IJMCM
International Journal of Molecular and Cellular Medicine
p-ISSN: 2251-9637 e-ISSN: 2251-9645
Babol University of Medical Sciences
Cellular and Molecular Biology Research Center

Current Perspectives and Future Prospects of mRNA Vaccines against Viral Diseases: A Brief Review

Vasavi Koppu[1]*, Deepa Poloju[1], Bhavani Puvvala, Kavitha Madineni, Shilpa Balaji, Chandana Muraleedharan Pillai Sheela, Sri Sai Charan Manchikanti, Satpathy Moon Moon

Department of Veterinary Microbiology, ICAR-IVRI, Bareilly, Uttar Pradesh, India.

Article type:
Review Article
Received:
2022.08.12
Revised:
2022.12.25
Accepted:
2023.01.21

ABSTRACT

The mRNA vaccines replace our conventional vaccines (live-attenuated and inactivated vaccines) due to their high safety, efficacy, potency and low cost for their manufacturing. Since these many years, the use of these mRNA vaccines has been restricted as they are unstable and their low efficiency in *in-vivo* delivery. But now, these problems have been solved by recent technological advances. Many studies conducted in animal models and humans demonstrated the good results for the mRNA vaccines. This review provides you a detailed overview of mRNA viral vaccines and considers the current perspectives and future prospects.

Keywords: mRNA, vaccine, virus, lipid nanoparticle, codon optimization

Cite this article: Koppu. **V**. Current Perspectives and Future Prospects of mRNA Vaccines against Viral Diseases: A Brief Review. *International Journal of Molecular and Cellular Medicine*. *2022; 11(3):260-272.*

DOI: 10.22088/IJMCM.BUMS.11.3.260

© The Author(s).
Publisher: Babol University of Medical Sciences
This work is published as an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (http://creativecommons.org/licenses/by-nc/4). Non-commercial uses of the work are permitted, provided the original work is properly cited.

Introduction

An mRNA vaccine consists of mRNA encoded by an antigen of interest and is given along with an adjuvant or through some delivery system which finally enters the cells of the body and codes for the protein, that may be generally produced by a pathogen molecule i.e., virus or it may also be produced by a cancer cell. The protein molecules which are the building blocks of amino acids produced by the foreign antigen produce an adaptive immune response and it informs the body to identify and remove the virus or cancer cells.

mRNA vaccines have wide advantages and many uses over live attenuated virus vaccines, killed vaccines, subunit vaccines, and DNA-based vaccines. First of all is safety, as it does not integrate into the cell's DNA (non-integrating), they do not infect the cells (non-infectious) and they do not cause mutations on insertion (insertional mutagenesis). As we know mRNA is generally highly unstable and they are

***Corresponding Author:** Vasavi Koppu
Address: Current Perspectives and Future Prospects of mRNA Vaccines against Viral Diseases: A Brief Review.
E-mail: koppuvasavivet@gmail.com

[Downloaded from ijmcmed.org on 2026-10-10]
[DOI: 10.22088/IJMCM.BUMS.11.3.260]

destroyed by normal cellular functions and processes, so its stability and half-life in the body can increased through many modifications and by different delivery systems. The safety profile can be further increased via the down-modulation of the immunogenicity of the mRNA. The second point to be noted is that the higher the modifications/ changes, the higher the stability and translatability of the mRNA. The mRNA molecule along with the antigen of interest induces an immunogenic response that should be delivered efficiently by incorporating it into the appropriate carrier molecules so that it can be immediately taken up by the cells and allows its expression in the cytoplasm of the cells. mRNA vaccines should be administered repeatedly. Lastly, mRNA vaccines can be rapidly produced at low cost with high yields.

Timeline and Progression of mRNA Vaccines

In 1961, messenger RNA was discovered at the earliest by Sydney Brenner, Francois Jacob, and Matthew Messelson. mRNA was transfected into the cell successfully initially in 1989 by packing it with the liposomal nanoparticle (1). Artificially mRNA is made in the laboratory which is not packed with any material that was injected intramuscularly into the mice one year later, this study showed that genetic information is delivered into the living cells also with in-vitro transcribed mRNA along with the desired gene of interest and this led to approach of mRNA vaccine development.

In 1993, mRNA with the gene of interest is encapsulated with liposome that activated T-cells after entering into the body of mice. In the same year, there was the development of self-amplifying mRNA that consists of viral antigens along with a gene that encodes replicase enzyme. The same procedure was applied in mice and this induced both antibody-mediated and cell-mediated responses against the foreign antigen. In 1994, mRNA that codes for tumor antigens was injected into mice, which induced cell-mediated and humoral immune responses against the cancer cells.

Types and Formation of mRNA

The mRNA vaccines were found to be effective for the transfer of genes directly into the cells. At present, there are two types of mRNA established. The first type is Non-amplifying/Conventional mRNA vaccines and the other type is self-amplifying mRNA vaccines. The latter ones are obtained from positive-stranded RNA viruses. Although the concept and proposal of mRNA vaccines had already been made in the early 1990s, these mRNA vaccines are not widely used as they are highly unstable and degraded by cellular RNases which are present in the cells normally and lack production at large scale.

In 1995, Ross and group proved that stability and transfection efficiency both can be increased by Codon optimization and modification of nucleosides.

Conventional mRNA vaccines consist of one open reading frame encoding for the antigen of interest, flanked on either side by untranslated regions, and also contains poly $(AAAA)_n$ tail at last. These vaccines cause the expression of antigens and induction of immune response immediately after transfection into the cells.

The self-amplifying mRNA vaccines are engineered/derived from the genome of positive-stranded viruses Sindbis virus, semliki forest virus, Kunjin virus, and others, the most frequently used ones are Alphaviruses. Generally, these mRNA vaccines construct contain structural proteins ,but they are replaced by a gene of interest, untranslated regions flanked on both sides, 3^1 poly-A tail, and non-structural proteins for self-replication, so-called self-amplifying mRNAs, and they replicate through an enzyme called RNA-

[Downloaded from ijmcmed.org on 2026-10-10]

[DOI: 10.22088/IJMCM.BUMS.11.3.260]

dependent RNA polymerase, resulting genomes are called as replicons. (Figure 1) (2) After the entry into the cytoplasm of the host cells, they produce multiple copies of the gene of interest resulting in the amplification of gene expression, inducing both cellular and humoral immune responses. This is similar to the generation of immune response by the virus *in vivo.*

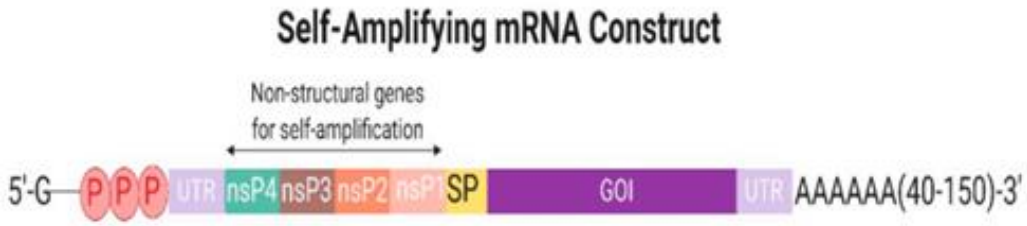

Fig.1. Self-amplifying mRNA construct.

The procedure for the formation of self-amplifying mRNAs is almost similar to conventional mRNAs and they can be produced in laboratories. These self-amplifying mRNAs are approximately 9-12kb in size. As the replicons enter the cytoplasm of host cells, they are highly translated by the presence of RNA-dependent RNA polymerase enzyme. When self-amplifying mRNAs are compared with Non-amplifying mRNAs while vaccinated, these are having higher levels of gene expression with time-lapse, but it persists for many days in the body. They do not produce an infection in the body as there is a lack of structural proteins for the virus. It gives the same level of protection at a much lower dose (3). Both the mRNAs do not integrate into the host genome. All these attributes denote that both these vaccines are much better and safer than DNA vaccines, subunit vaccines, and peptide vaccines.

Engineered mRNAwith Potent Efficiency

Weissman proposed that for any mRNA vaccine to be efficient, the two properties that the mRNA vaccine should have are stability along with high efficiency of translation. During the translation process, mRNA stability is lost, mostly due to degradation by cellular RNases and also due to dsRNAs contamination, which interrupts the translation machinery, thus decreasing the yield of protein and its expression. The translation process can be increased efficiently by removing the dsRNAs.

Purification is needed to be done to remove the several short-stranded RNAs and many double-stranded RNAs. Originally, LiCl is used for purification, later on, it is not used as it does not remove dsRNAs completely. Chromatographic techniques like Fast Protein Liquid Chromatography (FPLC) and high-performance liquid chromatography (HPLC) and RNAse III treatment are used for the complete removal of dsRNAs for the production of mRNA at large scale and to increase the translation efficiency. On either side of the open reading frame, there are 5^1 and 3^1 untranslated regions, which do not code for any gene, called non-coding sequences. The regulatory elements which are present in 5^1 UTR / Kozak sequence/ 5^1 caps and 3^1 UTR/ poly $(A)_n$ tail stabilizes the mRNA and increases the translation efficiency, thus efficient protein production.(Figure 2) (4). Nowadays another method called codon optimization is used to increase the mRNA strength, durability, and efficiency during the translation.

Although these vaccines are highly efficient in the expression of antigens, protein translation is inhibited for several reasons, especially due to the recognition of secondary structures and sequence

[Downloaded from ijmcmed.org on 2026-10-10]

[DOI: 10.22088/IJMCM.BUMS.11.3.260]

structures formed by mRNA by a large number of innate immune sensing receptors. There is a huge development in mRNA vaccines to know how the RNA works inside the cell. Many different methods are developed to increase the effectiveness of mRNA vaccines, by codon/sequence optimization(increasing the G+C content), modification of nucleosides, the addition of synthetic caps(cap 1), poly-A tails, and modulation of target cells. Modified nucleosides such as pseudouridine (Ψ), and 5-methylcytidine (5mC), decrease innate immune activation and increase the translation process efficiently. Double-stranded mRNAs, immature mRNA, and short strands of mRNA all inhibit the translation process by inducing innate immune receptor activation while doing the in-vitro transcription. Chromatographic techniques like fast protein liquid chromatography (FPLC) and high-performance liquid chromatography (HPLC) are used to avoid these drawbacks.

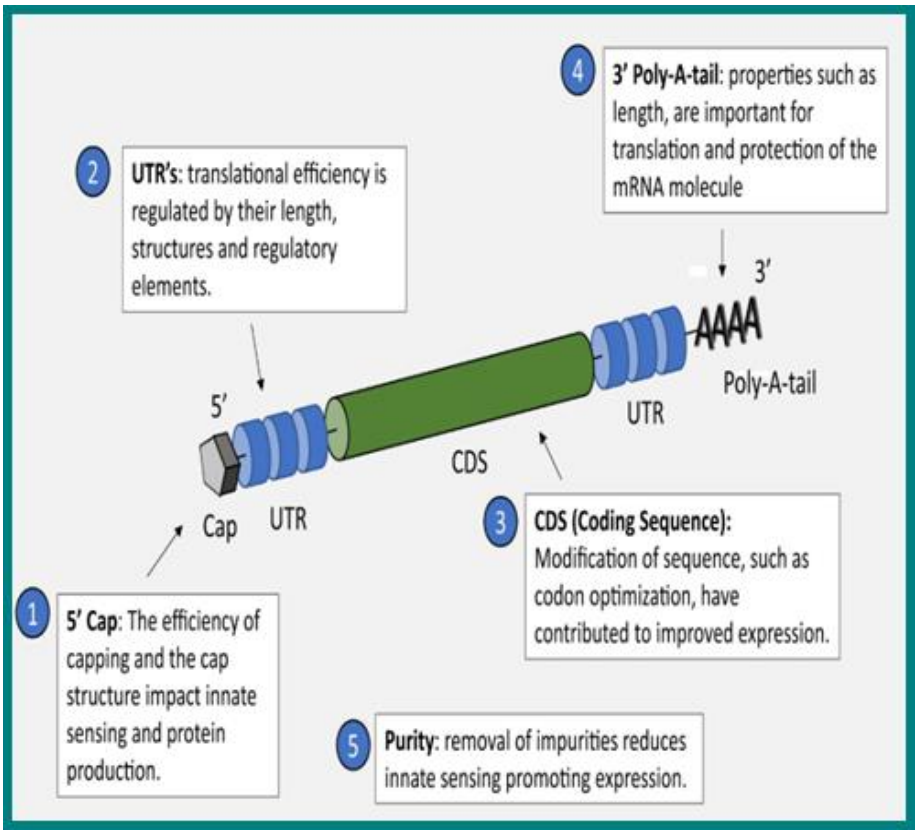

Fig.2. Strategies for optimizing mRNA pharmacology.

Design of mRNA Vaccine

5' CAPPING METHODS

There are two classes of capping methods, the first class includes enzymatic capping which will make the RNA generally purify and uses vaccine virus enzymes and the other one includes co-transcriptional capping and there are 3 generations of capping including Cap and mCap, ARCA(anti-reverse cap analog) and Clean Cap™ Cap Analogs, respectively.

Enzymatic capping is a method where caps are integrated with the opposite orientation, as reverse caps poorly bind to Eukaryotic initiation factor 4E (eIF4E). After the completion of the transcription process, it

[Downloaded from ijmcmed.org on 2026-10-10]

[DOI: 10.22088/IJMCM.BUMS.11.3.260]

utilizes the Vaccinia virus capping enzyme complex and 2^1 O-methyl transferase enzymes for capping in a different set of reactions. It can potentially achieve 100% capping. It yields a natural, unmodified cap structure. It uses an unstable temperature-sensitive co-factor s- adenosyl methionine (SAM). Cap 1 structure may be less immunogenic in vivo. Capping enzymes are very costly and significant batch-to-batch variations are observed. For the significant capping, we need 5^{1-}end accessibility. This method involves more steps in the process of manufacturing if uncapped mRNA transcript is used for purification before enzymatic capping.

Anti-reverse cap analogs (ARCA-3´-O-Me-m7G (5) ppp (5) G), this method prevents cap insertion in the wrong orientation. It yields cap O. Here, 3 prime hydroxyl group which is present closer to 7-methyl-guanosine (m7G) is replaced with the methoxy group. This Cap analog competes with GTP for the initiation of transcription. Capping efficiency and reproducibility is 80%. There will be a significant amount of Transcriptional stuttering. This cap contains an unnatural 3^1 O-methyl group. The remaining 20% cannot be translated due to 5^1triphosphates. The transcript must start with G for this capping. Generally, we include the phosphatase step to remove residual triphosphate, then it can be immunogenic.

Our natural mRNA consists of three types of caps. They are Cap-0 (m7G (5) pppN1pN2p) has N-7 methyl guanosine connected to the 5^1 nucleotide through a 5^1 to 5^1triphosphate linkage, Cap-1(m7G (5) pppN1mpNp) has a methylated 2^1 hydroxyl group on the first ribose sugar and Cap-2(m7G (5) pppN1mpN2mp) has methylated 2 hydroxy groups on the first two ribose sugars. The difference in cap structures is due to methylation at the last but one and 3rdthe last nucleosides. All our standard mRNA transcripts have a Cap-1 structure. All these cap analogs are used to increase translation efficiency and mRNA stability. There will be higher levels of transition only when the ARCA is inserted in the actual position.

CleanCap technology is a co-transcriptional capping method. Capping efficiency and reproducibility will be 90-99%. All standard mRNAs use Cap 1 analog. It can produce Cap 0, Cap1, or Cap 2 structures, and it yields a natural unmodified cap 1 structure. It allows any of the bases (A, C, G, or U) at the 5 prime ends. It also allows m6A or $m6A_m$ at the 5 prime ends. It has the potential for producing a cap 0 or cap 1 structure in a single pot reaction. This clean cap technology is more cost-effective than enzymatic capping. Easily scalable for therapeutic production requirements.

Chemical modification of mRNA is achieved through addition of pseudouridine (Ψ), 5-methylcytidine (5mC), N1methylpseudouridine, 5'-methoxyuridine in place of uridine and by codon optimization

The untranslated regions (UTRs) at both ends of mRNA are responsible for the lifespan of mRNA inside the cell. Generally, alpha globin 3 prime UTRs and beta-globin 5 prime UTRs are used. Examples of 5 prime untranslated regions include TEV (tobacco etch virus) of plants and human HSP 70(heat shock protein.

Poly A tail should be added to the transcribed mRNA by recombinant poly A polymerase enzyme leading to the formation of Polysome. The addition of adenine (poly-A) should not exceed 150 nucleotides.

[Downloaded from ijmcmed.org on 2026-10-10]

[DOI: 10.22088/IJMCM.BUMS.11.3.260]

Mechanism of Action of mRNA Vaccines

All the vaccines do one basic thing is they imitate a disease-causing agent or pathogen, intending to eliminate it from the immune system. Then our immune system recognizes and responds to that pathogen accordingly.

mRNA vaccines represent a departure from the way we have introduced pathogens in the past. Most of the time we have used vaccines/antigens in the form of killed pathogens or living ones that have been weakened or just pieces of viral proteins. In any case, our immune system recognizes these antigens (5). Each antigen has a shape unique to the particular pathogen. When we introduce a vaccine into the host's body, the immune system recognizes these antigens and triggers the formation of memory cells as well as antibodies that disable the pathogen if it tries to invade in the future. But instead of pieces of pathogen or any other strategy that we have used in the past that contain mRNA, we can use mRNA used as a vaccine.

mRNA vaccine is a coded genetic message to host cells. Instead of antigen, they contain the mRNA template to build an antigen. For example, In the case of our Covid-19 mRNA vaccines, the message codes for an antigen called spike protein. These spike proteins detach from the virus and infect the cells in the host. When the vaccine is injected intramuscularly into the upper arms of a human, these mRNA molecules are phagocytosed by the dendritic cells of the immune system (6) and use the host cell machinery (ribosomes) to read the message and translate the message and host cells display the finished spikes on their surface (7). Now, the host immune system recognizes these viral antigens and induces both cell-mediated and humoral immune responses. First of all, these antigens are chopped into minimal pieces by proteosomes, then the generated antigenic peptide epitopes are transported into the endoplasmic reticulum and loaded onto the major histocompatibility complex class I molecules (MHC I) (1). The loaded MHC I-peptide epitope complexes are presented on the surface of cells, eventually leading to the induction of antigen-specific $CD8^{+}$T cell responses after T-cell receptor recognition and appropriate co-stimulation (Figure 3) (8). From here the action is similar to traditional vaccines. The immune system makes memory cells and neutralizes antibodies against the target antigen (spike protein in case of Covid-19), thus resulting in immunity.

Conventional vs mRNA Vaccines

Conventional vaccines can be live viruses that have been weakened or altered or they can be made from inactivated or killed organisms, inactivated toxins, or containing some part of that pathogen, it may be subunit or conjugate vaccines. So, conventional vaccines use some part of the virus whether it be live or killed or a piece of it to make vaccinations. While mRNA vaccines include just the code for the mRNA once in the cell can then make the protein that it codes.

The production of conventional vaccines takes months or even years depending on which type of vaccine we are making. For example, the measles virus vaccination that we use today was isolated from a child with measles disease in 1954. Live attenuated vaccine is made for measles which takes a lot longer to make because it needs to be passed through several cultures to be weakened. It took almost 10 years of passing the virus through several different cultures to transform it from a wild-type virus that causes disease into an attenuated vaccine virus. The production time for mRNA vaccines is much shorter because RNA can be synthesized in the laboratory. It takes only a little time sometimes as little as a week to produce an

[Downloaded from ijmcmed.org on 2026-10-10]

[DOI: 10.22088/IJMCM.BUMS.11.3.260]

experimental batch. For example, Moderna, a pharmaceutical and biotechnological company, took only two days to design the mRNA-1273 vaccine for COVID-19 which is a pandemic virus.

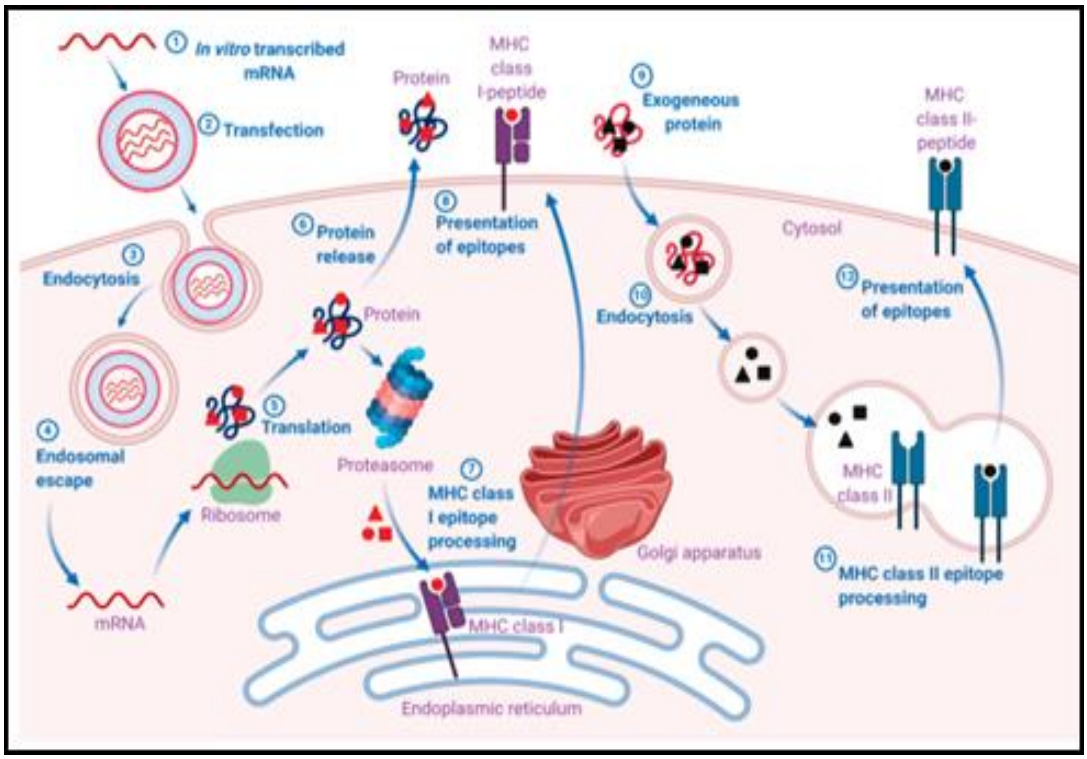

Fig.3. Mechanism of action of mRNA vaccines.

In conventional vaccines, we need to grow a lot of viruses which leads to potential safety hazards having to grow all that virus in the laboratory, whereas with mRNA vaccines no virus is needed but only to initially get that protein to make the mRNA against, once we are synthesizing and growing it in large quantities, we no longer use virus, we only use the mRNA. A potential issue of live attenuated vaccines is that they could have the ability to revert to their original pathogen from which it causes the disease. But in the case of mRNA vaccines, it only includes mRNA that codes for protein and as it produces antigens inside the cell, these vaccines stimulate the adaptive immunity.

Carrier-Based mRNA Vaccines

Lipid-Based Delivery

Among the different varieties of delivery systems for mRNA vaccines, lipid nanoparticles are the most widely used carrier-based system and also the first clinical trial was done with this delivery system (9, 10). Lipid nanoparticles are spherical vesicles made up of ionizable amino lipids, which are positively charged at low pH. These ionizable amino lipids are responsible for mRNA release and endosomal escape by interacting with the endosomal membrane.(11).It also contains polyethylene glycol(PEG), which prolongs the circulation time as it inhibits the binding of mRNA with plasma proteins and the phospholipids and cholesterol which will integrate into the lipid nanoparticle firmly (12). There are some benefits for lipid nanoparticles as a delivery system, one of which includes the protection the mRNA from degradation by enzymes in the endosome (13).

[Downloaded from ijmcmed.org on 2026-10-10]

[DOI: 10.22088/IJMCM.BUMS.11.3.260]

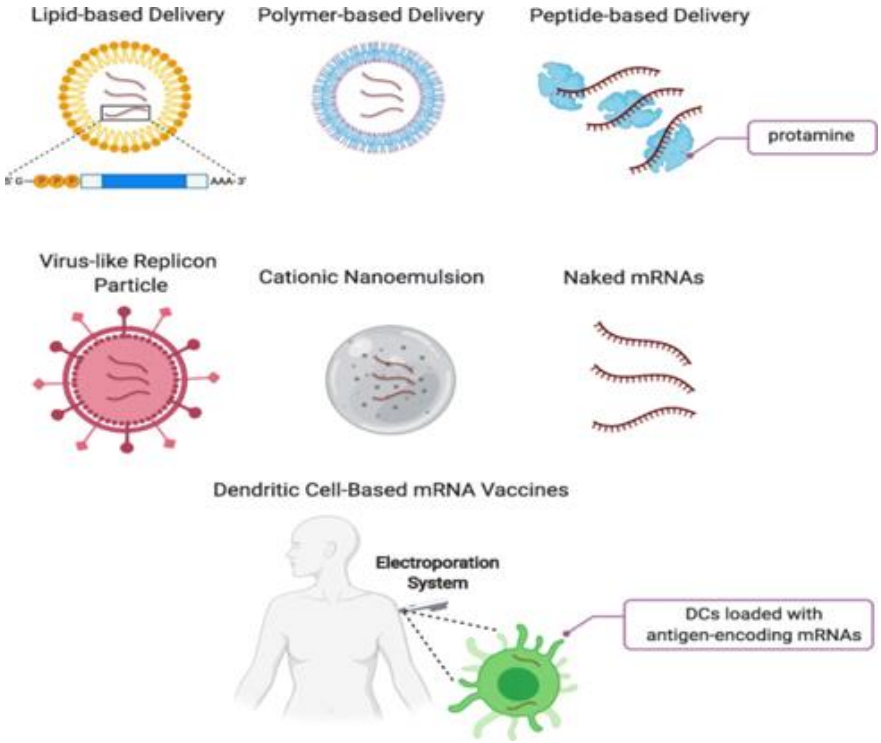

Fig.4. Delivery systems of mRNA vaccines.

The efficacy of lipid nanoparticles depends on the composition of lipids and modifications in the lipid components. Most importantly, there are ionizable lipids or cationic lipids which are used for delivering mRNA. They include N-[1- (2,3-dioleoyloxy) propyl]-N,N,N- trimethylammonium chloride (DOTMA) (14), dilinoleylmethyl-4-dimethylaminobutyrate (Dlin-MC3-DMA), N,N-Dimethyl-2,3-bis [(9Z,12Z)-octadeca-9,12-dienyloxy]propan-1-amine (DLinDMA) , 1,2-dioleoyl-sn-glycerol-3-phosphoethanolamine (DOPE), 1,2-dioleoyloxy-3-trimethylammonium propane chloride (DOTAP) and N^1,N^3,N^5-tris(3-(didodecylamino)propyl)benzene-1,3,5-tricarboxamide (TT3).

The distribution and expression of lipid nanoparticles depend on the method of administration of mRNA vaccines. They are administered locally through the intradermal route, subcutaneous route, intramuscular route, and intranodal route. At present, lipid nanoparticle is a potential vaccine candidate for delivering mRNA. It has good biocompatibility with high delivering efficacy.

Polymer-Based Delivery

Broadly, polymers are classified into cationic polymers and anionic polymers. polyethyleneimine (PEI), polyamidoamine (PAMAM) dendrimer, and polysaccharide (15) are three cationic polymers. As self-amplifying RNAs (saRNAs) are usually degraded by RNase enzyme and are taken up by the dendritic cells. The mRNA is mixed with a polyethyleneimine polymer complex to overcome this problem. For example, mRNA which codes for haemagglutinin and nucleocapsid of the influenza virusis coated with polyethyleneimine complex. After entering into the cytosol, this complex releases mRNA, then translation takes place which stimulates the immune system to induce both cell-mediated and humoral immunity (16).

[Downloaded from ijmcmed.org on 2026-10-10]

[DOI: 10.22088/IJMCM.BUMS.11.3.260]

Peptide-Based Delivery

One of the best examples of cationic peptidesis protamines, which deliver mRNA. Two characteristic features make it a potential vector. One of the features is that it can protect the mRNA from degradation by RNase in the serum (17). Although there is a temperature change, the immunogenicity of the mRNA vaccine remained constant as mRNA is stabilized by protamine. Another characteristic feature is that protamine itself acts as an adjuvant.

A study conducted by (18) that the mRNA which is combined with protamine activated the dendritic cells and monocytes and secretes interferon-alpha (IFN-α) and tumor necrosis factor-alpha (TNF-α). Protamine-formulated mRNA also activated immune cells by TLR-7/TLR-8-mediated recognition. The mRNA which is formulated with protamine has similarities in structure when compared to condensed RNA present in RNA viruses, they commonly share among them.

The second variety of peptide-based delivery includes Cationic cell-penetrating peptides (CPPs), which are made up of 8-30 amino acids. The characteristic features that make it an excellent delivery vehicle for mRNA, they incude the degradation the cell membrane for the escape from the endosome, which is useful for protein synthesis and these CPPs are coupled with low charged densities. The three Cationic cell-penetrating peptides namely RALA, LAH4, and LAH4-L1, the comparison was made among them by (19). These peptides are injected into the body, taken up by dendritic cells, and induce an innate immune response.

Virus-Like Replicon Particle

Virus-like Replicon Particles (VRPs) can deliver the self-amplifying mRNA into the cytoplasm of the cell as same as the virus do. For this, we have to make structural proteins in the laboratory and followed by encapsulation of self-amplifying mRNA. Most alpha viruses have the property of self-replication. The spike protein of SARS coronavirus along with the self-amplifying mRNA has been encapsulated in lipid inorganic nanoparticles (LIONs). Mostly, viruses that are attenuated have the property of self-replication.

Cationic Nanoemulsion

One of the non-viral delivery systems includes Cationic Nanoemulsion (CNE), which binds to self-amplifying RNAs thus increasing the potency of mRNA vaccines. The major component of CNE includes a lipid, which is cationic namely 1, 2-dioleoyl-sn-glycerol-3-phosphocholine (DOTAP). It has been used in clinical trials along with an emulsified adjuvant MF59. This DOTAP has some features which make it to use, due to squalene solubility, cationic at certain pH and they are easily available. These cationic lipids can form nanoemulsions in a pH-dependent manner.

Naked mRNA

During the 1970s, mRNA was injected directly into the cells by penetration called Microinjection (20). Some of the membrane permeabilization techniques include mechanical membrane disruption, electroporation, biochemical membrane disruption and gated channels (21), thermal membrane disruption, and optoporation.

Ringer's solution and lactated Ringer's solution, are commonly used solutions for naked mRNA. Both solutions are enriched with calcium as it is useful for the easy uptake of mRNA (22). As of now, both these solutions are mostly used in many clinical trials. In the first trial, mRNAs are mixed and dissolved in 1.0 milligrams in 1ml Ringer's solution, then followed by injection into the lymph nodes (23). Later, 80

[Downloaded from ijmcmed.org on 2026-10-10]

[DOI: 10.22088/IJMCM.BUMS.11.3.260]

micrograms of mRNA vaccines were mixed and dissolved in lactated ringers' solution followed by injection into the backs of C57BL/6 mice. Finally, after injecting into the mice there was an activation of immune cells and production of cytokines along with the enhanced immune response.

There are string of benefits of the naked mRNA and they include that the naked mRNA does not integrate into the genome and after the entry into the cytosol directly combines with ribosomes present in the cytosol, as they do not enter the nucleus and thus initiates the process of translation without any delay by combining with ribosomes present in the cytoplasm of the cell, the later one indicates that a rapid immune response after injecting mRNA into the cell. Wherever the mRNA reaches the last point in the cell cytosol that is the location where it determines its protein expression. One of the advantages of mRNA over DNA is that mRNA has low toxicity and immunogenicity (24).

Dendritic Cell-Based mRNA Vaccines

A dendritic cell is a target for most vaccines. It is well-known that dendritic cells are antigen-presenting cells that take up the antigen and will process and present it to immune cells, thus leading to the generation of the humoral and cell-mediated immune response (25). In particular, it does not only result in the expression of major histocompatibility complex (MHC) molecules along with antigens (26), but secondary signals are provided by co-stimulators and production of numerous cytokines for proliferation and differentiation of T-cells and development of cytotoxic T-lymphocyte (27,28), but also the secretion of chemokines for T cell recruitment (29). A good example of a dendritic cell mRNA vaccine for viral diseases is the HIV-1 vaccine. This vaccine is injected into the body through electroporation. In many cases, we inject mRNA vaccines through electroporation as it delivers mRNA with high efficacy.

Covid-19 mRNA Vaccines

One of the well-known vaccines is BNT162b1, a lipid nanoparticle-formulated mRNA vaccine encoding SARS-CoV-2 spike glycoprotein RBD. Local delivery of BNT162b1 is dose-dependent. The RBD-specific IgG and SARS-CoV-2 neutralizing titers increased after a second injection (30). Based on the curative effects, the phage I/II/III clinical investigation recruited 29,481 participants (NCT04368728).

Immune Responses in Mucosal mRNA Vaccines and their Challenges

Many emerging infectious agents are colonizing and transmitting by various mucosal surfaces, especially by oral or respiratory routes. Hence, sterilizing immunity at mucosal sites is critical for effective prevention and control. It is well established that mRNA vaccines provide good systemic humoral response on intramuscular administration (i.m). However, it is unclear whether they stimulate the mucosal immune response.

Recent studies on nasal and salivary mucosal immune response elicited by currently approved SARS CoV-2 mRNA vaccines have shown inconsistent results (31, 32, 33, 34). As there is lack of appropriate amount of sample collection from nasal swabs and the lack of validated assays for evaluating the mucosal immune response, this is most likely a case. Many studies reported that intramuscular administration of mRNA vaccines stimulates the detectable levels of secretory immunoglobulin A (sIgA) (major neutralizing Ig at mucosal sites) and IgG in saliva and nasal samples (35). However, it further needs to evaluate the role of this immune response in providing sterilizing immunity. Few studies argue that robust sterilizing

[Downloaded from ijmcmed.org on 2026-10-10]

[DOI: 10.22088/IJMCM.BUMS.11.3.260]

immunity at mucosal sites can be achieved only through the direct administration of the vaccine via the mucosal route and they recommend the booster dose with the mucosal vaccines after i.m priming (36).

Many mucosal surfaces are the target for vaccine delivery, namely oral, nasal, vaginal, conjunctival, and rectal. Already live attenuated, inactivated, and vectored nasal and oral vaccines are available in the market (37). However, there are no commercially approved mucosal mRNA vaccines. So far, very few studies have been carried out (38, 39, 40). As a result, more research on delivery mechanisms to overcome mucosal barriers for effective vaccine delivery to sufficiently stimulate the immune system is required.

Conclusions and Future Prospective

These mRNA vaccines replace conventional vaccines due to their safety, efficacy, potency, and lowcost for their manufacture. mRNA-based vaccines can fill the gap between emerging pandemic infectious diseases and a bountiful supply of effective vaccines. The clinical and preclinical trials have demonstrated that these vaccines provide a safe and long-lasting immune response in animal models and humans (41).Optimization of the 5′-untranslated region (5′-UTR) of mRNA, whose secondary structures are recognized by PAMP molecules can maximize the translational yield of mRNA vaccines. Presently, lipoplexes and lipid-based nanoparticles are mostly used for delivering mRNA. Polymers and lipid-polymer hybrid nanoparticles offer great promise in terms of safety, stability, high transfection efficiency, and low price. Despite benefits, they are unstable at high temperatures, making packaging and distribution difficult. Their safety is lower than inactivated vaccines & effectiveness is lower than DNA vaccines. Further insights into the mechanism of action and potency are still needed for the full development of mRNA vaccines. Future improvements should increase antigen-specific immune responses and the magnitude of memory immune cell responses, including memory B and T cell responses. Continued advancement in mRNA formulation and delivery using different nanomaterial can improve the wider use of mRNA for the treatment and prevention of viral diseases.

References:

1. Xu S, Yang K, Li R, et al. mRNA Vaccine Era-Mechanisms, Drug Platform and Clinical Prospection. Int J Mol Sci 2020;21.

2. Versteeg L, Almutairi MM, Hotez PJ, et al. Enlisting the mRNA Vaccine Platform to Combat Parasitic Infections. Vaccines (Basel) 2019;7.

3. Stitz L, Vogel A, Schnee M, et al. A thermostable messenger RNA based vaccine against rabies. PLoS Negl Trop Dis 2017;11:e0006108.

4. Jackson NAC, Kester KE, Casimiro D, et al. The promise of mRNA vaccines: a biotech and industrial perspective. NPJ Vaccines 2020;5:11.

5. Batty CJ, Heise MT, Bachelder EM, et al. Vaccine formulations in clinical development for the prevention of severe acute respiratory syndrome coronavirus 2 infection. Adv Drug Deliv Rev 2021;169:168-89.

6. Schlake T, Thess A, Fotin-Mleczek M, et al. Developing mRNA-vaccine technologies. RNA Biol 2012;9:1319-30.

7. Pardi N, Hogan MJ, Porter FW, et al. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov 2018;17:261-79.

8. Wadhwa A, Aljabbari A, Lokras A, et al. Opportunities and Challenges in the Delivery of mRNA-based Vaccines. Pharmaceutics 2020;12.

[Downloaded from ijmcmed.org on 2026-10-10]

[DOI: 10.22088/IJMCM.BUMS.11.3.260]

9. Shin MD, Shukla S, Chung YH, et al. COVID-19 vaccine development and a potential nanomaterial path forward. Nat Nanotechnol 2020;15:646-55.

10. Lokugamage MP, Gan Z, Zurla C, et al. Mild Innate Immune Activation Overrides Efficient Nanoparticle-Mediated RNA Delivery. Adv Mater 2020;32:e1904905.

11. Ramishetti S, Hazan-Halevy I, Palakuri R, et al. A Combinatorial Library of Lipid Nanoparticles for RNA Delivery to Leukocytes. Adv Mater 2020;32:e1906128.

12. Samaridou E, Heyes J, Lutwyche P. Lipid nanoparticles for nucleic acid delivery: Current perspectives. Adv Drug Deliv Rev 2020;154-155:37-63.

13. Lou G, Anderluzzi G, Schmidt ST, et al. Delivery of self-amplifying mRNA vaccines by cationic lipid nanoparticles: The impact of cationic lipid selection. J Control Release 2020;325:370-9.

14. Felgner PL, Ringold GM. Cationic liposome-mediated transfection. Nature 1989;337:387-8.

15. Stefan J, Kus K, Wisniewska A, et al. The antiatherogenic effect of new biocompatible cationically modified polysaccharides: chitosan and pullulan - the comparison study. J Physiol Pharmacol 2018;69.

16. Demoulins T, Milona P, Englezou PC, et al. Polyethylenimine-based polyplex delivery of self-replicating RNA vaccines. Nanomedicine 2016;12:711-22.

17. Hoerr I, Obst R, Rammensee HG, et al. In vivo application of RNA leads to induction of specific cytotoxic T lymphocytes and antibodies. Eur J Immunol 2000;30:1-7.

18. Scheel B, Teufel R, Probst J, et al. Toll-like receptor-dependent activation of several human blood cell types by protamine-condensed mRNA. Eur J Immunol 2005;35:1557-66.

19. Coolen AL, Lacroix C, Mercier-Gouy P, et al. Poly(lactic acid) nanoparticles and cell-penetrating peptide potentiate mRNA-based vaccine expression in dendritic cells triggering their activation. Biomaterials 2019;195:23-37.

20. Brachet J, Huez G, Hubert E. Microinjection of rabbit hemoglobin messenger RNA into amphibian oocytes and embryos. Proc Natl Acad Sci U S A 1973;70:543-7.

21. Stewart MP, Langer R, Jensen KF. Intracellular Delivery by Membrane Disruption: Mechanisms, Strategies, and Concepts. Chem Rev 2018;118:7409-531.

22. Probst J, Weide B, Scheel B, et al. Spontaneous cellular uptake of exogenous messenger RNA in vivo is nucleic acid-specific, saturable and ion dependent. Gene Ther 2007;14:1175-80.

23. Sahin U, Derhovanessian E, Miller M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature 2017;547:222-6.

24. Wang Y, Zhang Z, Luo J, et al. mRNA vaccine: a potential therapeutic strategy. Mol Cancer 2021;20:33.

25. Eisenbarth SC. Dendritic cell subsets in T cell programming: location dictates function. Nat Rev Immunol 2019;19:89-103.

26. Kambayashi T, Laufer TM. Atypical MHC class II-expressing antigen-presenting cells: can anything replace a dendritic cell? Nat Rev Immunol 2014;14:719-30.

27. Zhang G, Guo B, Wu H, et al. A delivery system targeting bone formation surfaces to facilitate RNAi-based anabolic therapy. Nat Med 2012;18:307-14.

28. Ding Y, Guo Z, Liu Y, et al. The lectin Siglec-G inhibits dendritic cell cross-presentation by impairing MHC class I-peptide complex formation. Nat Immunol 2016;17:1167-75.

29. Qiao J, Liu Z, Dong C, et al. Targeting Tumors with IL-10 Prevents Dendritic Cell-Mediated CD8(+) T Cell Apoptosis. Cancer Cell 2019;35:901-15 e4.

[Downloaded from ijmcmed.org on 2026-10-10]

[DOI: 10.22088/IJMCM.BUMS.11.3.260]

30. Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and T(H)1 T cell responses. Nature 2020;586:594-9.

31. Azzi L, Dalla Gasperina D, Veronesi G, et al. Mucosal immune response in BNT162b2 COVID-19 vaccine recipients. EBioMedicine 2022;75:103788.

32. Francavilla B, Nuccetelli M, Guerrieri M, et al. Importance of nasal secretions in the evaluation of mucosal immunity elicited by mRNA BNT162b2 COVID-19 Vaccine. EBioMedicine 2022;79:104006.

33. Tang J, Zeng C, Cox TM, et al. Respiratory mucosal immunity against SARS-CoV-2 after mRNA vaccination. Sci Immunol 2022;7:eadd4853.

34. Tsamadou C, Ludwig C, Scholz J, et al. Differentially induced immunity in buccal and nasal mucosae after vaccination for SARS-CoV-2: Prospects for mass scale immunity-screening in large populations. Front Immunol 2022;13:999693.

35. Guerrieri M, Francavilla B, Fiorelli D, et al. Nasal and Salivary Mucosal Humoral Immune Response Elicited by mRNA BNT162b2 COVID-19 Vaccine Compared to SARS-CoV-2 Natural Infection. Vaccines (Basel) 2021;9.

36. Sheikh-Mohamed S, Isho B, Chao GYC, et al. Systemic and mucosal IgA responses are variably induced in response to SARS-CoV-2 mRNA vaccination and are associated with protection against subsequent infection. Mucosal Immunol 2022;15:799-808.

37. Huang M, Zhang M, Zhu H, et al. Mucosal vaccine delivery: A focus on the breakthrough of specific barriers. Acta Pharm Sin B 2022;12:3456-74.

38. Li M, Zhao M, Fu Y, et al. Enhanced intranasal delivery of mRNA vaccine by overcoming the nasal epithelial barrier via intra- and paracellular pathways. J Control Release 2016;228:9-19.

39. Patel AK, Kaczmarek JC, Bose S, et al. Inhaled Nanoformulated mRNA Polyplexes for Protein Production in Lung Epithelium. Adv Mater 2019;31:e1805116.

40. Qiu Y, Man RCH, Liao Q, et al. Effective mRNA pulmonary delivery by dry powder formulation of PEGylated synthetic KL4 peptide. J Control Release 2019;314:102-15.

41. Zhang C, Maruggi G, Shan H, et al. Advances in mRNA Vaccines for Infectious Diseases. Front Immunol 2019;10:594.

[Downloaded from ijmcmed.org on 2026-10-10]

[DOI: 10.22088/IJMCM.BUMS.11.3.260]