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Recent Innovations in Lipid Nanoparticle Formulations for mRNA Vaccine Delivery Against Emerging Infectious Diseases

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ABSTRACT

Messenger RNA (mRNA) vaccines have demonstrated remarkable efficacy during the COVID-19 pandemic, largely owing to advances in lipid nanoparticle (LNP) delivery systems. This review explores the recent innovations in LNP formulation, including novel lipid components, ionizable lipid structures, and stability enhancement strategies, aimed at improving mRNA delivery efficiency, tissue targeting, and immune response modulation. We also discuss the challenges of scale-up manufacturing, storage stability, and regulatory pathways for LNP-mRNA vaccines addressing infectious diseases beyond COVID-19, such as influenza, Zika, and respiratory syncytial virus (RSV).

Keywords: Drug Delivery, Emerging Pathogens, Infectious Diseases, Lipid Nanoparticles, LNP Formulation, mRNA Vaccines, Vaccine Development

1 Introduction

IN response to the COVID-19 pandemic, there has been an increased interest in the development of lipid nanoparticle (LNP)-formulated RNA-based vaccines for protection against (re)emerging infectious diseases. Messenger RNA (mRNA) vaccines are promising alternatives to conventional vaccine technologies. LNPs, consisting of ionizable lipids, phospholipids, cholesterol and poly (ethylene glycol)-lipids, have been extensively studied as non-viral vectors for the delivery of mRNA to target cells [1]. Recently, due to the success and widespread use of LNP-formulated mRNA COVID-19 vaccines, a growing number of research groups and pharmaceutical companies have focused on developing LNPs and lipid formulations for the delivery of RNA therapeutics, such as mRNA or small interfering RNA (siRNA), to treat a broad range of diseases, including (re)emerging infectious diseases and cancers [2-4].

Protective immunity against infectious diseases often requires an appropriate vaccine platform that can elicit both humoral and T cell immune responses. For this purpose, new vaccine technologies based on viral vectors and nucleic acids, such as DNA and mRNA, have been developed in recent years [5]. DNA vaccines have entered early clinical trials but have been shown to produce weak immunogenicity. During their delivery, the DNA vaccines must cross the nuclear membrane, become transcribed to mRNA, and then translated into proteins [6, 7]. As a result, DNA vaccines are often combined with adjuvants to

enhance immunogenicity, which enhances local inflammation, posing a risk of autoimmune diseases [8]. mRNA vaccines are capable of direct translation of antigens and can be delivered for antigen expression without any requirement to cross the membrane barrier of the nucleus [9]. The source of mRNA vaccines could be either synthetic mRNA or in vitro transcribed (IVT) mRNA, which can be generated quickly and economically [10]. The efficient delivery of vaccines to target cells is the first critical step in order to induce immunogenicity. As naked liposomal mRNA is rapidly degraded, and even LNPs-mRNA can be taken up by antigen-presenting cells (APCs), forming small particles, LNPs-mRNA must be designed specifically to translate the encoded antigen effectively in vivo [11]. Different mRNAs, LNPs, and co-delivery approaches have been rationally designed to minimize the risk of immune-related adverse effects. Engineering of lipids to alter their charge and structure could optimize LNPs-mRNA in terms of in vivo delivery efficiency [3]. mRNA sequences could modulate the translation kinetics of coded proteins, further enhancing immune responses, and modification of LNPs and mRNA integrity could improve organ distribution and stability [12].

2 Overview of mRNA Vaccines

Messenger RNA (mRNA) carries the directions for the synthesis of proteins needed by the cell to maintain homeostasis. mRNA is translated into proteins via a series of steps involving transcription and translation; however, like all nucleic acids, it is highly susceptible to degradation by RNases in the environment [13]. Thus, the discovery of mRNA did not lead to tangible therapeutic benefits until recent advancements addressed these challenges. The development of bioinspired nanomaterials, by which proteins, peptides, nucleic acids, and even whole cells can be delivered to target sites, has revolutionized RNA therapy [1]. However, the unusual physicochemical properties of RNA introduce additional challenges for systemic delivery [14] . Since the discovery of interferon in virally infected cells, mRNA for vaccines has remained a focal point in virology and immunology research [5]. Notably, with recent advancements in RNA production and purification technologies, even inflammatory mRNA can now be produced at gram-scale purity for clinical applications [15]. As the second messenger in protein biosynthesis, mRNA's potential for vaccines was historically overlooked. Yet, mRNA offers distinct advantages over traditional vaccines—such as targeted delivery to professional antigen-presenting cells, the ability to induce both cellular and humoral immunity, and the absence of infectious risks linked with viral-vector-based platforms [16].

The diversity of platform technologies—including cross-linked polymer systems, lipid-based vesicular systems, proteins, polymers, and inorganic nanomaterials—has been meticulously tailored to formulate self-amplifying mRNA vaccines [17]. Lipid nanoparticles (LNPs), mainly composed of ionizable lipids, phospholipids, sterols, and polyethylene glycol-conjugated lipids, remain the most widely employed carriers for nucleic acid delivery [1, 12]. As early as 1990, solid lipid nanoparticles were fabricated using surfactants via high-shear homogenization, a nanoemulsion fabrication strategy [18]. mRNA delivery via LNPs has been widely explored with a focus on lipid composition and formulation protocol optimization [9, 19].

3 Lipid Nanoparticles: Structure and Function

Various materials have been developed to deliver messenger RNA (mRNA), the most important of which are lipids, lipid-like substances, polymers, and protein derivatives. The goal of developing these materials was to overcome the challenges of mRNA delivery, leveraging their ability to enhance cellular uptake of mRNA. Therefore, lipid nanoparticles have gained prominence and have been the subject of extensive studies to deliver small molecules and achieve more stable structures and properties, including small interfering RNA drugs and mRNA. COVID-19 vaccines have received emergency use authorization, bringing lipid nanoparticles into the spotlight. Due to the urgent need during the critical phase, LNP-mRNA vaccines have been developed. However, significant challenges remain, including improving the stability and properties of LNP-mRNA formulations [1]. Lipid nanoparticles are considered good delivery vehicles due to their ease of preparation, ability to condense large nucleic acids, and biocompatibility. Lipid nanoparticles are composed of lipids that form a self-assembling structure, including Knob-1 and Knob-2 lipids, cholesterol, and LA-5. A positively charged ion adsorbs mRNA to the LNPs, while a neutral lipid helps stabilize the mRNA-loaded lipid nanoparticles. The molecular weight of the LNPs can affect the efficiency and stability of mRNA encapsulation, which impacts vaccine efficacy [20]. Furthermore, mRNA-1273 and BNT162b2 are the two approved COVID-19 vaccines that utilize lipid nanoparticle technology to deliver antigen-specific mRNA. Various types of lipid nanoparticle formulations with mRNA have been developed [3]. The recent emergence of the COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has intensified the need for highly effective preventative medicines. Therefore, a brief overview is provided of lipid nanoparticles (LNPs) used in conjunction with mRNA as an advanced delivery system. In vitro, transcription of mRNA can be formulated with lipid nanoparticles (LNPs) for delivery to tissues or cells expressing the encoded proteins. Different LNP formulations containing the same mRNA exhibit significant differences in their in vitro and in vivo efficacy, including biodistribution, safety, and immunogenicity [21].

3.1 Composition of Lipid Nanoparticles

Nucleotides in RNA molecules comprise ribose-3-phosphate and ribonucleosides, allowing for free hydroxyl (OH) groups, making them intrinsically unstable. mRNA is prone to degradation by nucleotide bases, phosphatases, or by thermal or oxidative depurination. mRNA needs to be protected against ribonucleases [13]. The enzymatic step is prevented through the encapsulation of RNA inside a lipid layer, which forms protective lipid nanoparticles (LNPs). Lipid nanoparticles composed of ionizable lipids, pegylated lipids, cholesterol, and phospholipids are most widely used to formulate RNA vaccines [1]. LNPs used in the design of RNA-based vaccines require a specific type and proportion of lipid components in addition to specialized manufacturing procedures. Ionizable positive lipids are formed from at least two types of components, including the cationic head group and hydrophobic tails consisting of long carbon-chain alkyl and unsaturated carbon chains. There are 28% to 39% phospholipids in vaccines. The most important phospholipid component for assisting self-assembly structuring and minimizing toxicity is DSPC. Otherwise, cholesterol with high hydrophobicity must be present not only to promote self-assembly but also to protect RNA, thereby improving the stability and half-life of the vaccine [22]. Other lipid components include polyethylene glycol (PEG)-lipids, especially PEG 2000, which prolongs the circulation half-life, reduces liver uptake, and decreases blood clearance of LNPs while enhancing their uptake by target cells and tissues [23].

Recent studies have estimated that, for each mol%, approximately 12 to 25 LNPs or lipids are needed to formulate one mRNA based on mathematical modeling. A significant amount of effort has been focused on non-structural proteins of specific coronavirus gene regions. By encapsulating RNA through electrostatic interactions, LNPs facilitate cellular uptake via endocytosis. The instability of LNPs leads to the endosomal escape of RNA through fusogenic activity, thereby preventing RNA degradation [3, 9].

3.2 Mechanism of Action

mRNA is encapsulated into LNPs by mixing lipids and mRNA, often in saline buffers, using microfluidic rapid-mixing systems. LNPs with monovalent mRNA encoding a single antigen protein can be used as vaccines, while LNPs with multivalent mRNAs encoding several antigen variants are promising against rapidly mutating viruses [24]. LNPs-mRNA vaccines against SARS-CoV-2 have shown strong efficacy and safety in clinical use. LNPs deliver mRNA into cells through endocytosis, followed by mRNA release within endosomes by mechanisms not yet fully understood. The released mRNA is then translated into proteins by ribosomes, inducing immune responses [1]. Second-generation LNPs-mRNA vaccines are being developed to improve upon the first generation by including mRNAs encoding multiple protein variants and incorporating optimized 5′ and 3′ untranslated regions (UTRs) to enhance stability and translation efficiency [10].

3.3 Emerging Infectious Diseases: A Global Perspective

Emerging infectious diseases such as those caused by coronaviruses, influenza A viruses, and filoviruses represent a global health threat. The COVID-19 pandemic caused by SARS-CoV-2 highlighted the urgent need for pandemic preparedness and rapid vaccine development [5]. The emergence of variants with increased transmissibility, immune escape, and resistance to therapeutics poses ongoing challenges [25]. Prior to COVID-19, influenza pandemics and filovirus outbreaks underscored the importance of swift vaccine and therapeutic development [3].

Chemical modification of mRNA and the lipid composition of LNPs are a promising avenue for improving mRNA vaccine efficacy. For example, incorporation of 5-methylcytidine (5mC) nucleosides into mRNA suppresses pro-inflammatory responses [13]. Optimizing the mRNA cap structure and untranslated regions further enhances translation efficiency and immunogenicity. The LNP composition is critical for self-assembly, in vivo distribution, and immune activation [12].

mRNA vaccines offer several advantages over conventional vaccines: rapid production once a pathogen sequence is known, induction of both neutralizing antibodies and cellular immunity, and no need for live virus, which improves safety and manufacturing ease. Examples include the BNT162b2 and mRNA-1273 COVID-19 vaccines, as well as vaccines under development for Marburg virus (MARV) [10, 26].

3.4 Impact of Infectious Diseases on Public Health

Infectious diseases continue to cause significant morbidity and mortality globally, with the ongoing emergence of new pathogens [27]. Vaccine strategies for diseases like Ebola, Zika, and SARS-CoV-2 face challenges such as slow production, limited durability of immunity, and risks associated with live attenuated vaccines. There is strong interest in platforms like mRNA vaccines, which can be rapidly produced, adapted to various viruses, induce durable immunity, and possess excellent safety profiles [28]. Following vaccination, mRNA is translated into protein mainly in the cytosol. White blood cells shuttle between blood and tissues to provide immune surveillance. Monocytes, for example, can process antigen and present it to CD8+ T cells [1]. LNPs are formulated via microfluidic mixing to achieve particle sizes around 100–200 nm, optimizing delivery [29].

Recent studies engineered maltose-sucrose functional

groups into lipids to improve delivery efficiency. Intranasal delivery of LNPs-mRNA vaccines encoding SARS-CoV-2 spike protein induced both systemic IgG and mucosal IgA, activating T follicular helper cells and germinal center responses in mice. These vaccines elicited broad neutralizing antibodies effective against Beta and Omicron variants, demonstrating potential against emerging virus threats [30].

3.5 Recent Outbreaks and Challenges

The global outbreak of COVID-19 has led to unprecedented efforts to streamline the processes for vaccine development and rollout in a record time of one year [31]. Nevertheless, despite effective vaccines becoming available quickly, the pandemic is far from over, given the continual emergence of novel variants [32]. This emergence is unsurprising, given the selective pressures imposed by widespread vaccination and the lower prevalence of natural infection [33]. The resulting vaccine-resistant strains raise alarming questions related to the durability of vaccine-induced immunity against breakthrough infections, as well as the correlation between the breadth and robustness of such immunity [34]. More broadly, the emergence of new variants of global concern highlights the challenges facing multiple frontiers of vaccines that bypass existing immunity [25].

Thus, the lessons learned from the challenges posed by the COVID-19 pandemic have raised the priority of preparedness against emerging infectious diseases (EIDs) [35]. The development of new effective tests, treatments, and vaccines should be accelerated and made more affordable and accessible in many countries around the world. While considerable progress has already been made toward pandemic preparedness, there remain many challenges [36].

The emergence of new EIDs is gradual, with their associated risks most likely ebbing and flowing over years. New viruses that jump hosts to the human population emerge at a frequency of 1–2 per year, but many disappear quickly without clinical detection or epidemiological impact [37]. Some of these viruses, however, display several features that may predispose them to emerge as dangerous pathogens, and prioritization is required for surveillance [38]. While genomic sequencing and bioinformatics approaches for infectious disease surveillance have advanced significantly over the past 20 years, substantial increases in human and environmental sampling capacity are still necessary to enhance sequencing efforts and surveillance coverage [39].

Memory responses induced by non-zero-sum pan-variant vaccines can provide cross-protective immunity against diverse EID-causing viruses [40]. However, despite major advances in vaccine platforms, including highly optimized RNA vaccines, general solutions are still urgently needed against EIDs that infect human cells through tropisms and receptors very different from those of known human viruses [41].

4 Advancements in Lipid Nanoparticle Technology

Recent advances in mRNA delivery systems and drug delivery have shown great potential for therapeutic and biomedical applications, including protein replacement therapies, vaccines, and cell reprogramming. To achieve maximum therapeutic benefits, mRNA molecules must be protected from degradation while being delivered to selected target cells to produce the desired proteins. However, some key challenges facing mRNA delivery systems remain, including targetability and stability [42]. Developments of lipid nanoparticles (LNPs) designed for drug or vaccine delivery systems have led to significant improvements in safety and stability. Lipid nanoparticles have been extensively studied for the delivery of nucleic acids due to their biocompatibility, biodegradability, and ability for selective chemical modifications [43]. Recent mRNA-based COVID-19 vaccines encapsulated in lipid nanoparticles (LNPs) have demonstrated exceptional clinical efficacy. Following these promising results and rapid development, several novel LNP formulations and processes have been studied to improve particle quality and versatility [42].

Following these successes, several new LNP formulations and processes have been developed to enhance particle quality and versatility [44]. These particles are formed spontaneously when a cationic, ionizable lipid, co-phospholipid, cholesterol, and polyethylene glycol lipid are mixed with an aqueous phase at a specific ratio, such as in a microfluidic mixer. The LNP- mRNA formulations are cleaned, and residual ethanol and lipid molecules are removed using membrane filtration methods [20, 45].

LNPs have been introduced into living cells using EPR, and lymphoma-targeted LNPs have been used. LNPs bioconjugated to human IgG have been used to bind to the nucleus of dendritic cell membranes internally. Otherwise, LNPs alone are insufficient for potential vaccine candidates, and several issues must be addressed. [46]. First, the SPF conditions required for animals are more stringent, which significantly increases the cost of animal studies. Second, some studies question whether LNPs mediate mRNA uptake into cells and subsequent protein translation. LNP-mRNA uptake is inhibited by disrupting dynamin or actin polymerization [1, 47]. Third, some LNPs, such as C12-200, have shown significantly higher cytokine production efficacy compared to dendritic cells (DCs) in the dermis [48]. Several methods exist for assessing mRNA and confirming the presence of protective cytokines in organs, such as flow cytometry using trichrome nanoparticles; however, the process is much more complex. Fourth, other RNA formulations, such as nitrogen-doped carbon quantum dots with mRNA, are an effective option [49].

4.1 Nanoparticle Design and Engineering

Lipid nanoparticles (LNPs) are spherical nanoparticles with a diameter of 30–100 nm. The LNPs build a lipid monolayer for anchoring genetic drugs, such as mRNA and DNA, into lipid-encapsulated nanoparticles. In addition to structural lipids (lipids forming the lipid bilayer), lipids in LNPs for mRNA delivery feature a poly (ethylene glycol) (PEG)-lipid with a PEG moiety, along with ionizable lipids, which are positively charged at low pH but rapidly become neutral at physiological pH. As mRNA delivery vehicles, LNPs intercalate with the lipid membranes of endosomes through a membrane fusion mechanism and cause mRNA release. Currently, most available genetic drugs are mRNA and DNA therapeutics with applications spanning across in vivo and in vitro gene expression to vaccines [50].

Incorporation of LNP formulations into liposome fabrication is based on micelle methods utilizing a strong pH gradient, a microfluidic mixing process, and the ethanol dialysis method for batch production of LNPs with rigorous control over their physico-chemical properties [51]. The LNPs in an ethanol aqueous phase transition to a single-membrane structure and can be further optimized with regard to their swelling, stability, and RNA condensation using ethanol concentration and micellar composition [52]. Subsequently, several new systems for loading RNA have been fabricated in different fabrication modes with inherent advantages in a benchtop size [16]. Over the past two decades, various strategies have been designed to improve mRNA vaccine performance, notably the discovery of ionizable lipids, which have been instrumental in the development of mRNA delivery systems that have shown therapeutic value in preclinical studies and human clinical trials [53].

4.2 Scalability and Production Techniques

Two major challenges in the production of lipids for LNPs-mRNA vaccines are scalability and advancement of analytical technologies with the desired capacity, resolution, and throughput [54]. Current technologies dominate for de novo lipid synthesis (>100 g scale per batch), and slower reaction chemistries such as hydrolysis are also available but lack scalability [55]. An overview of lipids produced in significant quantities for LNPs-mRNA recently reported on their excellent safety and efficacy profiles, solidifying tapered lipids as prime candidates for infectious disease treatment [56]. By using a series of mPEG-lipid co-monomers, it is possible to produce polymer-lipid brushes with surface fusions targeted to specific organs. However, due to their inherent complexity, understanding where these lipids go in vivo requires newer, more powerful lipid analytics that can disentangle the composition, sizes, and conformations of heterogeneous populations [57]. Commercialization efforts by pharma-industry collaborations require a strong scientific basis to form a successful strategy for supply chains. These include technology choices such as lipid selections and their physicochemical properties, formulation approaches, and biopharmaceutical strategies to maximize the stability of vaccines [58].

The Restore technological platform includes all of the technology components required for the design and manufacture of products such as LNP-encapsulated mRNA vaccines and siRNA therapeutics [59]. Multiple COVID-19 vaccines worldwide use this platform. The platform was developed to be modular and adaptable; biopharmaceutical companies already using some parts of the platform could readily integrate others into in-house operations [60]. To demonstrate scalability, each of the production steps was scaled to at least one-tenth of the batch size proposed for production at the mega facility. Safety testing on the production of these larger batch sizes and stability testing of the larger vial and formulation batches are ongoing. Product characterization of mRNA-free vaccine lots will also be critical. Analytical testing throughput will need to be increased, and some equipment and all processes will need to be validated and transferred to new facilities during tech transfer [61].

5 Enhancing mRNA Delivery Efficiency

Sphingomyelin (SM) is a major lipid component of mammalian cell membranes and the myelin sheaths of the nervous system. SM has been used in lipid nanoparticle (LNP) systems since 2013, when it was initially introduced with cholesterol and cationic lipids for the encapsulation of mRNA. The physicochemical properties of SM have been studied regarding the degree of unsaturation, acyl chain length, and amine presence or terminal groups on cationic lipids. LNPs assembled from pure SM demonstrated altered size, zeta potential, and encapsulation efficiency. These effects either plateaued or diminished when 25 mol% cholesterol was added to the formulation [62].

Lipid volume (or hydrophobicity) defined by the cross-sectional area, number of carbon atoms, and degree of unsaturation has been shown to significantly influence lipid behavior within bilayers. A study examining 37 lipids with varying hydrophobicity revealed that, in addition to particle size and zeta potential, lipid density affects the intracellular localization of LNPs. Highly hydrophobic lipids were found to enhance endosomal escape but also increased cellular toxicity due to biodistribution and organ accumulation [63].

5.1 Targeting Mechanisms

An essential component for the successful application of mRNA—whether in vaccines or nanomaterials (NMs)—is the interaction motif between mRNA molecules and their delivery systems. The two primary mechanisms by which mRNA is introduced into cells are passive uptake and endocytosis [1]. Unlike viral vectors, synthetic mammalian

delivery vehicles primarily rely on passive uptake mechanisms, including diffusion-based entry pathways [5].

Passive uptake is typically associated with the diffusion of large nanomaterials, where only small particles with diameters ≤10 μm are suitable for intravenous or pulmonary delivery. Airborne mRNA delivery requires a finely tuned architecture capable of creating localized nanostructures from minimal peptide polymer starting materials. Beyond lipid-based carriers, new types of molecular complexes (MCs) based on amino acids or carbohydrates have emerged for the delivery of proteins and antimicrobial RNAs [4]. All synthetic carriers must avoid free reactive groups that might degrade RNA or interfere with cationic interactions required for complex formation. Furthermore, the design and screening of nano-delivery systems are still constrained by their cell-penetration efficiency and cytotoxicity [64].

5.2 *Stability and Release Profiles*

The stability of LNPs-mRNA vaccines remains a central concern in ensuring long-term storage, efficacy, and manufacturability. Adjuvants have been shown to strongly influence the immunogenicity of mRNA vaccines, particularly in the early phases of development [3]. While current clinical formulations—such as those used in COVID-19 vaccines—are now commercially available, significant challenges remain related to RNA integrity, lipid nanoparticle (LNP) composition, and formulation environment [5].

LNPs-mRNA systems are inherently complex due to the physicochemical characteristics of RNA and the dynamic structure of lipid components. Factors such as ionizable lipids, cholesterol content, helper lipids (e.g., SOPC), pH, ionic strength, concentration, lyophilization processes, and substrate interactions all play vital roles in stability and delivery [1].

For example, A18-Iso5p5-Phytanoyl, used in the BNT162b2 (Pfizer-BioNTech) formulation, showed optimal performance in various animal models and humans due to its homogeneous DOTAP-coated structure and size distribution. More recently, novel cationic lipids such as 5c14H and 5c14PH, synthesized from 1,2-amino alcohols, have demonstrated superior intracellular delivery and immune response compared to conventional LNPs. These newer formulations also showed improved thermal stability, with 5c14PH-BAC LNPs remaining stable at 37°C for over two weeks, highlighting their potential in next-generation affordable vaccine platforms targeting viruses like Zika and COVID-19 variants [65].

6 Case Studies: Successful Applications

The COVID-19 pandemic has highlighted the need for a fast, flexible vaccine platform that can keep up with emerging infectious diseases. To help meet this urgent need, a LNP-formulated mRNA platform has been developed over the last 10 years. Several safety studies were designed and conducted to evaluate the platform in mice and non-human primates, including 28-day repeat dose toxicology studies for a specific mRNA. Safety studies showed that LNP-formulated mRNA was well tolerated, with an acceptable safety profile [5, 66]. Further, the use of a proven LNP formulation, dosing, route, and schedule greatly expedited development, allowing initiation of testing in human subjects shortly after sequence identification [67].

A common exception to large vaccine programs is the "Subunit Vaccine" approach. These efforts in pathogen proteome or exoproteome characterization, muco-selective nanotool development and delivery-positive and negative screening approaches are immediately available to clinical and pre-clinical paths within a few months [68]. This advantage gives mRNA potentially commercial upside, particularly for recent emergent pathogens of zoonotic origin. Continued R&D and novel downstream vaccine formulations are a priority, where mRNA-based approaches on continued novel continuous emulsifier or voltage inlet mix design processes are promising [1]. To mitigate mRNA wastage post-harvest, a testing and waste management service for sample high-throughput screening lanes is urgently needed to steer mRNA reagents towards their best-priced future applications [3]. The future utility of mRNA vaccines looks bright with more companies entering the race. However, further prioritization of its use in countries with readily actionable infrastructure and islands with well-established systems is needed. New applications of hybrid and lipid-conjugated mRNA vaccines using oligonucleotide precursors of protein engineering could create a new generation of vaccines that trigger better T-cell pathways, hoping to also vaccinate responders producing antibodies with neutralization properties against zoonotic viruses. An initiative steering R&D and vaccine production of these challenges is urgently warranted. The possible successful applications of mRNA vaccines against highly contagious, lethal or economically problematic diseases underscore their importance [69].

6.1 *COVID-19 mRNA Vaccines*

The ongoing COVID-19 pandemic has posed an urgent worldwide health threat and unprecedented challenges to humankind. At the end of 2019, clusters of pneumonia cases of an unknown etiology primarily among patients who had visited the seafood market in Wuhan, China, were reported. In January 2020, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified as the causative agent, and its genome sequence was made publicly available on January 11, 2020 [70]. Since then, SARS-CoV-2 has spread globally and caused over 100 million confirmed cases and more than 2 million deaths as of January 25, 2021 [71].

Vaccines are the most effective approach to control and eradicate epidemics. Fortunately, there are safe and effective vaccine candidates to combat COVID-19, and mass vaccination campaigns are being conducted globally. This success is attributed to the genetic science of viruses, fundamental immunological knowledge, and advanced vaccine platforms [31]. Since the severity of the ongoing COVID-19 pandemic was recognized, over 200 vaccine candidates have been developed, most of which are based on conventional platforms comprising live-attenuated or inactivated viruses, recombinant proteins, and viral vectors [72].

Since the outbreak of the Zika virus, mRNA vaccines have come onto the public stage. Compared with conventional vaccine platforms, lipid nanoparticle (LNP)-mRNA formulations have outstanding features such as rapid design and manufacturing, high safety, versatility, and competitiveness that facilitate their rapid development and clinical assessment against emerging infectious pathogens after the design of mRNA antigens [73]. Shortly after the SARS-CoV-2 sequence was released, two clinical-grade LNP-mRNA vaccines against COVID-19, mRNA-1273 and BNT162b2, were developed and authorized for emergency use. LNP-mRNA formulations are currently one of the most advanced therapeutic platforms and vaccines, which warrant the careful investigation of their biophysical properties and formulation features prior to preclinical and clinical use [74].

6.2 Other Notable mRNA Vaccine Developments

In early 2022, PC7A-cholesterol LNPs were reported for the delivery of an mRNA vaccine encoding the D614G spike of SARS-CoV-2. Clinical trials revealed that the sIgG titre was boosted through the administration of a booster dose, while only a minimal titre was elicited by a single dose [75]. Later, a B.1.351 mRNA vaccination study using a similar LNP system was launched [76]. A comprehensive library of nano-sized lipid formulations was patented for nucleotide delivery, utilizing various cationic lipids based on alkylene-β-amino esters and N, N-dialkyl-4-oxopentanoyl amines. These formulations enabled potent induction of systemic immunity in mice after intramuscular injection, with C12-200/DOPE/cholesterol formulations producing superior responses [77]. They also established a conserved sequence in the mRNA, including a long hairpin structure and a poly(A) tail, to facilitate liposome encapsulation. The addition of a 10% molar excess of cationic lipids proved effective in treating hereditary ATTR amyloidosis (hATTR) [78].

Lipid-like nanoparticles are emerging colloidal carriers of mRNA vaccines against COVID-19. C12-200-like lipids, co-encapsulated with chitin and mRNA, stimulated both humoral and cellular immunity, offering surrogate neutralizing antibodies and protective efficacy [79]. A programmable design of lipid nanoparticles with ALK9 inhibitors enabled sequential mRNA delivery to treat hematologic malignancies [80]. Additionally, RGD-C166, a novel lipid vehicle, improved tumor targeting for mRNA delivery and suppressed disseminated cancer metastases. Bio-inert lipids bonded to peptides or antibodies allowed safe mRNA delivery in clinical treatments of cancer. Currently, most clinical ruptured LNP systems use cationic lipids with pKa > 6.5 or cationic ratios ≥1:2, supporting G7-encouraged immune mechanisms but raising safety concerns for in vivo translation [81].

7 Regulatory Considerations

The rapid development of LNPs-mRNA vaccines to counter the pandemic poses challenges to national and international regulators unlike any faced in peacetime. A key theme explored is the essential detail of the most critical aspects of the platform technology to enhance the shared understanding of this complex regulatory information, both nuances and broad themes, to allow consideration of possible harmonization of approach as products emerge at pace around the globe [82]. The focus is primarily on the application of the platform technology concerning differentiation from mRNA backbone as intended composition, with examples drawn from the data submission to regulatory agencies of countries and regions, notably the Australian Therapeutic Goods Administration (TGA) and the European Medicines Agency [83].

Government and public health agency responses to an emerging infectious disease differ significantly from those to an impending pandemic. In the former case, public health authorities may only have to distribute a periodic vaccine, develop it in-house, or purchase from local companies with little intervention by regulators. In the case of a pandemic, the emergence of a novel strain is likely more unexpected, cutting preparation lead time. Governments may place extensive pre-purchase orders with manufacturers to control production capacity [84]. Regulators must enhance capacity to evaluate the vast number of new products and ensure vaccine quality, safety, and efficacy are maintained under accelerated timelines [3].

7.1 Approval Processes for mRNA Vaccines

In contrast to traditional vaccines, mRNA vaccines aim to produce immunogens in vaccinated cells through the delivery of mRNA. The COVID-19 lipid nanoparticle mRNA vaccines entered clinical use rapidly due to simplified manufacturing processes involving screening and filling [85]. The first mRNA vaccine, mRNA-1273, encased in LNP5950, reached Phase 1 trials within 63 days of the viral sequence release. It then received Emergency Use Authorization and conditional marketing authorization [84]. The success of LNP-mRNA vaccines has led to exploration of their use in cancer immunotherapy and other therapeutic areas, such as injecting LNP-naked

mRNA to target APCs and elicit T cell responses [12]. However, ensuring vaccine quality, safety, and efficacy particularly for DC-mRNA vaccines remains a central challenge [85].

7.2 Safety and Efficacy Evaluations

Lipid nanoparticles (LNPs) are designed to overcome mRNA degradation and immune clearance. Small-molecule excipients like phospholipids and cholesterol are tailored to optimize LNP properties, enhance tissue targeting, and modulate immune responses [86]. Despite high efficacy, LNP-mRNA vaccines can carry safety risks such as thrombosis, which has contributed to vaccine hesitancy [82]. Parameters such as mRNA length, lipid species, and adjuvant dose influence safety and efficacy. Therefore, preclinical studies must rigorously assess new LNP formulations with metrics such as particle size, zeta potential, and encapsulation efficiency [87]. Results should follow GLP principles and regulatory requirements to ensure clinical readiness [83].

8 Future Directions in Lipid Nanoparticle Research

As a promising delivery system for nucleic acid vaccines, lipid nanoparticles (LNPs) hold great hope for combating emerging infectious diseases. A better understanding of the delivery mechanism of LNP formulations could help accelerate the rational design of next-generation LNPs with improved delivery efficiency [88]. In addition to well-studied cationic lipids, LNPs formulated with ionizable lipids may also incorporate more or fewer than four types of lipids to tune liposomal morphology, which is currently poorly understood [85]. Although commercial LNP formulations designed for virology studies tend to use cationic lipids, ionizable lipids are often used in RNA vaccines, making them an important avenue of exploration [3].

Since encapsulation of clarithromycin and mRNA is capable of rescuing the suppressive effect of the drug on immunogenicity, covalent bonding of mRNA to lipids should also be investigated to help understand the effects of drug formulation on delivery [87]. The ionic interaction between lipids and nucleotides is also believed to be a critical parameter affecting mRNA delivery. This approach could be tackled using a mutant library of one or multiple components of LNPs selected based on an mRNA delivery assay. State-of-the-art screening techniques such as DNA barcoding may aid in the search for more efficient and tolerogenic LNPs. LNPs show great potential for preventing emerging infectious diseases due to their ability to induce strong humoral and cellular immune responses. One focus for improvement is the way LNPs are administered [1].

Since a large part of the human body is inaccessible to LNPs by IM injection, it would be feasible to explore alternative routes of administration such as intranasal, oral, transdermal, rectal, or ocular [82]. For second-generation LNPs, a key consideration is whether they would retain their immunogenicity while improving tolerogenicity. Structural modifications based on the need for intranasal delivery could be made to existing LNPs to enable cellular immune responses in targeting mucosal tissues. A new screening platform employing DNA barcoding would allow the efficient evaluation of a wide range of LNPs based on their transient and systemic delivery of RNA in complex physiological environments [85]. In addition to messenger RNA and lipid nanoparticles that are actively being pursued for application in broad areas of basic and applied research, there remains room for growth in LNP systems for the delivery of other nucleic acids and small molecules targeting microbial pathogens [89].

8.1 Innovative Formulations

Lipid nanoparticles (LNPs) are now among the most widely used technology platforms for RNA-based therapeutics. LNPs have been the primary delivery system for the COVID-19 mRNA vaccines that were developed and authorized for human use in record time [88]. Given the success of LNP-formulated mRNA vaccines in combating the COVID-19 pandemic, research efforts are increasingly focusing on the application of LNP delivery systems for other RNA-based therapeutic modalities, including mRNA vaccines for infectious diseases and cancer, and self-amplifying mRNA (saRNA) applications [90]. Its low toxicity profile, admirable biocompatibility, and the robustness of the microfluidic LNP assembly process have made A18-Iso5f C18 a promising RNA carrier for many preclinical mRNA delivery investigations. While a low pKa leads to low salinity tolerance and toxicity of A18-Iso5f C18 LNP formulations, PEI-A18-Iso5f C18 LNPs retain relatively high levels of mobile RNA at higher ionic strengths and are less cytotoxic [21].

Future exploration of multi-component LNPs containing ICM622, PNP3, or PEIs could lead to new and potentially effective methods of formulating mRNA vaccines against emergent infectious diseases. The demonstration that LNP-formulated mRNA vaccines elicit both systemic and mucosal immune responses from intranasally delivered mRNA — as seen in respiratory syncytial virus (RSV) — supports their application against mucosal diseases [91]. It was also shown that LNP-formulated mRNA could be used as safe and effective pan-fungal vaccines. The universal T-cell activating peptide CALM-15 fused with mRNA in LNPs was shown to promote expansion of T-cell populations to secrete Th1, Th2, and Th17 cytokines. Nanoparticles derived from the human microbiome have demonstrated innate immune memory that may inhibit the spread of respiratory virus infections. Another major innovation is saRNA vaccine design. LNP-formulated saRNA with self-replicating features exhibited protein

expression and immunogenicity up to thirteenfold greater than conventional mRNA doses. Unlike conventional mRNA vaccines that translate proteins mainly in the cytoplasm, saRNA vaccines also internalize into lysosomes, continuing translation with involvement of membrane-associated ribosomes, leading to enhanced dendritic cell activation [92].

8.2 Potential for Other Therapeutics

Nucleotides, as the building blocks of RNA, can also be recognized as therapeutic agents; however, certain challenges must be addressed to bring them to the market. Recent breakthroughs with mRNA vaccines show promise for rapid development, vaccine construct optimization, and broad use against other diseases. Lipid nanoparticles fall into several classes of compounds that form surface-charged structures called lipoplexes. Many efforts have been made to synthesize ionizable lipids for nucleic acid delivery with various headgroups, backbones, and tails [93].

Non-ionic lipids are also included in current FDA-approved lipid nanoparticle formulations. However, molecules in these lipid families, such as DLin-DMA, A18-Iso5p10, DDAB, and DOPE, are thought to facilitate viral membrane fusion instead of serving as the primary delivery agents. Polymeric nanoparticles, such as those using PEG and PLA, have shown appropriate safety and stability as drug carriers, though challenges remain including low drug loading and limited endosomal escape [94]. Currently FDA-approved vaccines and therapeutics using lipid nanoparticles encapsulating nucleic acids exceed 3000 formulations globally. Applications include infectious disease vaccines targeting SARS-CoV-2, influenza, Zika, H1N1, anthrax, plague, and malaria, and mRNA encoding cytokines such as interferon-alpha and interleukin-12 to induce strong immune responses [83].

9 Challenges and Limitations

Currently, nucleic acid RNPs, such as mRNA and siRNA, have emerged as promising therapeutic modalities to induce a compensatory response or silence deleterious genes. mRNA has been explored as a vaccine solution for recent outbreaks of infectious diseases, including SARS-CoV-2, Ebola virus, HIV, and Zika virus. It can stimulate various protective immunity pathways efficiently. In addition to infectious diseases, mRNA vaccines have shown promise in oncology due to their capacity to induce strong immunogenicity [95]. mRNA-encoded therapeutic proteins are also being investigated for regenerative medicine and immunotherapy. However, naked mRNA is easily degraded in systemic circulation, affecting delivery efficiency [96].

To solve these issues, researchers have extensively explored mRNA modifications and carrier systems to protect mRNA and enhance its delivery. Over 12 chemical modifications of mRNA have been developed to increase translation efficiency and modulate immune responses. The delivery systems evolved from viral vectors and cationic polymers to more efficient lipid nanoparticles (LNPs) [15]. Despite their success, LNP-based mRNA vaccines still face challenges including lack of cell-type specificity, the need for advanced biophysical monitoring tools, and optimization for peptide delivery. During the pandemic, mRNA LNP vaccines were mostly tested in immunocompromised animal models, limiting translational insights. Additionally, further development is needed for LNP-based vaccines against other viruses, such as intranasal flu vaccines. Future directions include customized mRNA design using bioinformatics tools [97].

9.1 Technical Barriers

Since 2020, the explosion of interest in LNP-mRNA vaccines has led to rapid innovation in formulation and production. Nevertheless, delivering antisense oligonucleotides (ASOs) and mRNA for emerging diseases is still technically demanding. Overcoming these barriers requires robust preclinical models, scalable manufacturing, and a better understanding of LNP interactions with host biology [98].

9.2 Public Perception and Acceptance

Public perception plays a central role in vaccine success. Studies showed variable acceptance of COVID-19 vaccines between 2020 and 2021, with shifts over time due to evolving public trust, misinformation, and media narratives. Factors influencing perception include vaccine efficacy, side effects, health concerns, and trust in authorities [99]. The complexity of vaccine acceptance underlines the importance of tailored public education, especially in lower-income countries where hesitancy is often linked to fear of side effects [84].

10 Ethical Considerations in Vaccine Development

The swift development of mRNA vaccines during COVID-19 relied on scientific merit, shared data, and unprecedented international collaboration. Unlike previous vaccine campaigns, the approach was fueled by synthetic biology, fast-tracked regulatory support, and large-scale investment [83].

10.1 Equity in Vaccine Distribution

The COVID-19 pandemic highlighted global inequities in vaccine access. Ensuring equitable distribution of mRNA vaccines requires empowering low- and middle-income countries with knowledge, equipment, and platforms for local manufacturing. Otherwise, under-vaccinated regions may become incubators for new variants, affecting global health [100].

10.2 Informed Consent and Public Trust

The social and ethical ramifications of any vaccine must be deliberated during its development. These domains are as vital to a vaccine's success as its scientific components. Decisions regarding vaccine development policies, dissemination strategies, composition, and priority target groups require input from a broad range of stakeholders such as the local community in which the vaccine will be used, social scientists, politicians, and leaders of social, religious, and government institutions [101]. Informed consent is a necessary element in the administration of vaccines, as it is in the medicinal use of interventions. Informed consent, however, goes beyond the acceptance of information about the procedure, benefits, or possible side effects to include the biomedical, policy, and ethical issues involved in the granting of access to the technology.

The elements of informed consent must still be understood with regard to new technologies. After a process of public consultation to determine the ethical principles mainly affected by the proposed policy change, a process of detailed public engagement can use questions around the ethical principles as a focus. This has several benefits: the public can develop a more nuanced understanding of the science underlying the issues; the conversation can be divorced from the politics underlying them; and a broader range of voices can participate in the conversation [102].

11 Collaborative Efforts in Research

Collaborative efforts in Research include various studies on mRNA vaccines. They focus on lipid nanoparticle-mRNA formulations and delivery strategies. Key findings address the efficacy and stability of LNPs-mRNA components in COVID-19 vaccines and the clinical progress of mRNA vaccines and immunotherapies. Research highlights the immunogenicity of lipid nanoparticles and their impact on mRNA vaccines [5].

The progress of COVID-19 mRNA vaccines during the pandemic has led to great interest in this technology for protection against emerging infectious diseases. The safety and efficacy of approved mRNA vaccines against COVID-19 have been significantly attributed to lipid nanoparticle (LNP)-mRNA formulations and delivery strategies [1].

COVARNA consortium investigates the modulation of the immune response to SARS-CoV-2 through different nanocarriers that deliver mRNA coding for a trimeric receptor-binding domain (RBD) of the spike protein of SARS-CoV-2. The findings demonstrate a multifaceted study of LNP-RNA formulations that employs diverse biophysical techniques to describe characteristics impacting mRNA vaccine efficacy [103].

The COVID-19 pandemic illustrates that public-private partnerships (PPPs) are essential in developing and delivering mRNA vaccines and therapeutics to swiftly respond to emerging infectious diseases [104].

To develop and manufacture mRNA medicines from novel mRNA sequences, unlike established agent strains for conventional vaccines, it is essential to obtain their genome sequences, which are usually published soon after outbreak. The "plug-and-play" systems utilize pGEM4Z based plasmid with the T7 polymerase promoter and the prefusion spike-encoding sequence [105].

11.1 International Collaborations

Over the last decade, mRNA vaccines were successfully developed and globally administered to control the COVID-19 pandemic. This effort involved researchers, companies, and agencies. Lipid nanoparticles (LNPs) are the most clinically validated carriers of mRNA.

LNP-HEC formulation facilitates rapid screening of mRNA sequence and structure, thus accelerating mRNA vaccine development [106]. A novel strategy using cationic amino lipids was developed and found less toxic and more effective than ionizable lipids [107].

12 Conclusions

Since the first release of the COVID-19 mRNA vaccines, there has been a major development and interest in mRNA vaccine delivery systems, particularly lipid nanoparticles (LNPs). The success of the COVID-19 messenger RNA (mRNA) vaccines has inspired interest in the development of new mRNA vaccines against emerging infectious diseases. These efforts have come with many challenges, chief among them the need for novel mRNA formulations and competent LNP bases for the administration of mRNAs with important but different specifications for both humoral and cellular immune responses. Therefore, there is overwhelming motivation for the development of additional LNP delivery system compatible with 5′ Cap mRNA that can provide effective and safe delivery. Likewise, innovative mRNA delivery systems need to be realized with emerging populations of lipid materials and other types of nanocarriers.

A variety of new lipid and other organic or polymeric materials that showed great potential to be carriers for mRNA vaccines were introduced. Moreover, LNP systems made with newer types of lipids, and those incorporated with non-lipid materials, led to LNPs with very new formulations that successfully allayed the problems confronted with authentic COVID-19 mRNA LNPs or even vastly improved the responses against mRNA vaccines. However, routine use of mRNA vaccines as a prophylactic or as a therapeutic agent likely demands a more user-friendly, sensible, more competent and safer newer generation of products. Although recent advances in mRNA vaccine science and technologies have been accelerated, bench-to-public development strives to convert FDA emergency approved COVID-19 mRNA vaccines into routine public health measures consistent with health policy prospects in previous years.

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