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mRNA Vaccines and Their Potential to Control Foot and Mouth Disease (FMD)

Kashif Abdaal, MPhil, Scholar; Aneeza Batool, PhD Scholar; Abdul Aziz Khan, MPhil, Scholar; Muhammad Tariq Navid*; Muhammad Armaghan Khalid; Ali Asghar

Department of Biological Sciences, National University of Medical Sciences, Rawalpindi, Pakistan.

***Corresponding Author(s): Muhammad Tariq Navid**

Associate Professor, Department of Biological Sciences, National University of Medical Sciences, Rawalpindi, Pakistan.

Email: tariq.navid@numspak.edu.pk

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Abstract

Foot and Mouth Disease (FMD) poses significant threats to public health, the environment, animal welfare, and the economy, causing up to 100% morbidity and substantial economic losses globally. Traditional inactivated FMD vaccines, though widely used, suffer from short-lived immunity and stringent production requirements. The COVID-19 pandemic accelerated the development of mRNA vaccines, revolutionizing vaccine technology with their high potency, safety, and rapid, cell-free production. Despite challenges such as instability and inefficient in vivo delivery, mRNA vaccines offer a promising alternative to conventional vaccines. mRNA vaccines have shown significant promise due to their simplified development process, efficient scalability, and rapid manufacturing. However, they face challenges including potential side effects and degradation by RNase enzymes. The effectiveness of mRNA vaccines also depends on the administration route, with intramuscular injection being the most common and effective due to its rich blood vessels and immune cell presence. Current studies have demonstrated the effectiveness of an empty capsid vaccination against FMD using FMDV P1-2A capsid precursor and 3Cpro genes with baculovirus or vaccinia virus expression systems. Developing a synthetic thermostable RNA vaccine using these genes could protect against various FMD strains, eliminating the need for an infectious virus or cell culture. However, challenges remain in terms of stability, storage, and efficient delivery. Future research aims to enhance mRNA vaccine stability, explore new delivery methods, and develop targeted formulations to improve immunogenicity and accessibility. Collaborative efforts and continuous innovation are essential to fully realise the potential of mRNA vaccines in combating FMD and other disease.

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Introduction

Animal infectious illnesses pose considerable dangers to many sectors of the community, including the environment, animal welfare, public health, and the economy. These viral illnesses in animals can cause a variety of undesirable outcomes [11]. Foot and Mouth Disease (FMD) is one of the most easily transmissible illnesses that affect animals. The FMD virus reproduces swiftly and quickly spreads within the infected animal's body [18]. Airborne infections can spread up to 10 kilometers, posing great challenges for disease control efforts due to their wide-spreading nature. FMD has a 5% chance of mortality in adult animals, and every animal affected by FMD shows signs of disease, making the morbidity rate up to 100% [38]. The economic impact on a global scale has been significant, especially in regions where the disease is endemic. Researchers have estimated that the annual cost of FMD is $6.5 billion to $21 billion [32]. Currently, the commercially used vaccines globally for FMD are inactivated vaccines. This type of vaccine was among the first developed for FMD prevention and control [37]. The disadvantages of using inactivated vaccine formulations include a short period of immune protection, sensitivity to temperature fluctuations, and the need for an extremely regulated biological safety level III facility for preventing potential virus contamination during the vaccine manufacturing process. Furthermore, even in pure vaccine formulations, there might be occasional remnants of Non-Structural Proteins (NSPs) that can stimulate the formation of antibodies against NSPs [75].

Vaccination has significantly improved both human and veterinary medicine, representing a remarkable achievement [21]. By keeping a variety of infectious illnesses from spreading over the world and killing people, vaccinations are extremely effective in combating them [45]. One serious viral infectious disease, smallpox, has been eradicated globally with the help of vaccination [13,14]. Various approaches for developing COVID-19 vaccines have been explored simultaneously. Among this, mRNA vaccine technology has emerged as a groundbreaking innovation with a distinct impact on managing the COVID-19 pandemic [31]. The mRNA vaccine was first-time licensed for humans during COVID-19, which marked a historic milestone. The advent of mRNA vaccines has brought about a profound transformation in the field of vaccine development [46].

Exogenous mRNA must get through the protective lipid membrane barrier and enter the cytoplasm to be translated into a functional protein [34]. To facilitate the effective entry of mRNA vaccines into human cells presents a formidable hurdle. Naked mRNA is considered a foreign particle by the immune system and is swiftly broken down by enzymes in the body. To enhance the immunogenicity of mRNA vaccines, specialized delivery systems are essential. The lipid shell transfers the mRNA to the cytoplasm of the cell [15]. Once the mRNA vaccine is transported from the cell membrane, it enters the cytoplasm where ribosomes facilitate the translation of this mRNA into the specific protein needed, and it produces long-lasting immunity [60]. The ease of manufacture of mRNA vaccines over conventional vaccinations is one of its main advantages. In a cell-free system, mRNA is manufactured without the use of any ingredients sourced from animals. This approach ensures that there are no impurities or accidental contaminants in cells that make the production of mRNA vaccines safer [57].

mRNA vaccines are a strong alternative to conventional vaccinations because of their high potency, safety, effectiveness, the ability for quick clinical development, and the possibility for rapid, and low-cost manufacture. However, their uses have been limited due to the instability and inefficiency of mRNA distribution in vivo [16]. Table 1 lists the differences between traditional and modern vaccines.

Table 1: Demonstrates the difference between conventional and advanced vaccines.

	Conventional vaccines	Advanced vaccines	References
Composition	Whole microorganisms (killed or attenuated), protein fragments, polysaccharides, or recombinant proteins.	Synthetic RNA molecules contain viral gene sequences.	(Gote et al., 2023)
Mechanism of action	Expose the host to part(s) of the virus (to stimulate immunity against it).	Instruct the cell machinery to generate particular antigens that promote immunity.	(Pardi et al., 2018a)
Development duration	Month to years.	Weeks to several months.	(*Understanding Six Types of Vaccine Technologies*, n.d.)
Manufacturing procedure	Growth and modification of whole microorganisms	Synthesis of nucleotide sequences	(*How Vaccines Are Developed and Approved for Use | Vaccines & Immunizations | CDC*, n.d.)
Safety	Proven over decades, with few serious adverse effects.	Early safety evidence is strong, but long-term understanding is still developing.	(M. Zhang et al., 2023)
Immune Response	Depends on the type; typically, efficient at establishing humoral and cellular immunity.	Highly efficient and rapid adaptation to evolving varieties via updated designs.	(Gote et al., 2023)
Storage	Frequently require cold chain storage (-2°C to +8°C) depending on product requirements.	Generally, more stable under ordinary refrigeration temperatures (+2°C to +8°C) May have a longer shelf life than conventional versions.	(Pardi et al., 2018a)

History of mRNA vaccines

In 1961, Brenner and colleagues identified mRNA, a molecule in the cell's genetic machinery [19]. In 1978, scientists made a breakthrough by showing that human cells could use mRNA when it is enclosed in liposomes to make different proteins. In 1984, researchers successfully synthesized mRNA in the lab. Thus, as interest in the creation of mRNA vaccines increased. Martinon and his colleagues produced the first mRNA vaccine for influenza nucleoprotein in 1993 [68]. In 1989, Vical Incorporated, a San Diego biotech company, pioneered the unique use of mRNA as a possible treatment. They demonstrated that encapsulating mRNA in small lipid-based particles allowed for the successful delivery and introduction of mRNA into cells [58]. In early 2020, scientists worldwide worked quickly to create a safe and effective COVID-19 vaccine. One of the quickest accomplishments in the history of vaccine research. The Pfizer-BioNTech partnership produced the first licensed COVID-19 mRNA vaccine by December 2020. It was the first mRNA vaccination with excellent outcomes that was clinically licensed on an emergency basis (Chang Kim et al., 2022).

In vitro-transcribed (IVT) mRNA vaccine structure

Like eukaryotic mRNA, mRNA has an Open Reading Frame (ORF) in the middle that codes for a protein. Untranslated Translated Region (UTR) 5' UTRs and 3' UTR ends encircle the ORF from both sides, as shown in Figure 2. A. It also has a poly(A) tail after the 3' UTR shown in Figure 2. A 7-methyl guanosine 5' cap structure at the initiation site, which provides stability and aids in translation. Modification of these components allows for enhancements in mRNA (a) stability (b) translation efficiency (c) immune-stimulatory attributes. Different research focuses on identifying and incorporating favorable mRNA elements to optimize translation and stability for improved performance (Chang Kim et al., 2022). The structure of IVT mRNA is shown in Figure 2 A.

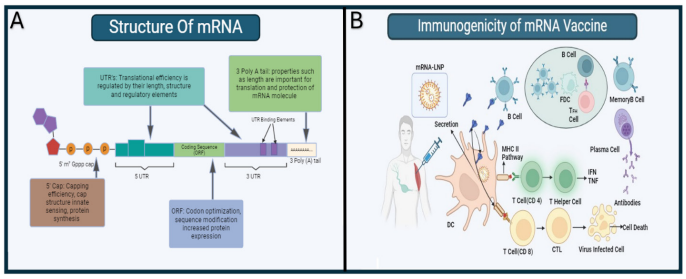

Figure 2: (www.biorender.com) (A) Structure of mRNA. Five different parts are In Vitro-Transcribed (IVT) mRNA: a 5' cap containing a 7-methylguanosine connected by a triphosphate bridge to a 2'-O-methylated nucleoside. The poly(A) tail is located at the end, along with the 5' and 3' *U*ntranslated *R*egions (UTRs) and a coding region called an *O*pen *R*eading *F*rame (ORF). Together, these elements guarantee that the mRNA performs as intended throughout biological processes. **(B) Immunogenicity of mRNA vaccine**. Following mRNA vaccination, cognate B-cells recognise the spike antigens that are produced. They produce both a robust germinal center reaction and a sizable neutralizing antibody reaction. By absorbing soluble spike antigens, Dendritic Cells (DCs) trigger antigen-specific CD4+ and CD8+ T cells through the cross-presentation and MHC II pathways. After DCs naturally generate spike proteins may also activate antigen-specific CD8 T-cells through the MHC I pathway.

5' Cap

To preserve mRNA's survival and enable effective protein synthesis, a 5' cap and a poly(A) tail have been added as post-translational modifications. For efficient mRNA translation, the 5' cap structure must be operating correctly. The component of this cap structure is a molecule called 7-methylguanosine (m7G), which is found in the cytoplasm of eukaryotic cells at the end of the 5' of normal mRNA. A triphosphate link to the primary nucleotide (m7GPPPN) comes next [34]. In eukaryotic cells, transcription involves the utilization of three different types of caps to modify the 5' end of mRNA molecules. These caps have distinct structures and go by labels such as Cap-0, Cap-1, and Cap-2. Cap-0 is denoted by m7GppXpYp, Cap-1 by m7GpppXmpYp, and Cap-2 by m7GpppXmpYmp. The fact that no unbound terminal phosphate groups remain at the 5' end of mRNA after the addition of any one of these three caps is a critical function of mRNA capping. Because of this characteristic, the mRNA is very resistant to the phosphate group-cleaving enzyme Alkaline Phosphatase (AKP). The free 2'OH-group on the phosphodiester link is prevented by the methyl groups on the two nucleotides that follow Cap-1 and Cap-2. The structural alteration improves the mRNA's stability against several ribonucleases like RNase, RNaseT1, and RNaseT2, which would otherwise decay RNA molecules [61]. To make sure that the methyl groups connect with the hydroxyl groups at the proper sites during transcription, researchers modified the anti-reverse cap analog (ARCA) molecule at either the C2 or C3 positions. This modified type of ARCA-capped mRNA has higher translation efficiency than its conventional cap counterpart. Recently, scientists have been working on additional alterations to the ARCA structure to boost mRNA characteristics [16]. During mRNA in Vitro Transcription (IVT), the researchers examined the impact of varying the first transcribed nucleotides (A, m6A, G, C, and U) either with or without the incorporation of 2'-O-methylation. When lipofectamine was used to transfer the mRNA encoding Am, A, or m6Am, the first nucleotide led to enhanced luciferase expression. Lower levels of luciferase expression were seen in mRNA containing G or Gm as the primary nucleotide. When examining mRNA translation in the JAWSII dendritic cell line, this variation was particularly apparent due to an eight-fold difference between the m6A and m6Am 5' caps. These results emphasize how crucial the 5' capping structure is for effectively identifying dendritic cells and inducing the best possible immune response [9].

5' UTR

The 5' Untranslated Region (5'UTR) is a critical component that regulates the translation process' efficiency in this region. Scientists have discovered various regulatory components that influence this process [72]. These include the m7G cap, secondary structures, Upstream Open Reading Frame (uORF), Internal Ribosome Entry Site (IRES), Zip code, Cytoplasmic Polyadenylation Element (CPE), and polyadenylation signal. UTRs are not bound by ribosomes, making them accessible to regulatory factors. The 5' UTR sequence determines ribosome initiation, efficiency, and site selection. Translation initiation involves a pre-initiation complex scanning the 5' UTR, but high Guanine (G) Cytosine (C) content can hinder this process. Predicting RNA structure in 5' UTRs considers factors like folding energy and GC content. Complex GC-rich structures in 5' UTRs can inhibit translation, as seen in guanine decarboxylase 36 mRNA [61]. The 5' UTR can be altered to improve the stability of mRNA and translation accuracy. This entails avoiding non-standard and

start codons, avoiding extremely durable secondary structures, and preferring shorter 5′ UTR sequences since they have been shown to enhance mRNA translation [21]. To enhance the effective translation, it is a common practice to incorporate the Kozak sequence adjacent to the 5′ UTR sequence [13].

3′ UTR

The 3C-Untranslated Regions (3C-UTRs) of the α- and β-globin mRNAs are found in many IVT mRNA molecules. These domains contain distinct genetic elements that support mRNA stability and translation (Chang Kim et al., 2022). Adenylate-uridylate-rich regions are important for mRNA breakdown. Changing these sequences in the 3′ UTR allows you to control the rate of mRNA degradation and the duration of the translation process [47]. These regulatory elements, which can originate from eukaryotic or viral genes, effectively enhance the stability and production of therapeutic mRNA molecules, greatly extending their half-life [8].

Open reading Frame (ORF)

The mRNA vaccine's Open Reading Frame (ORF) has great importance as it holds the essential code that gets translated into a protein. This region isn't as flexible as non-coding portions; improvement can be made by optimizing it. To enhance the translation process without changing the protein's composition, the open reading frame can be improved by replacing infrequently used codons with more commonly utilized codons that still code for the same amino acids [20]. The presence of UU or UA sequences within the ORF harms both mRNA protein expression and stability. This is thought to occur because these sequences are more exposed to endonuclease enzymes, which can lead to degradation of the mRNA and reduced protein production [36]. Optimal codons near the start of a gene enhance translation speed and protein production. Rare codons slow translation, potentially leading to mRNA degradation. High translation speed can disrupt protein folding, reducing protein activity, as seen in codon-optimized firefly luciferase mRNA [65].

Poly a tail

With a few exceptions, like histones, most cellular proteins that encode RNAs have poly(A) tails as a regular structural feature. Following transcription, these polys (A) tails are attached to mRNA in the cell nucleus and are found downstream of a specific genetic signal called the polyadenylation signal (AATAAA) [25]. These mRNAs are not stable without the poly(A) tail. The 60–150 nucleotide poly-A tail is critical for maintaining mRNA stability, facilitating translation, and enabling recognition by the Poly-A Binding Protein (PABP). In the mRNA molecule, PABP forms a structure resembling a loop by interacting with the translation initiation complex (eIF4G) [21]. During transcription, the poly(A) tail can be integrated into the template DNA. Alternatively, it can be added to mRNA enzymatically using a recombinant poly(A) polymerase. Recombinant poly(A) polymerase binds altered nucleotides to the poly(A) tail through the process of de-adenylation to prevent poly(A)-specific enzymes from breaking down the mRNA (Chang Kim et al., 2022). Translation efficiency declines when the Poly(A) sequence in mRNA is shorter than 20 nucleotides [16]. In mammalian cells, actively translated mRNAs normally include 100 to 250 adenosine residues in their poly(A) tails. It is important to uphold an ideal length for this poly(A) tail to enhance translation effectiveness and mRNA integrity. Research has indicated that the production of the protein that is encoded by that mRNA increases as the size of the poly(A) tail reaches about 120 base pairs (bp). However, further elongation does not lead to increased synthesis of the related protein after the poly(A) tail surpasses 120 bp. Other than their length, poly(A) tails can be created in a variety of ways [46].

Immunogenicity of mRNA vaccine

The administration of mRNA vaccines induces both innate and adaptive immune responses, as illustrated in Figure 2. B. The immunological response induced by the mRNA vaccination is not completely understood. Current research reveals that mRNA vaccination activates innate immunity by engaging with two separate types of RNA sensors: endosomal Toll-Like Receptors (TLRs) present inside the endosomal compartment and cytoplasmic Retinoic Acid-Inducible Gene-I-like Receptors (RLRs) [39]. An acute local inflammatory response is triggered upon injection of a Lipid Nanoparticle (LNP) mRNA vaccine into muscle tissue, mobilizing monocytes, dendritic cells, and neutrophils to the injection site shown in Figure 2 B. These recruited monocytes and dendritic cells absorb the LNP-formulated mRNA vaccine and travel to neighboring lymph nodes, where they deliver antigens to T cells. The stimulation of the innate immune system causes the creation of antibodies [54]. The TLR3 on dendritic cells, macrophages, and monocytes can detect single-stranded (ssRNA) and Double-Stranded RNA (dsRNA). TLR7, TLR8, and TLR9 are expressed by macrophages. TLR7 recognizes both dsRNA and ssRNA, but TLR8 only recognizes ssRNA. The RIG-I family, which includes RIG-I, MDA-5, and LGP2, enhances interferon production by detecting both single-stranded and double-stranded RNA [16].

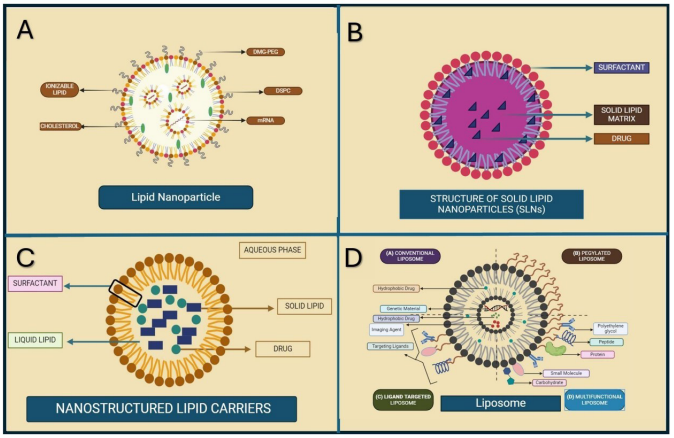

Figure 3: Nanoparticles (https://www.biorender.com/). (A) Lipid nanoparticle. To administer mRNA vaccines, lipid nanoparticles are used. They consist of (a)ionizable lipids, (b)cholesterol, (c) phospholipids, and (d) PEG-lipid. Ionizable lipids can bind to RNA when they are positively charged at low pH, and they can be safely delivered to cells when they are neutral at physiological pH. Structure and stability are provided by phospholipids and cholesterol, and an effective and secure profile for mRNA vaccine administration inside the cell is guaranteed by lipid-anchored PEGs, which inhibit nonspecific protein binding. **(B) Structure of Solid Lipid Nanoparticles (SLNs). (C) Non-structured lipid carriers**. NLCs are tiny particles that are made up of (a) a solid lipid, (b)a liquid lipid (oil), (c) an active drug ingredient, (d) a surfactant to stabilize the structure, and (e) an aqueous phase. These carriers provide an efficient way to deliver drugs into the cells. **(D) Structure of Liposome.** (a)Conventional liposomes are like tiny bubbles made of phospholipids. (b) Pegylated or stealth liposomes have an extra layer of Polyethene Glycol (PEG) to help them stay hidden from the body's defence system. (c) Targeted liposomes are designed with a special part that can lock onto cancer cells. (d) Multifunctional liposomes can both diagnose and treat solid tumours at the same time.

mRNA vaccine carriers

The primary hurdle in gaining clinical approval for mRNA vaccines lies in the complications of their delivery inside the cells. Due to its weakness in enzymatic degradation and by ubiquitous ribonucleases. So, mRNA faces instability under physiological conditions, which is not suitable for widespread therapeutic use [74]. In recent years, advances in nanoparticle technology have knocked down biological barriers at the cellular level. Nanoparticles increase drug solubility, allow mRNA evasion by the immune system, and extend the drug's half-life in the body. This also allows for accurate medication delivery to specific cells as well as regulated drug release for the best therapeutic effects [62]. Addressing these issues, such as reaching the correct target, stability, and endosomal escape, is critical for improving mRNA delivery methods. To address these issues, researchers have investigated several biocompatible and biodegradable components, including lipids, polymers, and protein-based derivatives, to create safe and effective mRNA medicines for mRNA-based therapeutics [64]. Some of these nanoparticles are shown in Figure 3.

Lipid nanoparticle

Lipid Nanoparticles (LNPs) carry a range of medicinal components. They have become more important in the pharmaceutical sector. Interestingly, they have gained a great deal of attention because of their vital role in the COVID-19 mRNA vaccines, in which they are mostly used to successfully preserve and transfer mRNA to cells [23]. A major turning point occurred in 1989 when the positively charged lipid DOTMA and its synthesized component DOTAP became the first lipids to be employed to transport mRNA into the cell [20]. Long-lasting protection can be achieved using LNP-based mRNA vaccines, which can shield the mRNA they carry from the host's environment and then progressively deliver it within the cell [71].

LNPs have many advantages over other nano-carriers. LNPs work better for large-scale industrial manufacturing approaches like microfluidics and are more economical and stable. Furthermore, compared to liposomes, LNPs show reduced cytotoxicity and immunogenicity [12]. LNPs are intricate lipid-based systems made up of several components, such as the PEG-lipids, cholesterol, phospholipids, and ionizable lipids shown in Figure 3A. Ionizable lipids play a key role in adhering to mRNA, generating the core structure shown in Figure 3. A. Helper lipids, such as cholesterol and phospholipids, surround this core. The nanoparticle's exterior layer is shown in Figure 3. A is shielded by the PEG lipids. Currently, little is known about the function of cholesterol in LNPs as well as how their shape affects cellular absorption and endosome escape [42]. Lipid colloidal carriers are another term for lipid-based nanoparticles. They are a particular class of colloidal particles consisting of a surfactant-stabilized lipid core. These nanoparticles range in size from 40 nanometers to 1000 nanometers and exhibit stability even at room temperature. Solid lipids, surfactants, and, if required, cosurfactants, in addition to the active chemicals they are intended to convey, are the essential components of LNP carriers. It is crucial to select the correct lipids for the formulation. Fatty alcohols, acylglycerols, fatty acid esters, and mixtures of acylglycerol esters are often utilized lipids for LNP production [41]. LNPs are created by introducing lipids that can ionize or carry a positive charge into liposomes. This allows them to encase oligonucleotides with a negative charge through electrostatic interactions [35].

Solid lipid nanoparticle:

Cosmetic active components with poor water solubility can be effectively transported by using Solid Lipid Nanoparticles (SLNs) [43]. During the 1990s, a novel drug delivery mechanism known as SLNs emerged. SLNs typically have a spherical structure with a diameter ranging from 50 to 1000 nanometers. These nanoparticles offer a promising solution by multiple advantageous characteristics found in polymeric nanoparticles. The low toxicity, targeted drug delivery, regulated release of the pharmaceutical components encased in it, large drug-carrying capacity, and protection against drug degradation are among the benefits of SLNs [35].

Common drawbacks of SLNs are the enlargement of lipid particles, a propensity to form gels, challenges in managing polymorphic transitions, and their limited ability to incorporate components effectively due to the crystalline nature of the solid lipid matrix [6].

Non-structured lipid carriers (NLC)

Nanostructured Lipid Carriers (NLC) are the second generation of lipid nanoparticles and were produced to increase the characteristics of SLNs [26]. NLCs represent an evolution of SLNs with a unique composition. They consist of both solid and liquid components, typically including oils, resulting in a semi-solid matrix without a defined structure. This composition serves to enhance both the stability and the capacity for loading active ingredients [6]. NLCs have found vast pharmaceutical applications in a wide range of medical conditions. These applications include hypertension, diabetes, Parkinson's disease, epilepsy, high cholesterol, cancer, hair loss, hormone imbalances, skin inflammation, eye-related issues, liver diseases, and fungal infections [28]. NLCs have certain limitations, including cytotoxic effects caused by both the matrix's composition and concentration. Certain surfactants cause irritation and sensitivity. There is also a deficiency in clinical and preclinical research in the formulation of NLCs [26].

Liposomes

At the Cambridge Babraham Institute, British haematologist Dr. Alec D. Bangham first described liposomes in 1961. G. Gregoriadis later proposed the novel concept of using liposomes as drug-delivery vehicles in the early 1970s [12]. Liposomes were created before LNPs, which are extremely versatile nanocarrier systems. They may carry both hydrophobic and hydrophilic items, such as tiny molecules, proteins, and nucleic acids. Liposomes were one of the first nanomedicine delivery technologies to go from theoretical considerations to real clinical uses [28]. Liposomes are tiny sacs consisting of a membrane composed of phospholipid bilayers, closely related to cell membranes on a microscopic scale shown in Figure 3D [12]. Liposome carrier systems showed some shortcomings like fast drug release, vulnerability to destabilization, and susceptibility to oxidation [35].

Liposomes are typically well-tolerated by living organisms, and utilizing liposomes as a vehicle for drug delivery offers numerous benefits over administering drugs in their free form. Liposomes enhance drug uptake by tumors and extend their circulation half-life in the bloodstream. Intertumoral administration of mRNA encapsulated in the liposomal complexes proves a remarkably efficient method for achieving localized tumor transfection within the tumor microenvironment [74].

Comparison of different mRNA vaccine carriers

mRNA serves as a template for the synthesis of proteins and is becoming an important active molecule in combating illnesses such as viral infection and cancer. However, the basic limitations, such as negative charge, and instability, make it unsuitable as a therapeutic agent. Allergies, renal failure, and heart failure remain risks; the mRNA vaccine may also break down fast after injection or trigger cytokine storms. This is a significant challenge to mRNA delivery. However, suitable carriers can prevent degradation while improving immune responses, effector presentation, biocompatibility, and biosafety [34,49,73]. Different types of mRNA vaccine carriers, their characteristics, pros, and cons are mentioned in Table 2.

Table 2: Different mRNA vaccine carriers.

mRNA vaccine carriers	Features	Advantages	Disadvantages	References
Lipid particles	Spherical vesicles with a lipid bilayer	Low toxicity Excellent biocompatibility mRNA protection	Low entrapment efficiency	(Liang et al., n.d.)
Cationic Lipid Particles	Like lipid particles but with a positive charge	Higher entrapment efficiency	Significant toxicity	(*Understanding Six Types of Vaccine Technologies*, n.d.)
Lipoplexes	Multilayered spherical form	Improved endosome escape Less toxic	Instability under nonfreezing conditions	(Zeng et al., n.d.)
Solid lipid nanoparticles	Solid core covered by a lipid coating	Enhanced physical stability	Limited availability of bigger mRNA molecules	(Zeng et al., n.d.)
Polymers	Charge interaction with mRNA	Biodegradable Diverse chemical options	Variable efficacy	(Pascolo et al., 2021b)
Peptides	Positive charge interacts with the negative backbone of mRNA	Good tissue penetration Presumably safe	Chemical engineering is complex Variable performance	(*Understanding Six Types of Vaccine Technologies*, n.d.)
Naked mRNA	Without additional carrier	Simple manufacturing	Fast breakdown Low bioavailability	(Pascolo et al., 2021a)

Types of mRNA vaccine

mRNA vaccines like the ones used against COVID-19 were the first to get approved on an emergency basis, but there is still a lot for improvement. There are three types of mRNA vaccines, which are discussed below.

Synthetic mRNA vaccine

Martinon and Colleagues 1993 were the first to use non-self-replicating RNA/synthetic RNA to generate vaccines [59]. Functional synthetic mRNA is synthesized by utilizing IVT with bacteriophage RNA polymerases like SP6 or T7 to transcribe from a cDNA template. To ensure systematic translation in eukaryotic systems, the minimal design includes integrating a 5′ cap and a lengthy 3′ poly(A) tail, with these elements acting synergistically to support the translation process [49].

Synthetic mRNA vaccines have many advantages, such as rapid and adaptable production, which makes them efficient in responding to new pathogens. They exhibit exceptional stability in vitro that allows them to be easily handled and stored even at room temperature. The transient nature of mRNA in the body ensures safe and well-defined therapeutics that make these vaccines safe and more controlled for repeated use [36].

Self-amplifying RNA (saRNA) vaccine

Self-amplifying RNA (saRNA) is an innovative platform for vaccine development, utilizing technology like mRNA but distinguished by its inherent self-amplifying capability. This unique feature allows saRNA to yield raised protein levels per dose, enabling the capacity for administration at lower concentrations compared to conventional mRNA therapies. This translates into less frequent and reduced higher dosage requirements and holds the promise of lowering costs and expanding the scope of applications, making saRNA vaccines more efficient and potentially quicker to develop, with a heightened immune response from smaller doses than their mRNA counterparts [10]. Replicons, the next generation of self-amplifying mRNA, emerge as the best vaccine platform since they boost cellular and humoral immune responses with few side effects after a single dosage. Replicons are transmitted by nonviral carriers such as lipid nanoparticles or liposomes, or by Virus-like Replicon Particles (VRPs) [40]. saRNA is noticeably more complicated than standard mRNA, with a bigger structure encompassing 9000 to 12,000 nucleotides. Due to this, adding additional components to the standard mRNA's core a cap, 5′ UTR, an ORF containing the Gene Of Interest (GOI), 3′ UTR, and a poly(A) tail became more difficult [51].

Circular RNA vaccine

In 1976, the first time, circular RNA was detected in pathogens and was reported as a viroid. In 1979, a second study was conducted that described that it does not contain any free ends, and its circular shape does not depend on associated proteins [80]. Over time, the advancement in RNA-sequencing technologies and bioinformatics applications has revealed that circular RNA (circRNA) is a prevalent characteristic within the human transcriptome and is widely present in other multicellular organisms. circRNAs have been shown in recent research to have a variety of activities, including that of protein scaffolds, miRNA sponges, and possible polypeptide translation [80]. The synthesis of circRNA has only been explained by three mechanisms so far: lariat-driven, RNA-Binding Protein (RBP)-mediated, and intron-pairing-driven. However, the mechanism of circular RNA synthesis remains mostly unknown [79]. The area of circRNA therapies is in its early stages, with numerous major hurdles that must be overcome for effective development. These problems include the requirement for creative circRNA design and optimization, assuring circularization efficiency, and building strong Chemical Manufacturing and Control (CMC) methods for circRNA production. For circRNAs to be used therapeutically in the medical field, several obstacles must be overcome [2].

Advantages and Disadvantages of mRNA vaccine

The pharmaceutical industry has invested much in the development of mRNA vaccines. However, like other vaccinations, mRNA vaccines might induce negative effects in the days following their administration. In recent years, significant progress has been achieved in mRNA vaccine development, particularly in personalized tumor vaccines. mRNA vaccines are a promising method since the manufacturing process is easy, the safety profile is superior to that of DNA vaccines, and mRNA-encoded antigens are easily produced in cells. However, mRNA vaccines do have some limitations [49,69,74].

Table 3: Advantages and disadvantages of mRNA vaccines.

Advantages	Disadvantages	References
Non-infectious	Naked RNA degradation by extracellular and intracellular RNases.	(G. Zhang et al., 2023)
Do not integrate into the genome	Only a few efficacy reports, and no long-term safety profile.	(Pardi et al., 2018b)
Rapid production	Delivery vehicle needed	(Zeng et al., n.d.)
Cell-free reaction for production	Cell or tissue-specific delivery	(Whitley et al., 2022)
Can be produced on a large scale	A strict cold chain is required	(*Understanding Six Types of Vaccine Technologies*, n.d.)
Stability for long-time		(Pardi et al., 2018c)
Induction of both B and T-cell responses		(Whitley et al., 2022)
Can easily be produced and engineered		(Zeng et al., n.d.)
Cost-effective		(G. Zhang et al., 2023)
More flexible than protein drugs		(Zeng et al., n.d.)

mRNA vaccine manufacturing

mRNA vaccines showed some distinct advantages over other conventional vaccines. Their simplified development process, efficient scalability, and rapid manufacturing. The manufacturing process for mRNA vaccine drug products follows a standard 3-step process

(a) upstream production

(b) downstream purification

(c) formulation of the mRNA drug substance.

Diagrammatically the mRNA manufacturing is shown in Figure 4.

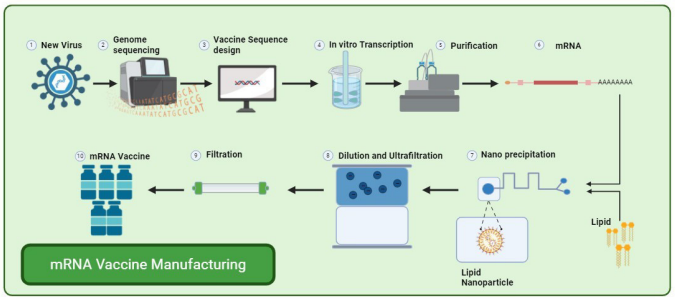

Figure 4: ***(https://www.biorender.com/)*** **mRNA vaccine manufacturing.** Scientists develop mRNA vaccines through different steps. (a) Once they come to know the genetic code of a virus, they design a specific sequence for the target antigen and place it in plasmid DNA. (b) This DNA is then transformed into mRNA using bacteriophage polymerases in a lab. (c) The resulting mRNA is purified using High-Performance Liquid Chromatography (HPLC) to get rid of impurities. (d) The purified mRNA is combined with lipids in a microfluidic mixer forming lipid nanoparticles as a protective coating for the mRNA. (e) To remove extra substances the nanoparticle solution undergoes dialysis or filtration. (f) The final filtered mRNA vaccine solution is stored in sterilized vials for use.

Upstream process

The starting phase of the upstream process consists of three key enzymatic stages. 1. plasmid linearization. 2. mRNA transcription. 3. DNA template digestion [56] The process of In Vitro Transcription (IVT) for mRNA synthesis utilizes T7, SP6, or T3 RNA polymerases to catalyze the conversion of a pre-prepared DNA template into the desired mRNA. This template is classically generated either through the linearization of a purified plasmid or PCR amplification of the specific region of interest. In addition to the linear DNA template, the essential components for IVT include an RNA polymerase, Nucleotide Triphosphates (NTPs) as substrates, Magnesium Chloride ($MgCl_2$) as a cofactor for the polymerase, and a pH buffer containing polyamine and antioxidants [13].

Downstream process

The downstream process involves addressing precipitation issues, selecting appropriate resins, the initial RNA reaction, and incorporating a chromatography step flanked by two Tangential Flow Filtrations (TFF) or Ultrafiltration (UF) and Diafiltration (DF)) to eliminate 1. Enzyme resides. 2. Residual DNA 3. Unwanted High Molecular Weight (HMW) species [56]. However, these techniques fail to eliminate abnormal transcription byproducts such as Double-Stranded RNA (dsRNA) and truncated RNA fragments. The presence of these abnormal products can trigger the host's innate immune responses that lead to inflammation and diminish the translation efficiency of the delivered mRNA. Previous investigations demonstrated a substantial enhancement in protein yield, ranging from 10 to 1000 times when employing purification methods like reverse phase HPLC, magnetic beads, anion exchange, ultrafiltration, or dialysis [14].

Formulation

To protect the mRNA molecules that have a negative charge from degradation and to enhance their activity and half-life, a lipid-based delivery system is employed for the drug. Among these systems, LNPs are the most valid carriers and FDA-approved non-viral carriers that carry mRNA vaccine inside the

cell. The formation of mRNA LNPs involves bringing on lipids in an organic phase and mixing them in an aqueous phase with mRNA. The common lipids present in the organic phase are ionizable lipids, cholesterol, helper lipids, and PEG lipids. The mRNA is dissolved at a low pH in a citrate or acetate buffer. The interaction between the protonated ionizable lipid and the anionic mRNA, coupled with hydrophobic interactions, drives the spontaneous self-assembly of mRNA-LNPs. This process is known as microprecipitation. To remove the non-aqueous solvent after LNP formulation dialysis is carried out. The utilization of microfluidic mixers is prevalent for achieving small-sized LNPs with low polydispersity and high mRNA encapsulation efficiency during both laboratory-scale formulation and GMP-level production. Precision NanoSystems' NanoAssemblr® platform is greatly employed for the development of LNP formulation and GMP production in controlled environments [5]. Adjusting NanoAssemblr® settings allows for easy control of aqueous and non-aqueous phase flow rates and volumes that enable the production of LNPs with desired size and distribution. Typically, a total flow rate of 12–14 mL/min and a 3:1 flow rate volume ratio of non-aqueous to the aqueous phase is employed for generating small, uniform LNPs. While Simple Harmonic Motion (SHMs) offers efficient LNP production. LNP use in GMP manufacturing is forced by solvent incompatibility that leads to potential deformation with prolonged exposure to ethanol therefore T-mixers are preferred for scale-up capable of handling higher flow rates (60–80 mL/min), and organic solvents like ethanol [66,77].

Challenges in mRNA vaccine stability and its storage and transportation temperature

An mRNA-based vaccine is a promising approach that can be quickly developed but its use is limited because mRNA can be degraded easily by RNase enzymes. To prevent mRNA-based vaccines from degradation the vaccine preparation, storage, and administration must be conducted in a very clean, separate, and RNase-free environment. It requires very sterile equipment throughout the development process to the administration process. While RNA is stable under certain conditions like heat or freezing. It can be damaged by RNase enzymes and is prone to hydrolysis at pH levels higher than 6 [30].

Pfizer has developed specialized thermal containers that contain dry ice to distribute its COVID-19 mRNA vaccine throughout the world. It originally required ultra-cold temperatures ranging from -90°C to -60°C for shipment and long-term storage. The alternative way to transport the vaccine is at temperatures between -25°C and -15°C but its storage time is up to 2 weeks at commonly -20°C. While transportation at 2°C to 8°C is possible it should be completed within 12 hours and unpunctured vials can be stored at this temperature for up to 1 month. Once vials are opened and mixed with the diluent, the vaccine must be used within 6 hours at room temperature and should not be refrozen after thawing. Its exposure to sunlight should be avoided strictly [17].

Moderna's COVID-19 vaccine shows that storing it at a temperature of -20°C ensures long-term stability for up to six months. Moreover, the company has also announced an extended shelf life for the vaccine when kept at refrigerated temperatures between 2°C and 8°C. This means that the vaccine remains stable for 30 days under these conditions and provides more flexibility in the storage, usage, and distribution of mRNA vaccine [44].

Administration routes of mRNA vaccine

The effectiveness of mRNA vaccines greatly depends on the administration route in the body. They are injected directly into the bloodstream for widespread impact or at a specific body site like the skin or muscles. The anatomical and physiological characteristics of the chosen location greatly influence the safety and efficacy of the mRNA vaccine [7].

Systemic mRNA vaccine transport and expression can occur through various routes that consist of tracheal inhalation, intravenous, intraperitoneal, and intramuscular injections [61].

Administration of mRNA vaccine through muscles

The most common way to inject mRNA vaccines is through intramuscular injection. Muscles have a lot of blood vessels and immune cells that help the vaccine work, and studies showed that the vaccine stays in the muscle and nearby lymph nodes for around 28 hours. This method has been effective in providing immunity against the SARS-CoV-2 virus [7].

Intravenous administration of mRNA vaccine

Intravenous injection of mRNA is highly effective in producing proteins inside the body especially when targeting the liver. Intravenous mRNA vaccines are less preferred due to their systemic side effects. They can induce adaptive immune responses by targeting the spleen. This approach is being explored in clinical trials for treating melanoma and triple-negative breast cancer, but still further optimization is required to enhance targeted delivery and minimize the side effects associated with it (*Advantages of Intranasal Vaccination and Considerations on Device Selection | Request PDF*, n.d.).

Intravenous injections are highly effective because they allow mRNA vaccines to quickly reach the target site via blood circulation and enable sustained protein expression in the liver for up to 4 days [61].

Intranasal administration of mRNA vaccine

Intranasal vaccines offer a convenient and less invasive way to protect against different infections. It stimulates strong immune responses through the nasal mucosa. This method is beneficial for groups like children, aged people, HIV-infected individuals, and those with multiple health conditions because it is too easy to administer and avoids the fear and discomfort often associated with injections [63].

Delivering IVT-mRNA to the lungs, especially through spray, presents great hurdles due to possible problems with the structure during aerosolization and complex interactions with the diverse biological components of the airway [33].

mRNA-based vaccine for foot and mouth disease (FMD)

Cloven-hooved Animals and 70 other species are susceptible to FMD [52]. The causative agent of FMD is the Foot-and-Mouth Disease Virus (FMDV), which is a member of the *Aphthovirus* genus within the *Picornaviridae* family. The FMDV virus has seven distinct serotypes that are found around the world: A, O, C, Asia 1, SAT 1, SAT 2, and SAT 3. Because there are differences in their antigenic composition, each serotype has some subtypes that are not cross-protective [24].

FMD is one of the biggest challenges to the livestock sector globally. Given the significant economic impact of FMD, many countries have established FMD control programs, including

vaccination campaigns, improved surveillance systems, and contingency plans. These measures help to minimize the risk of outbreaks, facilitate early detection, and support prompt and effective response strategies to mitigate the disease's impact on livestock production and international trade [70].

The efficiency of immune responses elicited by a particular FMDV strain against others is challenged by the significant variation in antigens found simultaneously between and within serotypes, potentially reducing cross-protection. Thus, to maximize vaccination strategies, it is crucial to assess the antigenic and immunogenic resemblance between the vaccine strain and field strains and make sure that the vaccine correlates with circulating field strains. This highlights the significance of vaccines having a wide antigenic coverage to improve cross-protection [3]. Baby Hamster Kidney (BHK) cells are used to cultivate the infectious virus to produce FMD vaccines in high-containment environments. After the viral particles are chemically inactivated by changing the viral RNA with Binary Ethyleneimine (BEI), non-structural viral proteins are removed by filtration. Before use, the vaccine is mixed with an adjuvant, which can be an aqueous solution or an oil that contains saponin and aluminum hydroxide [53]. There are several issues with the FMD vaccinations available today (a) Increased risk of virulent FMDV escaping from industrial facilities (b) Insufficiently immunogenic (c) The challenges associated with using serology to differentiate between vaccinated and diseased animals (d) Other major problems in the current vaccination formulations is heat instability [39].

Many attempts have been carried out over the past ten years to improve the safety and effectiveness of FMD vaccinations. An early 2010 study showed that mice genetically modified to express full-length FMDV mRNA strengthened their immune responses, pointing to the possibility of creating RNA-based vaccinations that would act within their natural host. These results demonstrated encouraging developments in the next-generation FMD vaccines, which had increased effectiveness and safety [55]. In a study, the researchers evaluated the antiviral capabilities of artificial non-infectious RNA molecules in FMDV-infected mice. Interestingly, they discovered widespread protection against viral infection from a few distinct serotypes by injecting transcripts matching the IRES [4]. Impressively, the mice did not produce neutralizing antibodies, suggesting that the innate immune response was initiated by IRES. RNA played a key role in safeguarding against FMDV. Different experiments revealed the immunomodulatory effects of these synthetic RNA molecules, as demonstrated by a significant increase in specific anti-FMDV antibody titers in mice co-administered with FMDV vaccine and RNA molecules.

The provided data offers a positive approach for advancing mRNA-based FMD vaccines but underlies the necessity for innovative approaches. Current studies have demonstrated the effectiveness of an empty capsid vaccination against FMD in its natural host, which is produced by co-expressing the FMDV P1-2A capsid precursor and 3Cpro utilizing baculovirus or vaccinia virus expression systems. It may be possible to develop a synthetic thermostable RNA vaccine using the FMDV P1-2A and 3Cpro encoding genes by focusing on the advancements made in COVID-19 mRNA vaccines. Such a vaccine holds promise for safeguarding livestock against different emerging strains of FMD. Notably, the mRNA vaccine presents advantages over traditional vaccines, except for the requirement for an infectious virus or cell culture, thereby offering a more versatile solution [39].

Future prospects

Continuous research in mRNA vaccine production aims to make the process continuous, reducing time and cost-effective than other conventional vaccines. Different Safety concerns are associated with mRNA vaccines, like adverse reactions and myocarditis. So, more research is needed in this area to improve vaccine confidence in the population. Strategies to boost vaccine effectiveness and produce long-lasting immunity include exploring booster schedules of vaccination. Understanding the whole mechanism behind how mRNA vaccines work guides us to improve memory immune responses. Future possibilities for FMD vaccines offer intriguing possibilities for research and development. First and foremost, efforts should be focused on improving the stability and storage conditions of mRNA vaccines. Advancements in storage technology might alleviate the existing constraints associated with ultra-cold storage requirements, making these vaccines more accessible in a variety of contexts. Furthermore, advances in nanoparticle technology, particularly LNPs, should improve the delivery efficiency of mRNA vaccines, assuring precise targeting and long-term effectiveness. Alternative administration methods, such as mucosal delivery, should be studied to improve the ease and efficacy of FMD immunization. Further study into the creation of new adjuvants and formulations targeted to FMD-specific antigens may improve the immunogenicity of mRNA vaccines. Long-term research to assess the durability of immune responses and potential adverse effects will be critical in determining the safety and efficacy of mRNA vaccines against FMD. Collaboration among virologists, immunologists, and nanotechnologists is critical for pushing the frontiers of mRNA vaccine design and production. Finally, as global vaccine distribution becomes more important, future research efforts should prioritize large-scale production, cost-effectiveness, and accessibility in resource-limited regions to fully realize the potential of mRNA vaccines against FMD.

Conclusion

mRNA vaccines have a great possibility to fight against cancer and different infectious diseases. Their adaptability, flexibility, and quick manufacturing process make them valuable from other conventional vaccines in preventing infectious diseases and providing treatments. All the vaccines up to now for FMD are inactivated. They have a great chance of reverting and other drawbacks. So, the mRNA vaccine will provide another gateway for strategic prevention of FMD.

Author declarations

Competing interests

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Authors contribution

Kashif Abdaal conducted the primary literature search, collected references, and contributed to drafting the sections on mRNA vaccine history and structure, while Aneeza Batool worked on the immunogenicity and carriers of mRNA vaccines and assisted in figure preparation and formatting. Abdul Aziz Khan drafted the sections on mRNA vaccine manufacturing, advantages and disadvantages, and future prospects, and also contributed to manuscript editing. Muhammad Armaghan Kha-

lid assisted in the literature review, compiled tables, and critically revised the manuscript for intellectual content, whereas Ali Asghar contributed to writing the section on mRNA-based vaccines for Foot-and-Mouth Disease (FMD) and helped refine the conclusion. Muhammad Tariq Navid conceived the idea for the review, supervised the overall work, provided critical guidance throughout manuscript preparation, and performed the final revision. All authors read and approved the final manuscript, agree to be accountable for their contributions, and ensure the accuracy and integrity of the work.

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