mRNA Vaccine: A New Era in Vaccine Development

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Abstract - The high potency, safety, and efficacy of mRNA vaccines, along with their potential for quick and inexpensive production, have made them a potent substitute for conventional vaccines. These vaccines have advanced from being just interesting to becoming the leading COVID-19 pandemic vaccines. Significant progress has been made in the field of nanotechnology for creating mRNA vaccine delivery systems. To improve the stability of the mRNA vaccine, scientists have altered the mRNA structure. In this review, we have summarized each and every aspect of mRNA vaccine. The article describe the mRNA structure, discussing its modifications and the subsequent manufacturing processes. This includes the upstream, downstream, and formulation processes, which are essential for the production of mRNA vaccines. Furthermore, the article provides an in-depth analysis of the mechanism through which mRNA vaccines function, storage condition for mRNA vaccine and its applications. mRNA vaccines can be used for much more than just preventing infectious diseases. They are being researched for use in cancer treatment, regenerative medicine, immunotherapy, and genetic disorders. In particular, mRNA vaccines have the potential to utilize immunotherapies, treat cancer, and manage infectious diseases.

Keywords : mRNA Vaccine, Infectious diseases (COVID-19), Lipid based nanoparticles, Immunotherapy, Cancer

INTRODUCTION

Vaccines represent one of humanity's greatest advancements in combating the spread of infectious diseases. The development of vaccines has evolved from using inactivated and weakened pathogens to employing subunits that include elements of the pathogen to stimulate the immune response[1]. We are now in the era of mRNA vaccines, as foundational research has been established for over thirty years[2]. An mRNA molecule is comprised of a single-stranded ribonucleic acid that conveys coding information necessary for the translation and formation of functional proteins[3]. mRNA-based nucleic acid vaccines were envisioned over thirty years ago with the aim of creating safe and adaptable vaccines that are simple to manufacture[4].

An mRNA vaccine is a kind of vaccine that utilizes a copy of a substance known as messenger RNA (mRNA) to trigger an immune reaction. The vaccine introduces mRNA molecules that encode antigens into cells, which then use this synthetic mRNA as a template to create foreign proteins typically produced by a pathogen (like a virus) or by cancer cells. These protein molecules provoke an adaptive immune response, teaching the body to recognize and eliminate the relevant pathogen or cancer cells[5]. The mRNA is delivered through a formulation that encapsulates the RNA within lipid nanoparticles, which safeguard the RNA strands and facilitate their uptake by the cells[6].

In recent decades, significant technological advancements and thorough research have focused on enhancing the overall quality of mRNA by increasing its stability through the implementation of capping, tailing, point mutations, and efficient purification methods. Enhancements in mRNA delivery have been achieved through the use of lipid nanoparticles. Modifications to nucleotides to lessen immunogenicity have led to the broad application of mRNA as a vaccine[2].

mRNA vaccines offer numerous significant benefits over traditional vaccines, such as those using live or attenuated pathogens, subunit-based, and DNA-based approaches. The first point is Safety, since mRNA does not combine with the host DNA and is not infectious. The second point is Efficacy, as alterations to the mRNA structure can enhance the vaccine's stability and effectiveness while lowering immunogenicity. The third point is the efficiency of manufacturing and scaling up, because mRNA vaccines are created in a cell-free setting, enabling quick, scalable, and cost-effective production[7].

In theory, mRNA vaccines offer numerous benefits compared to traditional vaccines. Unlike certain viral

vaccines, mRNA does not become integrated into the genome, alleviating worries about insertional mutagenesis[2]. mRNA vaccines can be produced without the use of cells, enabling quick, scalable, and cost-efficient manufacturing. For instance, a 5-liter bioreactor is capable of generating nearly one million doses of mRNA vaccine in a single reaction[7]. Additionally, one mRNA vaccine has the capability to encode several antigens, enhancing the immune reaction against stubborn pathogens and allowing for the targeting of various microbes or viral variants within a single formulation[8]. Luckily, several determined researchers and companies continued their efforts. In recent decades, by understanding mRNA pharmacology, creating efficient delivery systems, and managing mRNA immunogenicity, there has been a resurgence of interest in the clinical use of mRNA[9].

The effectiveness of this vaccine technology became evident with the development and approval of mRNA vaccines by Pfizer-BioNTech for the COVID-19 pandemic. These vaccines were created in an unprecedented timeframe of under a year after the world faced the SARS-CoV-2 virus infection, which led to hospitalizations and fatalities. The remarkable progress of Spikevax (Moderna) and Comirnaty (Pfizer – BioNTech), along with their extensive administration to millions, contributed significantly to managing the COVID-19 pandemic. The capabilities in development, approval, and manufacturing shown by the producers of these vaccines have confirmed that the mRNA platform serves as a safe and effective method for vaccination[10].

❖ mRNA Structure and its Modification

mRNA, or messenger RNA, is a single-stranded structure made up of nucleotides linked by a sugar-phosphate backbone. It features four nitrogenous bases: adenine (A), guanine (G), cytosine (C), and uracil (U). Fully processed mRNA includes a 5’ cap, a 5’ untranslated region (UTR), a coding region comprised of codons, a 3’ UTR, and a poly(A) tail.

Composition:

Nucleotides :- mRNA is made up of nucleotides, which are the basic building blocks of RNA. Each nucleotide consists of a sugar (ribose), a phosphate group, and one of the four nitrogenous bases (A, G, C, or U).

Backbone :- The nucleotides are linked together through phosphodiester bonds between the sugar and phosphate groups, forming the sugar-phosphate backbone.

Key Regions:

5’ Cap :- A modified guanine nucleotide attached to the 5’ end, crucial for mRNA stability and ribosome binding.

5’ UTR :- The region between the 5’ cap and the start codon, which may contain regulatory sequences.

Coding Region (Open Reading Frame) :- The portion of the mRNA sequence that is translated into protein, consisting of codons (triplets of nucleotides) that specify amino acids.

3’ UTR :- The region between the stop codon and the poly(A) tail, which may contain regulatory sequences.

Poly(A) Tail :- A string of adenine nucleotides added to the 3’ end, which increases mRNA stability and facilitates translation.

Processing:

Transcription :- mRNA is synthesized from a DNA template during transcription.

Splicing :- In eukaryotes, non-coding regions (introns) are removed from the primary mRNA transcript, and coding regions (exons) are joined together.

Capping and Polyadenylation :- The 5’ cap and poly(A) tail are added to the mRNA molecule, respectively[11].

The key element of an mRNA vaccine is its mRNA composition. The mRNA that is synthesized in vitro is derived from a modified plasmid DNA, which contains an RNA polymerase promoter along with a sequence that corresponds to the mRNA composition. By using T7 phage RNA polymerase in conjunction with the plasmid DNA, the mRNA can be produced in the laboratory. The effectiveness of the vaccine relies on the stability and structure of the engineered mRNA[2].

The mRNA transcribed in vitro contains the same structural elements as the natural mRNA found in

eukaryotic cells. It features a 5' cap, a 5'-untranslated region (UTR) as well as a 3'-UTR, an open reading frame (ORF) that encodes the specific antigen, along with a 3'-poly(A) tail. By altering these various elements of the synthetic mRNA, both the stability and translation capability of the mRNA can be improved, which consequently enhances the effectiveness of the vaccine[12].

mRNA can be enhanced by incorporating synthetic 5'-cap analogues, which boost its stability and promote protein translation. Likewise, adjustments to regulatory elements within the 5'-untranslated region and the 3'-untranslated region, along with optimizing the length of the poly(A) tail, can further stabilize the mRNA and elevate protein synthesis. Modifications to the mRNA nucleotides can reduce innate immune responses and prolong the mRNA's lifespan within the host cell. The sequence of nucleic acids and codon choice affect protein translation. Increasing the guanine-cytosine content within the sequence enhances mRNA stability and longevity, leading to improved protein production. Additionally, substituting infrequent codons with synonymous codons that are commonly utilized by the host cell boosts protein output[2].

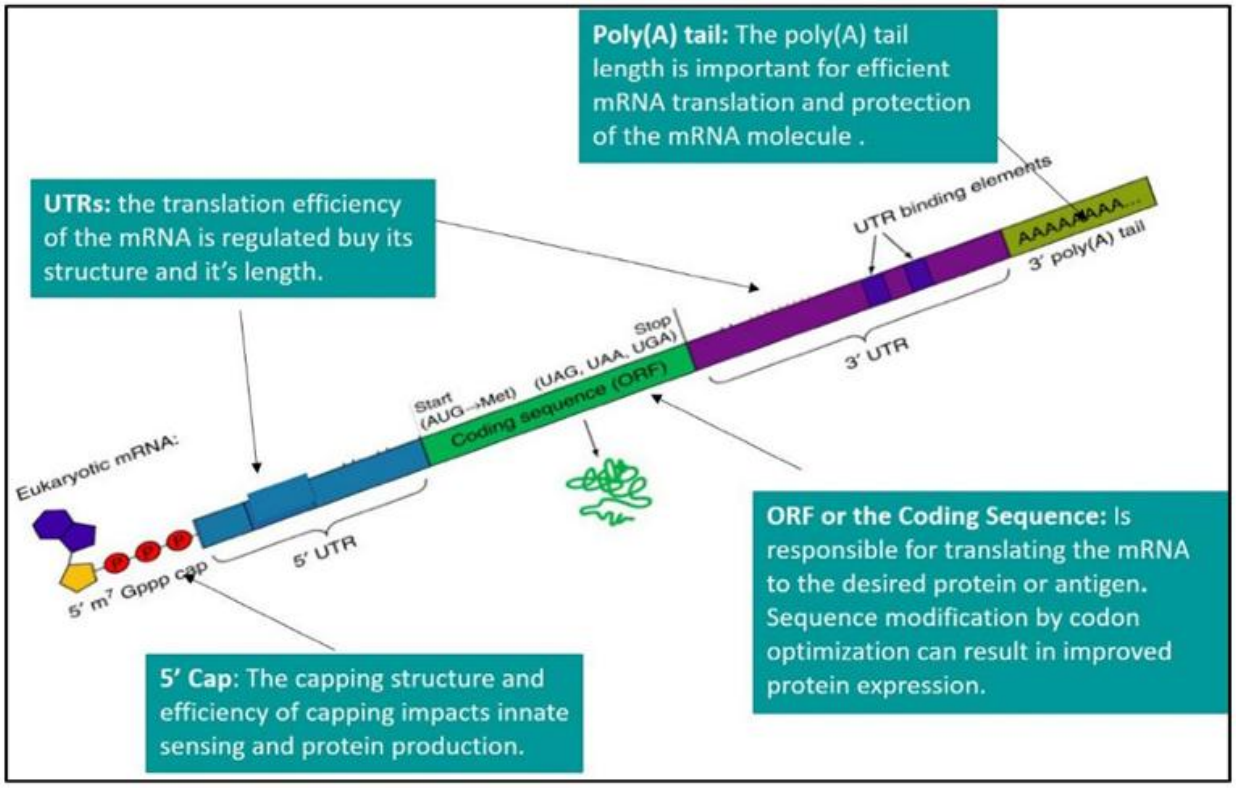

Fig. Structure of mRNA

❖ Development of mRNA Vaccine

Upstream manufacture, downstream purification, and formulation of the mRNA drug substance are the three standard processes that mRNA vaccine medication products go through in their manufacturing process, much like other vaccines. These processes and more recent advancements in each phase to expedite the manufacture of mRNA vaccines will be covered in this section.

1.Upstream production :-

The process of creating the mRNA transcript from the plasmid containing the desired gene is known as upstream production of mRNA vaccines. The in vitro transcription reaction (IVT) is the name given to this reaction. RNA polymerase enzymes like T7, SP6, or T3 are necessary for the IVT enzymatic reaction to occur. The target mRNA is synthesized from the linearized DNA template containing the desired gene by the RNA polymerase enzymes. The cleavage of a plasmid containing the desired gene by restriction of endonuclease enzymes results in a linearized DNA

template; alternatively, PCR amplification of the desired gene can also yield mRNA molecules.

RNA polymerase, which converts DNA to RNA, inorganic pyrophosphatase (IPP), guanylyl transferase, which adds GMP nucleoside to the 5′ end of mRNA, Cap 2′-O-Methyltransferase (SAM), which adds a methyl group at the 2′ position of the 5′ cap of the mRNA, DNase I, an endonuclease used to remove contaminating genomic DNA from RNA samples, and poly(A) tail polymerase, as well as modified and unmodified nucleoside triphosphates (NTPs), are essential enzymes of an IVT reaction. From a plasmid containing the desired gene, these enzymes aid in the upstream development of the mRNA transcript.

In the sphere of mRNA capping, there exist two primary enzymes: SAM and guanylyl transferase, which catalytically generate a 5’ cap at the commencement of the mRNA. Simultaneously, the poly(A) tail polymerase tailing enzyme constructs the poly(A) tail. An alternative approach to 5’ capping involves the co-transcriptional method, during which the 5’ cap is prefabricicated, and subsequently, this cap is appended to the mRNA in a non-enzymatic fashion. This co-transcriptional reaction can be executed with CleanCap® Reagent AG[12].

2.Downstream Purification

In the upstream production phase, the IVT reaction produces mRNA, which is subsequently isolated and purified in the downstream processing step through a series of purification procedures. Remaining NTPs, enzymes, erroneously generated mRNAs, and DNA plasmid templates are among the impurities present in the IVT reaction mixture. Methods based on DNA removal by DNase enzyme digestion and lithium chloride (LiCl) precipitation are used in the lab-scale purification of IVT mRNA. The full elimination of aberrant mRNA species, such as dsRNA and truncated RNA fragments, is not possible with lab-based techniques. To obtain a pure mRNA product that exhibits its intended efficacy and safety profile, these impurities must be eliminated.

The mRNA vaccine product may have an undesirable immunostimulatory profile and reduced translation efficiency as a result of an ineffective purification process. For instance, purifying modified mRNA using reverse-phase HPLC before delivering it to dendritic cells resulted in a 10–1000-fold increase in mRNA transfection and associated protein production. A popular and widely accepted purification method in the biopharmaceutical sector for the purification of vaccines and biologic drug products is chromatography.

3.Formulation

mRNA molecules should be designed in a lipid-based drug delivery system because they are negatively charged. This will prevent mRNA degradation and increase the transfection efficiency and half-life of the molecule. Lipid-based non-viral carriers (LNPs) are the most dependable, trustworthy, and FDA-approved method of delivering mRNA vaccine drug substances in the United States. By precipitating lipids dissolved in an organic phase and combining them with mRNA in an aqueous phase, mRNA LNPs are created. In the organic phase, helper lipids, PEG-lipids, cholesterol, and ionizable lipids are the most often utilized lipids. In the meantime, a pH 4 citrate or acetate buffer is used to dissolve the mRNA. Electrostatic attraction between the ionizable protonated lipid and the anionic mRNA results from protonating the ionizable lipid by mixing the aqueous and non-aqueous solutions. Together with the hydrophobic interactions of other lipids, this interaction promotes the mRNA-LNPs’ spontaneous self-assembly, with the mRNA enclosed within the nanoparticles’ core. We also refer to this process as microprecipitation. The non-aqueous solvent, typically ethanol, is removed by dialyzing them after LNP formation, bringing the pH of the solution up to physiological levels.

Microfluidic mixers make it possible to create tiny LNPs with a high mRNA encapsulation efficiency and a low polydispersity index. For mRNA LNP formulation at the lab scale and GMP level, microfluidic mixing is the most widely used technique[12].

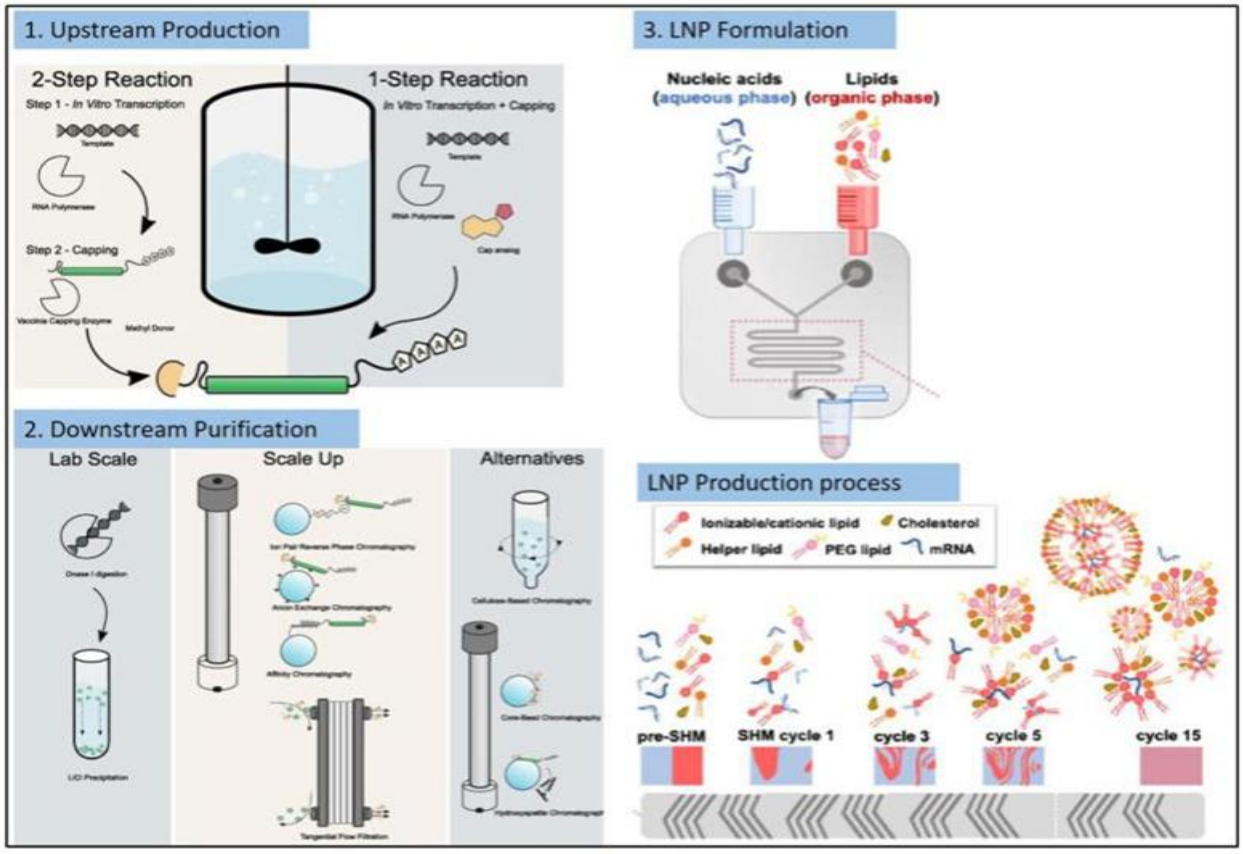

Fig. The steps and stages involved in production of mRNA vaccines

❖ Mechanism of mRNA Vaccine

The purpose of a vaccine is to activate the adaptive immune system to produce antibodies that specifically attack that specific pathogen. The components on the pathogen that the antibodies focus on are referred to as antigens[13]. Conventional vaccines provoke an antibody response by administering either antigens, a weakened (attenuated) virus, a dead (inactivated) virus, or a harmless carrier virus that contains an antigen transgene (recombinant antigen-encoding viral vector) into the body. These antigens and viruses are cultivated and prepared externally[14].

In contrast, mRNA vaccines deliver a temporarily existing synthetically produced segment of the RNA sequence from a virus to the vaccinated person. These mRNA segments are taken in by dendritic cells through the process of phagocytosis[15]. The dendritic cells utilize their internal components (ribosomes) to interpret the mRNA and create the viral proteins that it encodes[2]. The body breaks down the mRNA segments within a few days after they are introduced[16]. While non-immune cells can also potentially take up vaccine mRNA, synthesize proteins, and present those proteins on their surfaces, dendritic cells are significantly more efficient at absorbing the mRNA particles[17]. The mRNA segments are translated in the cytoplasm and do not interact with the cell's genomic DNA, which is located separately in the nucleus[5].

After the host cell generates viral antigens, the typical processes of the adaptive immune system commence. Proteasomes break down the antigens, after which class I and class II MHC molecules bind to the antigen and carry it to the cell membrane, thereby "activating" the dendritic cell[18]. Once activated, dendritic cells travel to lymph nodes, where they present the antigen to T cells and B cells[19]. This stimulation leads to the production of antibodies that are specifically aimed at the antigen, ultimately resulting in immunity[13].

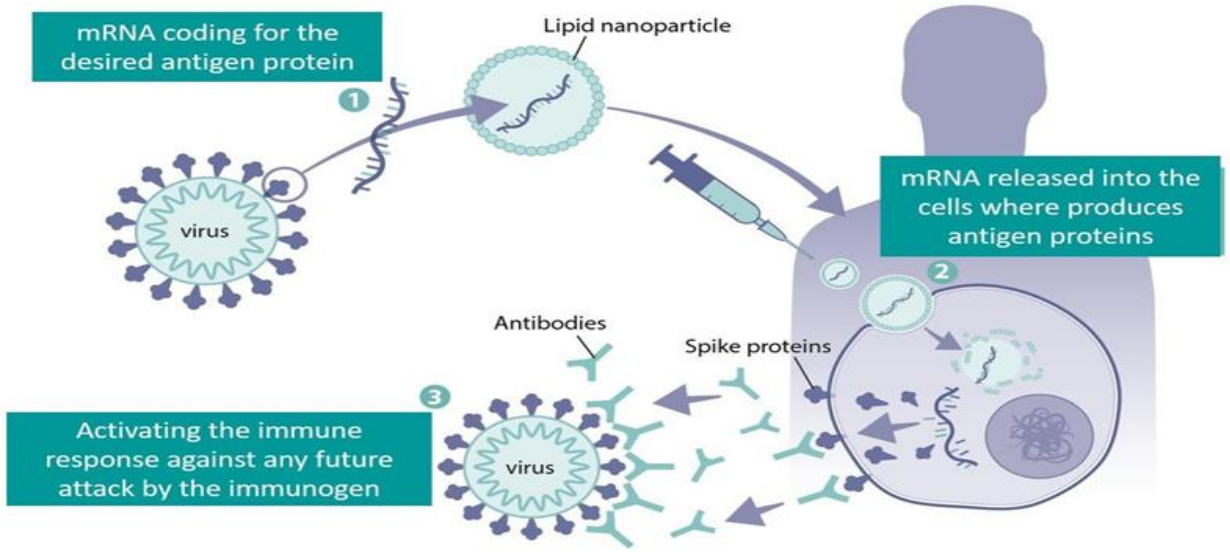

Fig. The image shows that the way mRNA vaccine works.

❖ Delivery Systems for mRNA Vaccine

For a vaccine to be effective, a sufficient amount of mRNA needs to enter the host cell cytoplasm to trigger the production of the specific antigens. However, the entry of mRNA molecules encounters several challenges. Not only are mRNA molecules too large to pass through the cell membrane via simple diffusion, but they are also negatively charged, similar to the cell membrane, which leads to a mutual electrostatic repulsion. Furthermore, mRNA is prone to degradation by RNAases present in the skin and blood.

Numerous approaches have been established to address these challenges in delivery. Vaccine delivery methods can generally be categorized based on whether mRNA is introduced into cells within the organism (in vivo) or outside it (ex vivo)[18].

1.Ex vivo :-
Dendritic cells present antigens on their surfaces, facilitating interactions with T cells to trigger an immune response. These cells can be harvested from patients, modified with the desired mRNA, and then reintroduced into the patients to elicit an immune reaction[20].

The most straightforward method for ex vivo dendritic cells to absorb mRNA molecules is via endocytosis, a relatively inefficient process in a laboratory context that can be greatly enhanced through electroporation[18].

2.In vivo :-
Following the discovery that administering in vitro transcribed mRNA directly results in antigen expression within the body, researchers have explored in vivo strategies. These methods provide certain benefits over ex vivo techniques, especially by eliminating the need for isolating and modifying dendritic cells from patients and by simulating a natural infection[18].

Various injection methods, including into the skin, bloodstream, or muscles, yield different levels of mRNA absorption, making the selection of the delivery route an essential factor in in vivo applications. One investigation found that injecting mRNA into lymph nodes produced the most significant T-cell response when comparing the various routes[21].

3.Naked mRNA injection :-
Naked mRNA injection refers to the administration of the vaccine solely in a buffer solution[22]. This method of mRNA uptake has been recognized since the 1990s. The first global clinical trials utilized intradermal injections of naked mRNA for vaccination purposes[23]. Various techniques have been employed to administer naked mRNA, including subcutaneous, intravenous, and intratumoral injections. While the delivery of naked mRNA does elicit an immune response, it is relatively mild, and the mRNA is usually quickly broken down after injection[18].

4.Polymer and peptide vectors :-
Vectors of polymers and peptides mRNA can be combined with cationic polymers to create polyplexes, which are protective coverings. These help the recombinant mRNA enter cells and shield it from ribonucleases. Protamine, a naturally occurring cationic peptide, has been utilized to encapsulate vaccine mRNA[24].

5.Lipid based nanoparticles :-
Lipid-based nanoparticles are the most clinically developed for the mRNA delivery vehicles. Lipid nanoparticles (LNPs) are used in all SARS-CoV-2 mRNA vaccines that are either in research or have been authorized for clinical use as of June 2021. Easy formulation, flexibility, biocompatibility, and a huge mRNA payload capacity are just a few of the many advantages that LNPs provide for mRNA administration.

When the FDA authorized the first siRNA medication, Onpattro, in 2018, it authorized the use of lipid nanoparticles as a drug delivery method for the first time. A significant advancement in the development of effective mRNA vaccines was the encapsulation of the mRNA molecule in lipid nanoparticles, which addressed several significant technical obstacles to the mRNA molecule's delivery into the host cell[25]. Studies on the delivery of siRNA to cells via lipids served as a basis for studies on the delivery of mRNA via lipids. However, because mRNA strands are significantly longer than siRNA strands, new lipids have to be developed in order to encapsulate them[26].

The lipid primarily offers a barrier against deterioration, enabling stronger translational output. Furthermore, by altering the lipid's outer layer, specific cell types can be targeted via ligand interactions. Numerous studies, however, have also emphasized the challenge of researching this kind of delivery, showing that the cellular intake of nanoparticles applied in vitro and in vivo differs[27]. There are several ways to deliver the nanoparticles to the body, including intravenously and through the lymphatic system[25].

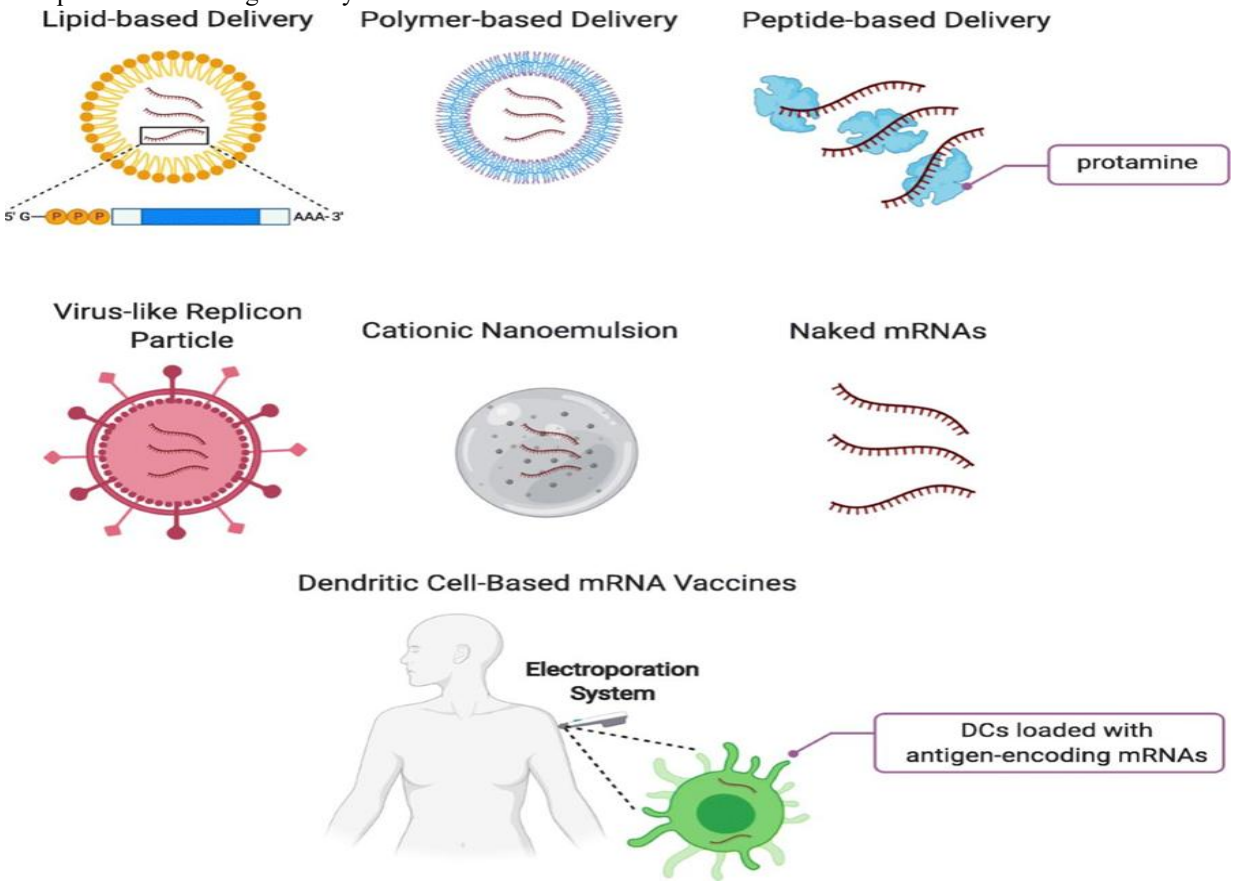

Fig. Methods of delivery and carrier molecules for mRNA vaccine

❖ Storage Condition for mRNA Vaccine

The mRNA component in certain vaccines is delicate, necessitating low storage temperatures to prevent degradation and the subsequent reduction in effectiveness for the recipient. The Pfizer–BioNTech BNT162b2 mRNA vaccine necessitates storage between -80°C and -60°C (-112°F and -76°F), according to. Moderna's mRNA-1273 vaccine can be stored between -25°C and -15°C (-13°F and 5°F), as reported by the company, which is comparable to a household freezer, and it remains stable between 2°C and 8°C (36°F and 46°F) for up to 30 days[28].

❖ Applications

Currently, mRNA therapeutics are being used in the most sophisticated ways in infectious disease vaccines. The use of mRNA-based therapy is anticipated for a number of illnesses that are not responding to present therapies, including cancer, infectious diseases, metabolic genetic disorders, cardiovascular disorders, and cerebrovascular disorders. Therefore, mRNA vaccines have already shown themselves to be a secure and successful method of stopping the spread of COVID-19.

The application of mRNA-based therapies for numerous illnesses, including cancer and AIDS, will necessitate additional study and development despite the field's quick advancements[29].

Infectious diseases :- COVID-19 is one of the many infectious diseases that mRNA vaccines have been shown to be effective in both preventing and treating.

Cancer :- By encouraging the immune system to identify and eliminate malignant tumors, mRNA vaccines are being developed to target and treat cancer cells.

Immunotherapy :- mRNA vaccines are helpful in immunotherapies because they can be used to improve the immune system's capacity to identify and combat foreign cells.

Genetic Disorders :- The application of mRNA vaccines could potentially involve the delivery of therapeutic RNA to rectify genetic anomalies within cells, potentially offering a cure for specific genetic disorders.

Regenerative Medicine :- It is conceivable that mRNA vaccines may serve to stimulate the proliferation of novel cells or tissues, which could prove beneficial in the field of regenerative medicine.

CONCLUSION

As a noteworthy new technology, the mRNA platform has already revolutionized vaccine development. The ability to stimulate broadly protective and long-lasting humoral and cellular immunity is a key feature of mRNA vaccines, which are also easily developed and quickly implemented, reproducible, and reasonably priced to produce.

mRNA vaccines are an incredible tool for fighting pandemics and current infectious diseases, thanks to decades of development and research in mRNA design and delivery technology. Unexpectedly quickly, the first two mRNA vaccines to fight SARS-CoV-2 were created. These vaccines have surpassed expectations and established a solid basis, which is crucial for the development of mRNA vaccines in the future. In the near future, mRNA technology may be used to treat a variety of cancers and create more potent vaccines against difficult-to-fight infections. However, for mRNA delivery to be safer and more effective, technological advancements will be needed. It is necessary to conduct more research on the effects of mRNA vaccines on innate immune responses. The scientific community is hopeful that mRNA therapeutics will revolutionize current biotherapeutic approaches to cancer immunotherapy, protein replacement therapy, and vaccination due to the wealth of positive safety and efficacy data for approved mRNA vaccines and a successful regulatory approval process.

REFERENCE

[1] Plotkin, S. History of Vaccination, Proc. Natl. Acad. Sci. USA 2014, 12283-12287.
[2] Pardi, N.; Hogan, M.J.; Porter, F.W.; Weissman, D. , mRNA Vaccine – A New Era in Vaccinology, Nat. Rev. Drug. Discovery, 2018, 261-279.
[3] Mascola JR, Fauci AS., Novel Vaccine technologies for the 21st century, Nat. Rev. Immunol. 2020; 87-88.
[4] Namit Chaudhary, Drew Weissman, Kathryn A. Whitehead, mRNA vaccines for infectious

diseases : principles, delivery and clinical translation, Nature Review, 2021, Vol.20.

[5] Park KS, Sun X, Aikins ME, Moon Jj, Non- Viral COVID-19 vaccine delivery system, Advanced Drug Delivery Review, 2020, 169.

[6] Kowalski PS, Rudra A, Miao L, Anderson DG, Delivering the messenger : Advances in Technologies for Therapeutic mRNA Delivery, 2019, 710

[7] Kis, Z.; Kontoravdi, C.; Dey, A.K.; Shattock, R.; Shah, N. Rapid Development and Development of High Volume Vaccines for Pandemic Response, 2020.

[8] Wu, K. et. Al. Variant SARS-CoV-2 mRNA Vaccines confer broad neutralization as primary or booster series in mice, bioRxiv, 2021.

[9] Sahin, U., Kariko, K., Tureci, O. mRNA based therapeutic developing a new class of drugs Nat. Rev. Drug Discovery, 13, 759-780 (2014).

[10] Vrinda Gote, Pradeep Kumar Bolla, Nagavendra Kommineni, Arun Butreddy, A Comprehensive Review of mRNA Vaccine, International Journal of Molecular Science, 2023.

[11] https://en.wikipedia.org.

[12] Jackson NA, Kester KE, Casimiro D, Gurunathan S, DeRosa F, The Promise of mRNA Vaccine : a biotech & industrial perspective, npj vaccines, 2015.

[13] Batty CJ, Heise MT, Bachelder EM, Ainslie KM (December 2020). "Vaccine formulations in clinical development for the prevention of severe acute respiratory syndrome coronavirus 2 infection". Advanced Drug Delivery Reviews. 169: 168–89.

[14] Kyriakidis NC, López-Cortés A, González EV, Grimaldos AB, Prado EO (February 2021). "SARS-CoV-2 vaccines strategies: a comprehensive review of phase 3 candidates". Npj Vaccines.

[15] Schlake T, Thess A, Fotin-Mleczek M, Kallen KJ (November 2012). "Developing mRNA-vaccine technologies". RNA Biology. 9 (11): 1319–30.

[16] Anand P, Stahel VP (May 2021). "Review the safety of Covid-19 mRNA vaccines: a review". Patient Safety in Surgery.

[17] Goldman B (22 December 2020). "How do the new COVID-19 vaccines work?". Scope. Stanford Medicine. Archived from the original on 30 January 2021. Retrieved 28 January 2021. Xu S, Yang K, Li R, Zhang L (September 2020). "mRNA Vaccine Era—Mechanisms, Drug Platform and Clinical Prospection". International Journal of Molecular Sciences

[18] Xu S, Yang K, Li R, Zhang L (September 2020). "mRNA Vaccine Era—Mechanisms, Drug Platform and Clinical Prospection". International Journal of Molecular Sciences

[19] Fiedler K, Lazzaro S, Lutz J, Rauch S, Heidenreich R (2016). "MRNA Cancer Vaccines". Current Strategies in Cancer Gene Therapy. Recent Results in Cancer Research. Fortschritte der Krebsforschung. Progres dans les Recherches Sur le Cancer. Vol. 209. Pp. 61–85.

[20] Benteyn D, Heirman C, Bonehill A, Thielemans K, Breckpot K (February 2015). "mRNA-based dendritic cell vaccines". Expert Review of Vaccines. 14 (2): 161–76.

[21] Kreiter S, Selmi A, Diken M, Koslowski M, Britten CM, Huber C, et al. (November 2010). "Intranodal vaccination with naked antigen-encoding RNA elicits potent prophylactic and therapeutic antitumoral immunity". Cancer Research. 70 (22): 9031–40.

[22] Immunisation Advisory Centre. 22 September 2016. Archived from the original on 26 January 2021. Retrieved 20 December 2020.

[23] Probst J, Weide B, Scheel B, Pichler BJ, Hoerr I, Rammensee HG, Pascolo S (August 2007). "Spontaneous cellular uptake of exogenous messenger RNA in vivo is nucleic acid-specific, saturable and ion dependent". Gene Therapy. 14 (15): 1175–80.

[24] Weide B, Pascolo S, Scheel B, Derhovanessian E, Pflugfelder A, Eigentler TK, et al. (June 2009). "Direct injection of protamine-protected mRNA: results of a phase ½ vaccination trial in metastatic melanoma patients". Journal of Immunotherapy. 32 (5): 498–507.

[25] Cooney E (1 December 2020). "How nanotechnology helps mRNA Covid-19 vaccines work". Stat. Archived from the original on 1 December 2020. Retrieved 3 December 2020.

[26] Cross R (6 March 2021). "Without these lipid shells, there would be no mRNA vaccines for COVID-19". Chemical & Engineering News. American Chemical Society. Archived from the original on 5 March 2021. Retrieved 6 March 2021.

[27] Paunovska K, Sago CD, Monaco CM, Hudson WH, Castro MG, Rudoltz TG, et al. (March 2018). “A Direct Comparison of in Vitro and in Vivo Nucleic Acid Delivery Mediated by Hundreds of Nanoparticles Reveals a Weak Correlation”. Nano Letters.

[28] Moderna TX, Fact sheet for Healthcare providers Administering vaccine (pdf), 2021.

[29] Yu-shiuan Wang, Monika Kumari, mRNA based vaccines and therapeutic : an in depth survey of current and upcoming clinical applications, Journal of Biomedical Sciences, 2023.