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Technical Breakthroughs and Safety Challenges of mRNA Vaccine Delivery Systems: Application and Optimization of Lipid Nanoparticles

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Abstract: This paper reviews the key technologies and research progress in mRNA vaccine delivery systems, with a particular focus on the pivotal role these systems play in enhancing mRNA stability, delivery efficiency, and safety. The effective delivery of mRNA relies on carrier systems, among which lipid nanoparticles (LNPs) have emerged as the most successful platform. LNPs form stable complexes by electrostatically binding ionizable lipids to mRNA, thereby protecting mRNA from degradation and facilitating cellular uptake. Various carriers—such as cationic lipid nanoparticles, solid lipid nanoparticles (SLNs), and non-lamellar lipid nanoparticles—each exhibit distinct characteristics. However, the immunogenicity and toxicity of delivery systems present key challenges for clinical safety. Cationic lipids and polyethylene glycol (PEG) modifications may trigger inflammatory or allergic reactions, necessitating component optimization to reduce these risks. Moreover, the stability and targeting capabilities of delivery systems directly influence vaccine efficacy; PEGylation and auxiliary lipids can extend circulation time and minimize immune clearance. This paper systematically summarizes technological breakthroughs and existing issues in delivery systems, providing theoretical insights for the application of mRNA vaccines in infectious disease prevention and tumor immunotherapy.

Keywords: mRNA vaccines, delivery systems, lipid nanoparticles

1. Introduction

In recent years, mRNA vaccine technology has achieved revolutionary breakthroughs in infectious disease prevention and cancer therapy due to its rapid development cycle and high immunogenicity. For example, the COVID-19 vaccines marked the first large-scale global application of mRNA technology, demonstrating its potential for rapid responses to emerging infectious diseases [1,2]. Nevertheless, the intrinsic vulnerabilities of mRNA molecules—such as their susceptibility to degradation by nucleases, difficulty in penetrating cellular membranes, and excessive recognition by the immune system—pose significant limitations to their practical use [3]. As a result, the development of efficient and safe delivery systems has become a core technical challenge in mRNA vaccine research.

Lipid nanoparticles (LNPs), currently the most advanced delivery platform, form stable complexes with mRNA through electrostatic interactions with ionizable lipids. This approach significantly improves both the stability and delivery efficiency of mRNA [4]. Despite these advancements,

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existing delivery systems still face multiple challenges. These include the cytotoxicity of cationic lipids, allergic reactions induced by PEG modifications, and off-target effects due to insufficient targeting capabilities, all of which may compromise vaccine safety and efficacy [5,7]. Additionally, issues related to the regulation of immunogenicity, optimization of long-term stability, and adaptation to personalized therapies require further investigation.

This paper aims to provide a comprehensive review of the key technological advancements in mRNA vaccine delivery systems. It analyzes the mechanisms of action, advantages, and limitations of various carriers—including lipid nanoparticles, liposomes, and solid lipid nanoparticles—and examines how delivery systems affect mRNA stability, targeting, and safety. To achieve this, the paper integrates multi-dimensional evidence from molecular design, in vivo and in vitro experiments, and clinical research, and explores future directions for optimizing delivery systems in line with the latest technological trends [4,8]. By systematically examining the scientific rationale and application bottlenecks of delivery technologies, this review seeks to offer theoretical support for the further development of mRNA vaccines in infectious disease prevention and tumor immunotherapy.

2. Properties of mRNA

mRNA is a single-stranded ribonucleic acid molecule that carries genetic information encoding specific proteins. Due to its simple structure, mRNA is highly susceptible to degradation by enzymes within the body and is easily recognized and eliminated by the immune system. Furthermore, mRNA is unstable in aqueous solutions, which makes it difficult to efficiently enter cells and perform its function of protein expression [1,3]. Therefore, protective strategies—such as the use of carriers or delivery technologies—are essential to prevent degradation during the delivery process. As a negatively charged molecule, mRNA requires complexation with positively charged lipids to form stable complexes, thereby preventing aggregation or precipitation during delivery [9].

mRNA is capable of encoding a variety of antigens restricted by major histocompatibility complex (MHC) phenotypes, which broadens its potential applications in vaccine development [9]. It can also encode tumor-associated antigens and tumor-specific antigens, along with related cytokines, to induce both humoral and cellular immune responses [10].

At one end of the mRNA molecule is a 5’ cap structure, which facilitates binding to ribosomes. Additionally, mRNA contains an open reading frame (ORF) that encodes proteins; this sequence is recognized by ribosomes, initiating the translation process. As a result, mRNA can be translated directly in the cytoplasm without the need to enter the nucleus. This enables more precise control over antigen expression and reduces the risk of prolonged antigen exposure [10].

3. Mechanism of the Delivery System

The successful application of mRNA vaccines is largely attributed to their unique delivery strategies. First, mRNA is combined with a carrier or delivery technology to form stable complexes, such as lipid nanoparticles or liposomes, which protect the mRNA from degradation and enhance its delivery efficiency. For example, lipid nanoparticles are composed of lipids and mRNA, forming stable spherical structures that effectively protect the mRNA and promote its cellular uptake. These complexes are then administered into the body via intravenous injection, intramuscular injection, or localized injection, allowing them to enter systemic circulation. The complexes interact with the membranes of target cells, either through lipid fusion with the cell membrane or electrostatic interactions. These interactions facilitate the entry of the complexes into cells and the subsequent release of mRNA [9,11]. Once inside the cell, mRNA is translated into proteins that serve as antigens. These antigens are degraded by enzymes within the endosome into peptide fragments, which then bind to major histocompatibility complex (MHC) molecules. Antigen-presenting cells, such as

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dendritic cells, present these antigens to T cells. Dendritic cells internalize antigens through various mechanisms, including phagocytosis and receptor-mediated endocytosis. After antigen uptake, dendritic cells present the antigens to T cells, thereby initiating an immune response [12]. A schematic diagram of mRNA delivery via lipid nanoparticles is shown in Figure 1.

As illustrated by the delivery process, there are three critical elements of the delivery system: the carrier, stability and efficiency, and targeting capability. The carrier is the core of the mRNA vaccine delivery system. It protects the mRNA from degradation, enhances delivery efficiency, and optimizes mRNA translation. Additionally, carriers must ensure the stability and effectiveness of mRNA during delivery to prevent degradation or loss of function. When carriers possess targeting capabilities, they can specifically bind to dendritic cells or other immune cells, thereby increasing delivery efficiency and ensuring that mRNA is delivered to the intended target cells.

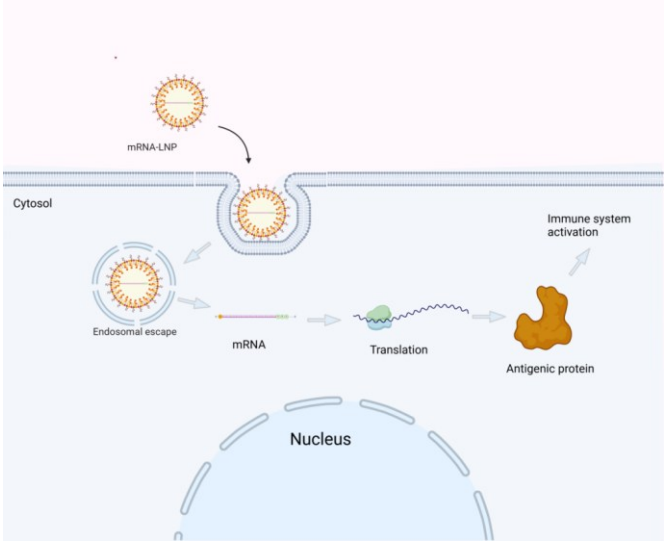

Figure 1: Schematic Diagram of mRNA Delivery Based on Lipid Nanoparticles

4. Common Types of Lipid Nanoparticles, Their Mechanisms of Action, and Advantages and Disadvantages

Liposomes are composed of a bilayer lipid membrane and can encapsulate both hydrophilic and hydrophobic drugs. The surface charge affects the distribution and cellular interactions of liposomes within the body, while their size controls their circulation time and targeting efficiency. Depending on their composition, liposomes can encapsulate various types of drugs and can achieve targeted delivery through ligands that bind to receptors on specific cell surfaces. Additionally, they can release drugs in a controlled manner through external stimuli such as temperature or pH changes. However, if there are strong interactions between the drug and the lipid or if the drug distribution within the liposome is uneven, the encapsulation efficiency may decrease, thus affecting the lipid's loading efficiency and the effective delivery of the drug. Some liposomes may aggregate in the body due to protein adsorption, and external factors such as enzyme degradation, temperature, and pH changes may influence their stability, leading to drug leakage. Due to the complex preparation methods required, large-scale production is difficult [1,12-13].

Cationic Lipid Nanoparticles consist of positively charged lipids and nucleic acids, such as siRNA or mRNA. The positive charge of cationic lipids interacts with the negatively charged nucleic acids, encapsulating them within the particles and facilitating their entry into cells. Based on this mechanism, cationic lipid nanoparticles can effectively deliver nucleic acids to cells and enhance their stability. However, some cationic lipids may be toxic because the positive charge of the lipids interacts with

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the negatively charged phospholipids that make up the cell membrane. The electrostatic attraction between the charges can cause lipid binding to the cell membrane, potentially disrupting its integrity, leading to cell damage or death. Moreover, cationic lipids may be recognized by the immune system as foreign substances, triggering an immune response, particularly with long-term or repeated administration, which may induce inflammation or cytotoxicity [1,5].

Solid Lipid Nanoparticles have higher physical stability compared to liposomes, making them more stable and less prone to aggregation. In the solid state, lipid molecules are restricted in their movement, which allows drug molecules to be more easily captured and embedded into the lipid lattice. The solid lipids form a tight crystalline structure, providing spatial confinement for the drug molecules and further promoting their embedding. Due to the tight packing of solid lipids, solid lipid nanoparticles exhibit higher loading capacity for hydrophobic drugs, but they may also affect the release of some drugs. In addition, the release rate of the drug can be controlled by adjusting the lipid composition. However, long-term storage may lead to drug crystallization, affecting drug release. The lower crystallinity, higher stability, faster drug release rate, and higher encapsulation rate of nanostructured lipid carriers make their bioavailability superior to that of solid lipid nanoparticles, making them a more ideal carrier for hydrophobic drug delivery systems [4].

Non-lamellar Lipid Nanoparticles are composed of non-lamellar lipid phases, such as cubic or hexagonal phases. Compared to liposomes, non-lamellar lipid nanoparticles have a larger surface area, making them suitable for higher drug load applications such as drug delivery and biosensing. However, their preparation methods are more complex [8]. Table 1 shows the advantages and disadvantages of common types of lipid nanoparticles.

Table 1: Advantages and Disadvantages of Common Types of Lipid Nanoparticles

Type	Advantages	Disadvantages
Liposomes [1,12-13]	- Can encapsulate both hydrophilic and hydrophobic drugs - Surface charge is adjustable, affecting distribution in the body - Specific delivery can be achieved through targeting ligands - Supports external stimuli to trigger drug release	- Interaction between drugs and lipids may lead to low encapsulation efficiency - Prone to aggregation in vivo due to protein adsorption, stability affected by environmental factors (enzymes, temperature, pH) - Complex preparation process, difficult to produce on a large scale
Cationic Lipid Nanoparticles [1,5,14]	- Highly efficient in delivering nucleic acids - Positive charge promotes cellular uptake and enhances stability	- Cationic lipids may disrupt the cell membrane, causing cytotoxicity - Long-term or repeated administration can induce immune responses and inflammation - Metabolic products may be toxic
Solid Lipid Nanoparticles [4]	- High physical stability, not prone to aggregation - Suitable for hydrophobic drugs, stronger drug loading capacity - Lipid lattice structure controls drug release rate	- Long-term storage may lead to drug crystallization, affecting release efficiency - Slower drug release rate (compared to NLCs) - Lower loading capacity for hydrophilic drugs
Non-lamellar Lipid Nanoparticles [8]	- Large surface area, high drug loading (e.g., cubic or hexagonal phases) - Suitable for macromolecule drug delivery and biosensing	- Complex preparation methods, higher cost - Stability affected by lipid phase structure, requires precise process control
Nanostructured Lipid Carriers [4]	- Low crystallinity, better stability than solid lipid nanoparticles - Faster drug release rate, higher bioavailability - Significant improvement in encapsulation efficiency and drug loading	- Requires optimization of lipid mixture ratio to balance drug loading and release - Long-term storage may cause lipid phase separation

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5. The Impact of Delivery Systems on the Safety of mRNA Vaccines

The safety of mRNA vaccines is one of the most important considerations in their clinical application. Although mRNA itself is a non-infectious and non-integrating platform, due to its unique delivery method and interaction with the immune system, there are still some potential safety risks. To date, although large-scale clinical trials of mRNA vaccines (such as the COVID-19 vaccines) have been conducted globally, no direct evidence has been found linking mRNA vaccine administration to thrombocytopenia syndrome (including severe cases and deaths). The main adverse reactions include pain at the injection site, fever, and fatigue. In related documents, both the World Health Organization and the U.S. Food and Drug Administration emphasize the importance of safety studies for different viral strains, particularly in the context of different populations and vaccine regimens.

5.1. Immunogenicity of Delivery Systems

mRNA inherently has high immunogenicity. The choice of delivery system can influence the immunogenicity of mRNA vaccines. Literature [15] mentions that delivery systems such as lipid nanoparticles and lipid complexes can enhance the stability and immunogenicity of mRNA. For example, lipid nanoparticles, through their unique structure of ionizable lipids, cholesterol, and phospholipids, can stably encapsulate mRNA and promote its release within cells, thereby enhancing the immune response. However, the delivery system itself may also trigger some immune responses, especially with repeated administration, potentially generating antibodies against the delivery system, which could affect the vaccine's efficacy and safety. In response to the immunogenicity of delivery systems, researchers are developing novel lipid components to reduce the immunogenicity of the delivery system. For instance, biodegradable lipids or adjustments to the lipid's charge distribution are being used to reduce immune system stimulation [6]. At the same time, literature [6] indicates that polyethylene glycol (PEG) modification can reduce the interaction between the delivery system and the immune system, thus prolonging its circulation time in the body. However, PEG may also trigger allergic reactions, so researchers are working on developing new PEG alternatives or reducing the amount of PEG used.

5.2. Toxicity of Delivery Systems

The toxicity of mRNA vaccines may stem from three sources, one of which is the delivery vectors currently used, such as cationic lipids and polymer nanoparticles. Repeated administration may lead to the accumulation of lipid metabolic products, potentially causing toxicity [16-17]. Cationic lipids are key components in lipid nanoparticles, used to bind to negatively charged mRNA and facilitate its delivery. Cationic lipids can trigger cytotoxicity, leading to cell apoptosis, cell membrane damage, and inflammatory responses [18]. For example, some cationic lipids (such as DOTMA, DOTAP) carry a positive charge and can interact with negatively charged cell membranes, disrupting membrane integrity and causing cell death [14]. The toxicity of nanoparticles is closely related to their size, shape, and surface modifications. For instance, gold nanoparticles penetrate cell membranes through physical interactions, accumulating in organelles like mitochondria and the endoplasmic reticulum, interfering with their normal function, and causing cytotoxicity, leading to cell apoptosis or necrosis [19]; silica nanoparticles have been shown to induce an increase in intracellular ROS levels, leading to cell apoptosis [20].

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6. The Impact of Delivery Systems on the Stability and Delivery Efficiency of mRNA Vaccines

The stability of mRNA vaccines is one of the key factors for their widespread clinical application. Unmodified naked mRNA is easily degraded in the body, significantly reducing its immunogenic efficacy. Therefore, scientists have modified the mRNA or used technologies such as lipid nanoparticles to improve its stability and prolong its half-life in the body. Lipid nanoparticles form a core-shell structure by encapsulating mRNA, preventing it from being degraded by nucleases in the bloodstream, thus affecting the stability and delivery efficiency of mRNA vaccines. For example, cationic lipids form stable complexes with negatively charged mRNA through electrostatic interactions, significantly enhancing the mRNA's resistance to enzymatic degradation. Adjuvants are crucial in enhancing the mRNA-LNP delivery efficiency. PEGylated lipids form a hydrophilic layer on the surface of lipid nanoparticles, reducing serum protein adsorption and the clearance by the mononuclear phagocyte system, thus prolonging their half-life in the bloodstream [1]. Studies have shown that the length and density of PEG chains directly affect the stability and immune evasion capability of lipid nanoparticles [7]. Longer PEG chains provide better structural protection, preventing nanoparticle degradation and thereby improving stability. However, excessively long or high-density PEG chains may interfere with the immune recognition of nanoparticles, leading the immune system to misinterpret them as viral particles, thereby triggering a stronger immune response [21]. Helper lipids, such as cholesterol, improve the physical stability of lipid nanoparticles by enhancing the tightness of the lipid bilayer structure. Cholesterol also prevents particle aggregation by modulating membrane fluidity [1,5]. Choosing the appropriate adjuvant is crucial for optimizing mRNA-LNP drug delivery systems. Adjuvants can enhance the stability and transfection efficiency of LNPs, reduce immunogenicity, and improve targeting, thereby improving therapeutic outcomes.

7. Conclusion and Outlook

With the rapid advancement of mRNA vaccine technology in recent years, especially the breakthroughs in its application during the COVID-19 pandemic, its prospects in fields such as infectious disease prevention, cancer immunotherapy, and gene therapy are vast. Clinical trials in multiple fields have already yielded preliminary results, and it is expected to play a significant role in these areas in the future. Moving forward, the research on mRNA vaccines will primarily focus on the following areas: First, optimizing the delivery system, particularly the design of lipid nanoparticles (LNPs), to improve mRNA stability and targeting while reducing side effects. Second, enhancing the translation efficiency and immunogenicity of mRNA through chemical modifications and codon optimization, while reducing the risks of immune reactions. Additionally, the development of personalized mRNA vaccines will become an important direction [22], especially in cancer treatment, by identifying tumor-specific antigens and providing customized treatment plans for patients. However, the mass production and clinical application of personalized vaccines still face technical and regulatory challenges. Finally, as mRNA vaccines are applied to more diseases, their long-term safety and efficacy still need further validation. Overall, the continuous innovation in mRNA vaccine technology will bring more groundbreaking progress to human health.

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