Research Advances in mRNA Vaccine Manufacturing Processes

Jianyao Sun
College of Agriculture, Yanbian University, Yanji City, Jilin, China

Keywords: mRNA Vaccines, Lipid Nanoparticles (LNPs), Manufacturing Process.

Abstract: The rapid development and deployment of mRNA vaccines have revolutionized the field of vaccinology, particularly highlighted by their pivotal role in combating the COVID-19 pandemic. This review systematically explores the technological advancements in mRNA vaccine production, emphasizing key processes such as plasmid DNA (pDNA) preparation, in vitro transcription (IVT), mRNA purification, and lipid nanoparticle (LNP) encapsulation. Innovations in nucleotide modification, codon optimization, and LNP formulation have enhanced mRNA stability, translation efficiency, and delivery precision. Despite these breakthroughs, challenges persist, including scalability limitations, batch variability in IVT and LNP production, intellectual property disputes, and the need for harmonized regulatory frameworks. Emerging delivery systems—such as polymer-based carriers, inorganic nanomaterials, and peptide derivatives—offer promising alternatives to LNPs, potentially improving tissue targeting and reducing immunogenicity. Furthermore, the application of mRNA technology extends beyond infectious diseases to cancer immunotherapy and protein replacement therapies. The review underscores the importance of standardized quality control protocols, sustainable supply chains, and global collaboration to address manufacturing bottlenecks and ensure equitable access. By resolving these challenges, mRNA vaccines are poised to become a cornerstone of 21st-century medicine, offering versatile solutions for evolving health crises.

1 INTRODUCTION

1.1 Type Area Introduction

The advent of mRNA vaccines represents a paradigm shift in biomedical innovation, combining rapid development cycles, precise antigen design, and adaptable manufacturing processes to address global health challenges. Since the discovery of mRNA's role as a protein synthesis template in the 1960s, decades of research have focused on overcoming its inherent instability and inefficient delivery. Early breakthroughs, such as Wolff et al.'s 1990 demonstration of in vivo protein expression via injected naked mRNA, highlighted its therapeutic potential but underscored the need for robust delivery systems (Wolff et al., 2020). The emergence of lipid nanoparticles (LNPs) in the 2010s revolutionized mRNA technology by enabling efficient cytoplasmic delivery while enhancing stability and reducing immunogenicity. These advancements paved the way for mRNA vaccines to transition from experimental platforms to clinical reality, exemplified by the rapid deployment of COVID-19 vaccines like BNT162b2 and mRNA-1273 during the pandemic (Teo et al.,2022.). Their success, however, hinged on establishing robust manufacturing frameworks capable of producing these genetic vaccines at unprecedented scales.Central to mRNA vaccine production is a multi-step process that begins with plasmid DNA (pDNA) template preparation. pDNA, amplified in bacterial hosts, undergoes linearization and serves as the blueprint for in vitro transcription (IVT). During IVT, RNA polymerase synthesizes mRNA strands incorporating modified nucleotides (e.g., pseudouridine) to minimize innate immune activation. Subsequent purification steps, including tangential flow filtration and chromatographic methods, remove enzymatic residues and ensure mRNA integrity. The final formulation relies on LNPs, which encapsulate mRNA via microfluidic mixing, optimizing particle size and encapsulation efficiency. Each stage demands stringent quality control, from verifying pDNA supercoiling ratios to assessing 5' capping efficiency and poly(A) tail length, as outlined by regulatory bodies like the FDA and NMPA (Verbeke et al., 2021). Despite having these advances, challenges still remain. Scalability

Sun, J.
Research Advances in mRNA Vaccine Manufacturing Processes.
DOI: 10.5220/0014499200004933

In *Proceedings of the 1st International Conference on Biomedical Engineering and Food Science (BEFS 2025)*, pages 458-464
ISBN: 978-989-758-789-4

remains hindered by the lack of standardized protocols for large-scale IVT and LNP formulation. Patent disputes over critical components, such as ionizable lipids, further complicate global accessibility. Additionally, long-term stability studies and harmonized regulatory frameworks for intermediate products are urgently needed to ensure batch consistency. Innovations in alternative delivery systems—such as polymer-based carriers or inorganic nanoparticles—and advancements in codon optimization algorithms promise to address these gaps. Beyond infectious diseases, mRNA platforms are being repurposed for cancer immunotherapy and protein replacement therapies, underscoring their versatility. This review examines the technological milestones in mRNA vaccine production, analyzes current bottlenecks, and explores future directions to enhance manufacturability, safety, and therapeutic scope.

2 mRNA VACCINES

2.1 Introduction to the Background of mRNA Vaccines

In 1961, research revealed that mRNA could serve as a direct template guiding protein biosynthesis (BRENNER et al.,1961, SMULL et al.,1962) In 1963, Isaacs et al. (ISAACS et al., 1963.) discovered that mRNA could induce the production of interferon. mRNA can induce the production of interferon; however, due to its inherent instability, it was not until 1969 that Lockard and Lingrel successfully synthesized proteins using isolated mRNA in vitro (Lockard et al.,1969). In 1990, Wolff et al. (Wolff et al., 1990) injected naked mRNA obtained through in vitro transcription directly into the skeletal muscle of mice, leading to the successful expression of the corresponding protein in the mice and the generation of an immune response. This groundbreaking experiment laid the foundation for further research into the therapeutic potential of mRNA.

2.1.1 Classification of mRNA Vaccines

mRNA can be classified into two categories based on genetic characteristics: non-replicating mRNA and self-amplifying mRNA (saRNA).

Non-replicating mRNA, in addition to containing a 5' cap structure, a 5' untranslated region (5'-UTR), a 3' untranslated region (3'-UTR), and a Poly(A) tail, solely encodes the target antigen.(Furuichi et al.,1975)mRNA technology primarily encompasses sequence design, delivery system construction, in vitro transcription (IVT), and formulation development. The complete molecular structure of mRNA includes an open reading frame (ORF) that encodes the target protein, flanked by 5'UTR and 3'UTR regions, a 5' cap structure (or its substitute), and a 3' sequence [such as a poly(A) tail].Each component has its own function, and the components can work in an organic and coordinated manner to jointly regulate the translation efficiency, stability, and immunogenicity of mRNA within the body (Zong et al.,2023, Sahin et al.,2014, Lu et al.,2024).

2.2 The Production Process of mRNA Vaccines

The production of mRNA active pharmaceutical ingredients (APIs) primarily involves two fundamental steps: upstream enzymatic processes and downstream chromatographic and ultrafiltration purification. The upstream enzymatic processes mainly include three enzymatic reactions: plasmid DNA linearization, mRNA in vitro transcription, and template DNA digestion. The downstream process begins with the collection of in vitro transcription (IVT) reaction products and primarily includes dilution with nuclease-free water, chromatographic purification, ultrafiltration diafiltration concentration, filtration, and filling. The production of mRNA formulations mainly involves the preparation and encapsulation of lipid nanoparticles (LNPs), followed by sterile filtration and formulation filling (Teo et al., 2022). The specific production process for mRNA vaccines can be divided into five steps: ① Preparation of plasmid DNA (pDNA), which includes the production and purification of pDNA. ② IVT and modification. First, linearize the pDNA, then use the linearized plasmid DNA as a template to transcribe and synthesize mRNA in a cell-free system. Nucleotide modification can be applied to enhance the functionality of the mRNA. ③ Filtration and purification of mRNA. This primarily involves exchanging the buffer to remove impurities such as enzymes and nucleotides. ④ Preparation and encapsulation of LNPs. Currently, microfluidic mixing technology is primarily used to precisely mix the mRNA bulk solution with LNPs in a specific ratio, thereby forming uniformly sized lipid nanoparticles that encapsulate the mRNA. After steps such as ultrafiltration and buffer exchange to remove impurities, the intermediate product of mRNA-LNPs formulation is ultimately prepared. ⑤ Filling of

mRNA vaccines. After the intermediate product of the mRNA-LNP formulation has passed the quality inspection, it is aseptically filled to obtain the finished product of the mRNA vaccine (Teo et al.,2022).

2.3 Delivery Vehicles

The main carriers for mRNA drug delivery include lipid-based materials, polymers, protein derivatives, and exosomes. As shown in Figure 1, intracellular delivery process of mRNA Vaccines (Using LNA-mRNA Vaccine as an Example): 1. Endocytosis: the LNA-modified mRNA vaccine, encapsulated in a lipid nanoparticle (LNP) composed of ionizable lipid, phospholipid, cholesterol, and PEG, is internalized by host cells via endocytosis. The ionizable lipid facilitates membrane interaction, while PEG stabilizes the nanoparticle and reduces immune recognition. 2. Endosomal Escape: within the acidic environment of the endosome, the ionizable lipid becomes positively charged, destabilizing the endosomal membrane. This promotes fusion of the LNP with the endosomal membrane, enabling the release of the LNA-mRNA into the cytosol.3. LNA-enhanced Stability and Translation: the incorporation of Locked Nucleic Acid (LNA) modifications in the mRNA backbone enhances its stability against enzymatic degradation and improves translational efficiency. This ensures prolonged availability of intact mRNA in the cytosol.4. Translation of Antigenic Protein: the released mRNA is recognized by ribosomes, which translate it into the antigenic protein (e.g., viral spike protein). No nuclear entry is required, as mRNA vaccines function entirely in the cytosol.5. Immune Activation: the antigenic protein is processed and presented on major histocompatibility complex (MHC) molecules,

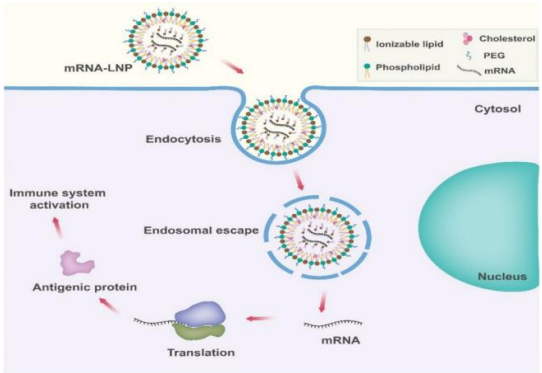

Figure 1: This Diagram Illustrates the Mechanism of mRNA-LNP Vaccines, Highlighting Key Steps from Cellular Uptake to Immune Activation.

activating adaptive immune responses (T cells and B cells). This primes the immune system to recognize and neutralize future pathogen exposure.

2.3.1 Liposomes and Lipid Complexes

Lipid nanoparticles (LNPs) are commonly used advanced mRNA delivery carriers. Their emergence is a key factor in advancing the approval of mRNA vaccines and has also provided a direction for other mRNA therapeutic fields (Kimura et al., 2020). Although mRNA-based therapies possess the a forementioned advantages, mRNA also faces challenges such as poor stability and difficulty in entering the cytoplasm to exert its function (Kubiatowicz et al., 2022). Therefore, an appropriate delivery carrier is crucial for mRNA therapy. Lipid nanoparticles (LNPs) are the most widely used carriers for nucleic acid drugs in clinical applications. It can deliver mRNA to the cytoplasm to express target proteins. Compared to other delivery carriers, LNP has many advantages such as high encapsulation efficiency, high transfection efficiency, simple preparation, stable structure, and good safety (Cullis et al., 2017, Eygeris et al., 2022). LNPs comprise four lipids: ionizable, helper, cholesterol, and PEGylated—each vital for stability, transfection, and safety (Zong et al., 2023). Ionizable lipids (primary component) are neutral at physiological pH but protonate in acidic conditions, enabling electrostatic mRNA encapsulation during self-assembly (Hald Albertsen et al., 2022). In serum (neutral pH), uncharged LNPs avoid protein binding, reducing macrophage uptake and prolonging circulation. This pH-responsive charge lowers toxicity versus permanent cationic lipids. In endosomes, acid-induced positivity enhances endosomal membrane interaction, driving mRNA cytoplasmic release and boosting transfection efficiency (Samaridou et al., 2020). Helper lipids (primarily phospholipids) stabilize LNPs and promote endosomal membrane fusion for mRNA release. Common phospholipids include DSPC (high phase transition temperature, enhancing stability) and DOPE (cone-shaped structure, driving hexagonal phase formation to boost mRNA transfection efficiency) (Ahmed et al., 2019). Cholesterol, the second most abundant LNP component, localizes in the outer shell. It improves stability, prolongs blood circulation, and increases membrane fluidity/permeability to facilitate mRNA release (Sebastiani et al., 2021).

2.3.2 High Molecular Weight Polymers

Polymer carriers themselves are easy to prepare, and fine-tuning their surface properties can achieve the desired effects, promoting cross-presentation of antigens. Modifying the surface of polymeric nano/microspheres can mediate targeted delivery to immune cells, enhancing antigen utilization and activating the immune system with lower antigen doses (Malla et al., 2024). Polymer nanocarrier materials can be categorized into synthetic macromolecular polymers and natural polymers. Synthetic polymers are polymer materials synthesized through chemical methods, which can be custom-designed according to specific requirements. Common synthetic macromolecular polymers include polyethylene, polypropylene, and polystyrene, among others. These materials are characterized by their ease of synthesis, low cost, and lack of immunogenicity (Charbe et al., 2020).

2.3.3 Protein/Peptide Derivative-mRNA Complex

Peptide/protein vaccines represent one of the most common types of vaccines, offering advantages such as well-defined antigenic sequences, low production costs, and ease of preparation (Mai et al., 2020). Lou et al. (Lou et al., 2019) developed a straightforward method for peptide-functionalized mRNA polyplexes to enhance dendritic cell presentation of mRNA antigens, demonstrating high cellular uptake and no cytotoxicity. Mai et al. (Mai et al., 2020) constructed a delivery carrier based on protamine, which prevents mRNA degradation, promotes antigen presentation by antigen-presenting cells, and simultaneously induces anti-tumor immune responses. Lin et al. (LIN et al.,2022) developed a novel compound by conjugating peptides with protoporphyrin for targeted therapy and photodynamic therapy. Such formulations provide a strong foundation for the development of mRNA vaccine delivery systems.

3 mRNA VACCINE PRODUCTION

3.1 Synthesis of mRNA Fragments

Currently, there are multiple methods for preparing DNA transcription templates for mRNA vaccines, such as templates produced using PCR technology or non-linearized plasmids containing terminator sequences. These drug templates can be amplified and expanded in host cells, such as E. coli. However, in all cases, each batch of DNA plasmids used for the production of mRNA vaccines must undergo release testing to confirm their sequence, purity, and quality (Naveed et al.,2023). For the quality control of transcription template plasmid DNA, specific requirements have been outlined in the latest guideline documents issued by China's NMPA, the WHO, and the U.S. FDA. The pre-release testing of plasmid DNA primarily includes five quality control aspects: identification, concentration, purity, safety, and others. Each aspect encompasses corresponding testing items. Regarding the detection of the percentage of supercoiled content for plasmid DNA purity, the second edition of the draft guidance issued by the USP in 2023 has included this item in the pre-release testing of plasmid DNA. However, the NMPA and WHO have not yet proposed requirements for this specific test. The guideline documents from various countries also recommend corresponding testing methods, summarized as follows: for the identification of plasmid DNA, methods include direct sequencing, next-generation sequencing (NGS), and restriction enzyme agarose gel electrophoresis analysis; The detection methods for the concentration and purity of plasmid DNA include ultraviolet spectroscopy (UV), among others.The detection methods for the superhelical percentage content include capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC), among others. The detection methods for host RNA residual content include agarose gel electrophoresis and HPLC, among others. The detection methods for host DNA template residual content include quantitative real-time PCR (qPCR) Baden et al.,2021, Liu et al., 2022, Verbeke et al., 2021, Cortese et al., 2024).

3.2 mRNA Production and Optimization

The plasmid DNA template is enzymatically linearized and purified, then used to synthesize mRNA through in vitro transcription in a cell-free system. After further purification, the mRNA stock solution is obtained. Due to the degeneracy of the genetic code, mRNA can be optimized in terms of codon usage to achieve more efficient translation and enhanced stability. Furthermore, modified nucleotides can be utilized to reduce the immunogenicity of mRNA, suppress innate immune activation, and mitigate adverse effects such as

inflammation (Maruggi et al., 2019). The 5' cap can be introduced co-transcriptionally by adding a capping reagent to the IVT (in vitro transcription) mixture. The 3' poly(A) tail can be added enzymatically or encoded directly within the DNA template. Therefore, the release testing of mRNA stock solutions should include specific assessments such as the 5' capping efficiency and the length of the 3' poly(A) tail (US Pharmacopeia et al., 2023).

3.3 The Intermediate Product of the Formulation

The NMPA (National Medical Products Administration) has introduced the concept of intermediate products in formulations and emphasized that the definition of these intermediates, as well as the establishment of quality standards for them, should be based on the actual conditions of the mRNA delivery system preparation process. These intermediates may include products resulting from the complexation of mRNA with positively charged polymer materials, nanoparticle intermediates, and others. The NMPA also pointed out that the testing of intermediate products is part of process control. Whether to define a product as an intermediate and the corresponding testing requirements should consider the following factors. ①Whether this stage is the most sensitive phase for the corresponding testing items. ②Whether subsequent production processes and formulation compositions have an impact on the active components, such as whether lyophilization is performed.③Whether subsequent process steps require testing at this stage, such as using the content of active ingredients to guide formulation preparation (Baden et al., 2021).

3.4 mRNA Vaccine Final Product

After precise mixing of mRNA original solution and lipid nanoparticles (LNPs) and other delivery systems in a certain proportion through jet impact mixing method, microfluidic mixing method and other technologies, nanoliposomes encapsulating mRNA with uniform particle size are formed. After that, through steps such as ultrafiltration and buffer exchange to remove impurities, and then adding buffer, sugars, and dispersants, followed by quality inspection, aseptic filling is carried out to obtain the final product of mRNA vaccine (Naveed et al.,2023).

4 APPLICATIONS OF mRNA VACCINES

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, continues to persist, exerting a significant impact on human health and the global economy. Vaccines represent the primary approach to combating the COVID-19 pandemic. According to data released by the World Health Organization on January 24, 2023, there are over 300 COVID-19 vaccine candidates globally, with 176 of them having entered the clinical trial phase. Leveraging the advantages of mRNA vaccines in preventing infectious diseases, COVID-19 mRNA vaccines were rapidly developed and iterated during the pandemic, making significant contributions to the fight against COVID-19. The Biologics License Application (BLA) was also approved in the US in August 2021, making it the first mRNA vaccine to be launched globally. Moderna's mRNA-1273 (Spikevax) was granted an Emergency Use Authorization (EUA) in the US in December 2020, with its Biologics License Application (BLA) being approved in the US on February 1, 2022, becoming the second mRNA vaccine to be launched globally. AWcorna, co-developed by the Academy of Military Medical Sciences, Suzhou Abio Biology, and Yunnan Walvax Biology, was granted an EUA in Indonesia in September 2022, marking the first EUA obtained by China overseas. SYS6006 from China Pharmaceutical Group was granted an EUA in China in March 2023, becoming the first of its kind in the country (Cheng et al., 2020).

5 CHALLENGES AND PROSPECTS IN mRNA VACCINE PRODUCTION

With the advancement of technology, researchers have developed some new quality control methods and strategies. For instance, Packer et al., created a kinetic model for predicting the shelf life of mRNA vaccines and utilized this model to estimate the expiration period of mRNA vaccines (Packer et al., 2021). BioNTech has developed a ribozyme-based method combined with denaturing polyacrylamide gel electrophoresis or liquid chromatography and mass spectrometry to quantitatively analyze and detect the capping efficiency of in vitro transcribed mRNA （Vlatkovic et al., 2022）. The production

of mRNA vaccines, from plasmid templates to final filling, lacks standardized scale-up and quality control, posing challenges in manufacturing. In China, mRNA vaccine development focuses on COVID-19, with expansion into vaccines for other infectious diseases (e.g., RSV, influenza) and cancer. Patent limitations in sequence design and delivery systems, along with the need for localized raw materials and equipment, hinder progress. Despite these challenges, mRNA vaccines show promise in infectious disease prevention, cancer vaccines, and protein replacement therapies. In summary, mRNA vaccines have significant potential in immunotherapy, relying on stable synthesis, immunogenicity regulation, and effective delivery systems. They enhance our ability to combat infectious diseases and offer new approaches in cancer treatment by optimizing existing therapies (Packer et al., 2021). However, addressing ethical, regulatory, and societal acceptance issues is crucial to ensure their safe and responsible contribution to human health and scientific progress.

6 CONCLUSION

The evolution of mRNA vaccine manufacturing has been marked by transformative innovations, from nucleotide modifications to LNPs, enabling unprecedented speed and precision in vaccine development. The COVID-19 pandemic catalyzed the industrialization of these technologies, proving their viability in global health crises. However, scalability and standardization challenges—such as inconsistent IVT yields and LNP batch variability—highlight the need for automated, closed-loop production systems and universal quality benchmarks. Regulatory agencies must prioritize harmonizing guidelines for intermediate products, particularly mRNA-LNP complexes, to streamline commercialization. Future progress hinges on diversifying delivery platforms, such as peptide-based carriers or stimuli-responsive nanomaterials, to improve tissue targeting and reduce off-target effects. Equally critical is addressing intellectual property barriers and fostering open-access collaborations to democratize mRNA technology. Investments in sustainable raw material supply chains and localized manufacturing infrastructure will enhance global equity, particularly for low-resource regions. As mRNA platforms expand into oncology and genetic disorders, rigorous long-term safety assessments and public education initiatives are essential to build trust.

In summary, mRNA vaccines epitomize the convergence of molecular biology and bioengineering, offering a dynamic toolset against evolving pathogens and complex diseases. By resolving existing technical and logistical hurdles, this technology can fulfill its promise as a cornerstone of 21st-century medicine.

REFERENCES

Ahmed, K. S. 2019. Liposome: composition, characterisation, preparation, and recent innovation in clinical applications. Journal of drug targeting, 27(7), 742–761.

Baden, L. R. 2021. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. The New England journal of medicine, 384(5), 403–416. https://doi.org/10.1056/NEJMoa2035389

Brenner, S. 1961. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. Nature, 190, 576–581.

Charbe, N. B. 2020. Small interfering RNA for cancer treatment: overcoming hurdles in delivery. Acta pharmaceutica Sinica. B, 10(11), 2075–2109.

Cheng, Q. 2020. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. Nature nanotechnology, 15(4), 313–320.

Cortese, S. 2024. Psychopharmacology in children and adolescents: unmet needs and opportunities. The lancet. Psychiatry, 11(2), 143–154. https://doi.org/10.1016/S2215-0366(23)00345-0

Cullis, P. R. 2017. Lipid Nanoparticle Systems for Enabling Gene Therapies. Molecular therapy, 25(7), 1467–1475.

Eygeris, Y. 2022. Chemistry of Lipid Nanoparticles for RNA Delivery. Accounts of chemical research, 55(1), 2–12.

Furuichi, Y. 1975. A blocked structure at the 5' terminus of mRNA from cytoplasmic polyhedrosis virus. Nature, 253(5490), 374–375.

Hald Albertsen, C. 2022. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. Advanced drug delivery reviews, 188, 114416.

Isaacs, A., & Cox, R. A. 1963. Foreign nucleic acids as the stimulus to make interferon. Lancet, 2(7299), 113–116.

Kimura, N. 2020. Development of a Microfluidic-Based Post-Treatment Process for Size-Controlled Lipid Nanoparticles and Application to siRNA Delivery. ACS applied materials & interfaces, 12(30), 34011–34020.

Kubiatowicz, L. J. 2022. mRNA nanomedicine: Design and recent applications. Exploration, 2(6), 20210217.

Lin, H. X. 2022. An acid-targeting peptide can be used as a carrier for photodynamic therapy (PDT). Mater Today Commun, 31, 103659.

Liu, M. A. 2022. WHO informal consultation on regulatory considerations for evaluation of the quality, safety and

efficacy of RNA-based prophylactic vaccines for infectious diseases, 20-22 April 2021. Emerging microbes & infections, 11(1), 384–391.

Lockard, R. E. 1969. The synthesis of mouse hemoglobin beta-chains in a rabbit reticulocyte cell-free system programmed with mouse reticulocyte 9S RNA. Biochemical and biophysical research communications, 37(2), 204–212.

Lou, B. 2019. mRNA Polyplexes with Post-Conjugated GALA Peptides Efficiently Target, Transfect, and Activate Antigen Presenting Cells. Bioconjugate chemistry, 30(2), 461–475.

Lu, C. 2024. Safety, Immunogenicity, and Mechanism of a Rotavirus mRNA-LNP Vaccine in Mice. Viruses, 16(2), 211.

Mai, Y. 2020. Intranasal delivery of cationic liposome-protamine complex mRNA vaccine elicits effective anti-tumor immunity. Cellular immunology, 354, 104143.

Malla, R. 2024. mRNA vaccines and their delivery strategies: A journey from infectious diseases to cancer. Molecular therapy, 32(1), 13–31.

Maruggi, G. 2019. mRNA as a Transformative Technology for Vaccine Development to Control Infectious Diseases. Molecular therapy, 27(4), 757–772.

Naveed, M. 2023. A one-health approach to design an mRNA-based vaccine candidate against the lumpy skin disease virus as an alternative to live-attenuated vaccines. European review for medical and pharmacological sciences, 27(13), 6401–6413.

Packer, M. 2021. A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems. Nature communications, 12(1), 6777.

Sahin, U. 2014. mRNA-based therapeutics--developing a new class of drugs. Nature reviews. Drug discovery, 13(10), 759–780.

Samaridou, E. 2020. Lipid nanoparticles for nucleic acid delivery: Current perspectives. Advanced drug delivery reviews, 154-155, 37–63.

Sebastiani, F. 2021. Apolipoprotein E Binding Drives Structural and Compositional Rearrangement of mRNA-Containing Lipid Nanoparticles. ACS nano, 15(4), 6709–6722.

Shi, S. 2015. VEGFR targeting leads to significantly enhanced tumor uptake of nanographene oxide in vivo. Biomaterials, 39, 39–46.

Smull, C. E. 1962. Enhancement of the plaque-forming capacity of poliovirus ribonucleic acid with basic proteins. Journal of bacteriology, 84(5), 1035–1040.

Teo, S. P. 2022. Review of COVID-19 mRNA Vaccines: BNT162b2 and mRNA-1273. Journal of pharmacy practice, 35(6), 947–951.

Verbeke, R. 2021. The dawn of mRNA vaccines: The COVID-19 case. Journal of controlled release, 333, 511–520.

Vlatkovic, I. 2022. Ribozyme Assays to Quantify the Capping Efficiency of In Vitro-Transcribed mRNA. Pharmaceutics, 14(2), 328.

Wolff, J. A. 1990. Direct gene transfer into mouse muscle in vivo. Science, 247(4949 Pt 1), 1465–1468.

Yang, A. 2021. Hydrogel/nanoadjuvant-mediated combined cell vaccines for cancer immunotherapy. Acta biomaterialia, 133, 257–267.

Zong, Y. 2023. Lipid Nanoparticle (LNP) Enables mRNA Delivery for Cancer Therapy. Advanced materials, 35(51), e2303261.