscular dystrophy [43,44], and infantile-onset spinal muscular atrophy [45].
  - ASOs act through three mechanisms:
    - **RNase H1 Activation**: ASOs bind mRNA via Watson–Crick base pairing, recruiting RNase H1 to cleave the target RNA, a process known as gapmer function [46].
    - **Splicing Modulation**: ASOs can interfere with splicing machinery, promoting alternative splicing and increasing target protein expression [47].
    - **Translational Arrest**: ASOs can bind to the translation initiation codon of target mRNA, leading to downregulation of protein expression [48].
  - Chemical modifications of ASOs, such as gapmers with RNA-like and DNA regions, and other modifications like locked nucleic acids, impact their binding affinity and mechanism of action. These modifications can enhance pharmacokinetics, stability, and

immune response [50,51]. ASOs often have a phosphorothioate backbone to aid nuclear transport [52,53].

- **ADAR-Oligonucleotides**: A novel class of ASOs with engineered hairpin domains that recruit the RNA-editing enzyme adenosine deaminase acting on RNA (ADAR) for A-to-I editing. These oligonucleotides, with a molecular weight of 10–35 kDa, bind target mRNA and induce editing, representing an emerging approach for genetic disease treatment [56].

mRNA-Based Therapeutics

- **Overview and Applications of mRNA Therapies:**
  - **Protein Encoding and Therapeutic Functions:** mRNA therapeutics can encode proteins with therapeutic functions. Due to their size, mRNAs are synthesized in vitro and cannot yet be chemically modified with site-specific precision using solid-state synthesis (Fig. 1b) [57]. These mRNAs can serve various roles, including:
    - **Protein Replacement:** Replacing deficient or malfunctioning proteins [57].
    - **Protein Reduction:** Using Cas9-based methods to reduce levels of target proteins [58].
    - **Mutation Repair:** Employing base editing techniques to correct protein mutations at the DNA level [59,60].
- **Clinical Examples and Successes:**
  - **CRISPR-Based mRNA Therapies:** In 2021, a clinical study demonstrated that lipid nanoparticles (LNPs) encapsulating Streptococcus pyogenes Cas9 mRNA and a CRISPR guide RNA achieved an 87% reduction in blood transthyretin (TTR) levels in patients with hereditary transthyretin-mediated amyloidosis (hATTR) [58]. TTR mutations cause hATTR, a condition affecting vitamin A and thyroxine transport.
  - **mRNA Vaccines:** The successful FDA-approved mRNA vaccine against SARS-CoV-2 exemplifies the potential of mRNA-based therapies for viral infections [61,62]. Other clinical efforts include:
    - **Cystic Fibrosis:** Ongoing trials by Translate Bio for mRNA-mediated protein replacement, though improvements in lung function have been limited [63].
    - **Ornithine Transcarbamylase Deficiency:** Trials by Translate Bio were discontinued due to adverse pharmacokinetic and safety profiles [64].
    - **Arcturus Therapeutics:** Initiation of a Phase II trial for an mRNA therapeutic targeting ornithine transcarbamylase deficiency [65].
- **Immunological and Vaccine Applications:**
  - **Autoimmune and Vaccine Development:** mRNA therapies have led to immunological tolerance and potential treatments for autoimmune diseases in animal models, such as experimental autoimmune encephalomyelitis [66]. Conversely, mRNA vaccines aim to induce long-lasting immunity against specific antigens, with

research spanning viruses like Zika, HIV, and influenza, as well as cancers such as melanoma [67–71].

- **Cancer Vaccines:** BNT111, developed by BioNTech, targets a combination of melanoma-associated antigens and has shown partial responses and metastasis shrinkage in Phase I trials [73]. mRNA can also deliver immune checkpoint molecules like OX40L to treat solid tumors, with Moderna's mRNA-2416 showing promise in increasing OX40L expression and pro-inflammatory responses [74].

- **mRNA for Gene Editing and Nucleases:**
  - **Transient Expression and Gene Editing:** mRNA can transiently express nucleases, such as zinc finger nucleases, transcription activator-like nucleases, and CRISPR-Cas system components [75]. This transient expression is advantageous for creating long-lasting gene editing effects while minimizing risks associated with persistent nuclease activity [76,77].
    - **Clinical Trials and Delivery Challenges:** Trials using adeno-associated viral vectors for SaCas9 have been initiated [78]. However, mRNA-based nucleases might be preferred due to the risks of off-target effects and vector integration associated with persistent DNA nucleases [27,79].
  - **Cas Enzyme Improvements:** Cas enzymes can be modified in three primary ways to enhance their therapeutic potential:
    - **Design and Evolution:** Rational design or evolution of Cas enzymes to target diverse DNA sequences [80,81,82].
    - **Nickases and dCas9:** Modification of Cas enzymes to produce nickases or dead Cas9 (dCas9) for targeted applications [82].
    - **Functional Additions:** Fusion of Cas enzymes with domains for transcriptional activation, epigenome editing, base editing, and other modifications [83–89]. Cas12a enzymes, which require shorter guide RNAs and produce staggered cuts, are also noted [90].

- **RNA Nucleases and Delivery:**
  - **RNA Editing and Therapeutics:** RNA nucleases can bind and cleave RNA or be engineered with adenosine deaminase acting on RNA (ADAR) domains for RNA base editing [91–94]. These nucleases are suited for transient gene expression changes and are advantageous for short-term diseases and RNA pathogens [93,95,96].
  - **Delivery Strategies:** Effective delivery of CRISPR therapeutics requires concurrent delivery of Cas proteins and guide RNAs, addressed through various strategies including nanoparticle co-delivery, AAV-mediated sgRNA expression, and pre-complexed ribonucleoproteins [77,96–106]. Compact Cas enzymes like Cas12j (Casϕ), Cas12f, Cas13bt, and Cas13ct are being explored to simplify delivery [109–112].

Synthetic Vehicles for RNA Delivery

Challenges and Requirements for RNA Delivery:

- **Avoiding Clearance and Targeting:** RNA therapeutics must evade clearance by off-target organs, target the correct tissue, and interact with the desired cell type within a complex microenvironment [113].
- **Cellular Uptake and Endosomal Escape:** Successful delivery requires endocytosis and efficient endosomal escape, while minimizing immune responses [113].
- **Modifications and Delivery Vehicles:** Small oligonucleotide RNA therapeutics (e.g., antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), and ADAR-oligonucleotides) can be delivered using conjugates and stable chemical modifications. In contrast, mRNA and DNA-based therapeutics necessitate specialized delivery vehicles [113].

Lipids and Lipid-Based Nanoparticles (LNPs):

- **Key Components and Structures:** LNPs, crucial for drug delivery, are composed of lipids forming micelles, liposomes, or multilayered structures. FDA-approved LNPs for liver delivery of siRNA and mRNA vaccines include cationic or ionizable lipids, cholesterol, helper lipids, and PEG-lipids [19,61,62].
- **Lipid Structures and Delivery:** Variations in lipid structures influence LNP interactions with cells. Libraries of lipid delivery systems have been created using chemistries such as Michael addition, epoxide, and alcohol-based reactions [114,118,119].
- **Preclinical and Clinical Developments:**
    - **Hepatocyte Delivery:** Advances in lipid design reduced the dose required for effective hepatocyte gene silencing from 1.0 mg/kg to 0.002 mg/kg [121,122]. Key lipids include C12-200, cKK-E12, DLin-KC2-DMA, and DLin-MC3-DMA [120–123].
    - **mRNA Delivery:** LNPs have effectively delivered mRNA to the liver in various models. Recent LNPs, like LP0177 and Lipid H, have demonstrated efficacy in both preclinical and clinical studies [59,60]. LNPs used in vaccines and therapeutic trials include components such as DLin-MC3-DMA (Alnylam), SM-102 (Moderna), and ALC-0315 (Pfizer/BioNTech/Acuitas) [19,61,62].
    - **Modifications for Targeting:** Changes in cholesterol, PEG-lipid, or helper lipid structures can alter delivery efficiency and targeting specificity. For instance, modified cholesterol or PEG-lipids have enhanced delivery to specific tissues [124–139].

Polymers and Polymer-Based Nanoparticles:

- **Polymeric Systems:** Various polymers, including polyethylenimine (PEI), poly(l-lysine) (PLL), and poly(beta-amino ester) (PBAE), are utilized for RNA delivery due to their ability to form complexes with RNA [144,145].
    - **PLGA and Cationic Polymers:** PLGA, though commonly used for small molecules, requires modification with cationic groups for RNA delivery. PEI and PLL, which can be toxic in unmodified forms, are often modified for improved efficacy and tolerability [147–155].

- **PBAE Nanoparticles:** PBAEs, designed for better biodegradability and reduced cytotoxicity compared to PEI and PLL, have been used for delivering various RNA types [156–165].
- **Lipid-Polymer Hybrids and Dendrimers:** Lipid-polymer hybrids combine lipids with polymers to enhance stability and delivery. Dendrimers, such as PAMAM, offer another approach with well-defined structures for RNA delivery to various tissues, including the central nervous system [166–172].

Overall, the development of effective RNA delivery systems involves optimizing vehicle components to ensure targeted delivery, efficient cellular uptake, and minimal off-target effects.

Active vs. Passive Tissue Targeting in RNA Delivery

Passive Tissue Targeting:

- **Concept and Mechanism:** Passive targeting, or endogenous targeting, leverages the natural interactions between nanoparticles and serum proteins. This method does not require specific targeting ligands but relies on the adsorption of serum biomolecules onto the nanoparticle surface. This adsorption alters the nanoparticle's surface properties and affects how it interacts with tissues and immune cells [173].
- **Key Factors Influencing Passive Targeting:**
  - **Protein Corona:** When nanoparticles enter the bloodstream, they quickly adsorb proteins, forming a "corona" that modifies their behavior. For instance, apolipoprotein E (ApoE) can be critical for the delivery of certain LNPs to hepatocytes, whereas other LNPs may depend on different serum proteins like LDL or VLDL [178,179].
  - **Nanoparticle Size and Charge:** Size affects the surface area-to-volume ratio, influencing how nanoparticles interact with immune cells and target tissues. Smaller nanoparticles have a higher surface area relative to their volume, which can influence their interaction with biomolecules. Nanoparticle size and charge also affect delivery efficiency and tissue targeting [174,182,183].
  - **Example:** LNPs originally designed for liver delivery have been repurposed for targeting other organs. For example, altering the charge of LNPs has redirected their delivery from the liver to the spleen or lungs [142,125].

Active Tissue Targeting:

- **Concept and Mechanism:** Active targeting involves modifying the delivery system with ligands, antibodies, or aptamers that specifically bind to receptors on target cells. This approach enhances the precision of delivery by using these targeting moieties to direct the therapeutic agent to specific cell types or tissues [173].
- **Types of Active Targeting:**
  - **Ligand-Based Targeting:** Ligands like GalNAc bind to specific receptors (e.g., asialoglycoprotein receptor, ASGPR) on target cells. This method has been employed in FDA-approved drugs such as

givosiran and lumasiran, which use GalNAc–siRNA conjugates for targeted liver delivery [16,18].

- **Antibody-Based Targeting:** Antibodies or antibody fragments can be conjugated to RNA molecules or nanoparticles. For instance, anti-CD71 antibody fragments have been used to deliver siRNA to muscle tissues [197], and monoclonal antibodies have been used for long-term muscle silencing in preclinical models [198].
- **Aptamer-Based Targeting:** RNA aptamers, which fold into specific three-dimensional structures, can bind to receptors on target cells. An example is the use of an anti-PDGFRα RNA aptamer to deliver siRNA targeting STAT3, a key regulator in glioblastoma [196].
- **Nanoparticle Decoration:** mRNA, due to its large size, is often delivered using nanoparticles decorated with antibodies or aptamers. The ASSET platform uses monoclonal antibody-coated LNPs for targeted delivery to specific cell types or subsets [199–201].

Key Examples and Approaches:

- **Cholesterol and Lipid Conjugates:** Cholesterol-functionalized DNA–RNA heteroduplexes have shown promise in crossing the blood-brain barrier [194]. Hydrophobic conjugates have been used for liver delivery, while less hydrophobic conjugates improved delivery to extrahepatic tissues [192,193].
- **LNPs with Specific Antibodies:** LNPs conjugated with antibodies or antibody fragments can be targeted to specific receptors. For example, LNPs decorated with antibodies targeting plasmalemma vesicle-associated protein have been used for lung cell targeting [202].

The Pathway to Clinical RNA Delivery

1. Nanoparticle Discovery Pipeline:

- **Overview:** The discovery pipeline for RNA delivery systems involves several stages of preclinical testing before advancing to clinical trials. This pipeline begins with high-throughput screening of nanoparticles in cell culture, progresses to animal models, and culminates in non-human primate (NHP) studies if initial results are promising [Fig. 5a].
- **High-Throughput Screening:** Initially, thousands of nanoparticles are tested in vitro. Due to the limitations of in vitro models in predicting in vivo outcomes, this stage helps in optimizing nanoparticle traits, but not all nanoparticles will advance to the next stages.
- **In Vivo Testing:**
  - **Mouse Studies:** A smaller subset of nanoparticles is tested in mice to evaluate their in vivo performance. This step often involves testing thousands of nanoparticles, but logistical constraints limit the number of candidates.
  - **Rat and NHP Studies:** Nanoparticles that show promise in mice are then tested in rats and, subsequently, in non-human primates. NHPs are considered the closest model to humans, providing a better prediction of clinical outcomes.

- **Challenges and Improvements:**
    - **Species Variability:** Differences in metabolism, serum lipids, and organ size across species can affect nanoparticle delivery. For example, the liver size relative to body mass differs between mice, rats, and NHPs, which can impact nanoparticle targeting and efficacy [Fig. 5b].
    - **SANDS Approach:** Species-Agnostic Nanoparticle Delivery Screening (SANDS) is a method developed to address these challenges. It involves testing nanoparticles in various models, including mice with humanized livers, to improve predictions of clinical efficacy and safety.

2. Hallmarks of Clinically Relevant Delivery Systems:

- **Scalable Chemistry:** Successful clinical delivery systems are synthesized using scalable, often biodegradable chemistry. For instance, adding ester bonds to lipids can enhance safety and biodegradability [233].
- **Manufacturability:** The delivery system must be chemically simple enough to be manufactured at a large scale, complying with Current Good Manufacturing Practice (CGMP). For example, GalNAc conjugates are manufactured in large batches and conjugated to RNA or ASOs [234].
- **On-Target vs. Off-Target Delivery:** An acceptable ratio of on-target to off-target delivery is crucial. This involves measuring both biodistribution (where the delivery system travels) and functionality (where the payload affects cell function). For effective RNA delivery, the payload must reach its target cell and function correctly within the cell.
- **Dose and Safety:** The therapeutic dose should be much lower than the dose at which toxicity occurs. Non-human primate studies are preferred for assessing RNA toxicity due to their closer physiological resemblance to humans.
- **Consistency and Stability:** The delivery system should maintain consistent activity across batches and be stable during storage and shipping. Techniques such as lyophilization and cryoprotection are used to enhance the stability of mRNA-LNPs [237,238].
- **Re-dosing:** The ability to safely re-dose the RNA drug is important for maintaining therapeutic effects. Successful re-dosing has been demonstrated with siRNA and mRNA therapies, though the optimal dosing intervals need to be determined [19,21].

3. FDA and EMA Approved RNA Therapeutics:

- **GalNAc–siRNA Conjugates:** Drugs like givosiran, lumasiran, and inclisiran utilize GalNAc conjugates to target specific liver receptors. These conjugates have shown efficacy and safety in clinical trials [16,18,17].
- **Other Examples:** Fitusiran and vutrisiran are other GalNAc–siRNA conjugates with positive clinical outcomes. Fitusiran targets antithrombin mRNA to treat hemophilia, while vutrisiran treats hATTR amyloidosis [241,242].
- **Ongoing Trials:** Companies like Arrowhead, Silence Therapeutics, and Dicerna are exploring GalNAc–siRNA conjugates for various diseases, while Ionis Pharmaceuticals is using GalNAc to deliver ASOs [62,243].

The pathway to clinical RNA delivery involves a multi-stage preclinical pipeline, characterized by high-throughput screening, in vivo testing across multiple animal models, and eventual clinical trials. Success in this pipeline requires addressing challenges related to species variability, ensuring scalable and manufacturable delivery systems, and maintaining consistency, safety, and efficacy of the RNA therapeutics [244].

Conclusion

The advancement of RNA-based therapeutics has significantly impacted the treatment landscape for a variety of diseases, ranging from genetic disorders to viral infections. The efficacy of these therapies is highly dependent on overcoming challenges related to the delivery of therapeutic RNA molecules to their target cells. The review highlights the progress made in developing advanced drug delivery systems designed to address these challenges, focusing on both viral and non-viral strategies. Viral vectors have demonstrated substantial clinical success, but face limitations related to immunogenicity, production complexity, and payload capacity. In contrast, non-viral delivery systems, particularly lipid nanoparticles (LNPs) and polymer-based nanoparticles, offer promising alternatives. These systems have been instrumental in the success of several FDA-approved RNA therapeutics, such as mRNA vaccines and siRNA-based drugs. The ability to encapsulate RNA therapeutics within these delivery vehicles has been pivotal in protecting RNA from degradation and enhancing cellular uptake. Targeting strategies have also evolved, with advancements in passive and active targeting approaches improving the precision of RNA delivery. Passive targeting leverages natural interactions between nanoparticles and serum proteins, while active targeting employs specific ligands, antibodies, or aptamers to direct RNA therapeutics to precise cellular targets. These strategies have shown considerable potential in increasing the specificity of RNA therapeutics, thereby reducing off-target effects and improving therapeutic outcomes. Despite these advancements, significant challenges remain, including optimizing delivery efficiency, reducing off-target effects, and addressing the scalability and manufacturability of RNA delivery systems. Ongoing research and development are crucial to overcoming these hurdles and expanding the therapeutic applications of RNA-based technologies. Future efforts should continue to refine delivery systems, enhance targeting precision, and address the remaining limitations to fully leverage the potential of RNA-based therapeutics in clinical practice.

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