Delivery of mRNA Vaccine and its Application for COVID-19

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Abstract. mRNA vaccine has potential to bring a revolutionary change to conventional vaccines due to its high efficacy, minimal risk profile, less time needed for development, and potential to be manufactured substantially. This innovation comes to spotlight as it successfully demonstrated its capacity to treat severe pandemic like COVID-19. Over decades of research on mRNA vaccine, a crucial focus has been the efficient delivery of mRNA vaccines into the cytosol to facilitate protein synthesis, and thus several different methods of mRNA delivery have been developed. This passage reviews ways of mRNA delivery, especially lipid-based nanoparticle (LNP) and its application in treating COVID-19.

Keywords: mRNA vaccine, LNP, application.

1. Introduction

As conventional vaccines use attenuated and non toxic pathogens to trigger adaptive immune response, mRNA vaccines use mRNA to encode specific proteins presented on pathogen's surface, therefore triggers immune response. It is promising as mRNA vaccines have advantages comparing to conventional vaccines, including non-infectious nature, specificity and potential ability to treat severe diseases like cancer. mRNA vaccines could be categorized to two types: conventional mRNA and self-amplifying RNAs. Conventional mRNA encodes for antigen of interest, and self-amplifying, derived from virus, not only encoding proteins but they also encode viruses replicating machinery enabling them to self-replicate and elicit stronger responses. Though mRNA vaccine was first proved feasible in 1990s, it wasn't until more recently that their value was fully recognized, due to previous concerns about stability and technological hurdles.

Due to COVID-19 that outbreaks in 2020, draining the world into a sever global health crisis, many different types of vaccines are developed. mRNA vaccines emerged as most promising vaccines due to its low development time, eliciting strong immune response, and simple production process. A significant milestone was reached in August 2021 when the Pfizer-BioNTech COVID-19 vaccine received official approval from the U.S. Food and Drug Administration (FDA), marking a breakthrough of vaccine development.

mRNA's delivery from in vitro to in vivo is crucial part of its development and application. During research of mRNA delivery, several strategies have been developed, including naked mRNA, dendritic cell-based mRNA, cationic nanoemulsion, and the most mature one, also the only that have already proven successful and used in COVID-19 vaccines, lipid-based delivery. These delivery mechanisms can be introduced into the body through various routes, including intradermal (ID), subcutaneous (SC), intramuscular (IM), intranodal (IN), and intravenous (IV) injections. This discussion summarizes the diverse approaches to mRNA delivery, with a particular focus on the highly effective lipid- based method and its pivotal role in the deployment of COVID-19 vaccines."

2. Diverse Strategies for mRNA Delivery

2.1. Naked mRNA

Naked mRNA, suggested by name, is the direct transfer of mRNA to human body without any carrier or encapsulation. This is one of the most straightforward and original method of mRNA delivery, first successfully deliver to mice through IM injection. mRNA first need to be dissolved in buffer to be injected. Generally, two types of buffer are suitable for makes mRNA: Ringer's solution

and Ringer's lactate, both contain calcium which facilitate uptake of naked mRNA. How naked mRNA reaches cytosol is still under discussion as it cannot diffuse across plasma membrane, but two hypotheses has been developed to explain its feasibility: micropinocytosis and mechanical force. Micropinocytosis of macrophages and young dendritic cells are highly active and non-selective, which could serve functions of passing naked mRNA into the cytosol. Mechanical force refers to the hydrostatic force that formed when relatively large amount of mRNA is injected, which could send mRNA into cells by disrupting plasma membrane.

Naked mRNA has a remarkable advantage that it is easy to store. In presence of storage reagent, such as 10% trehalose, and suitable temperature of 4 degrees, it could be stored for up to 10 months. And it only needs to be dissolved in buffer to be usable. Its disadvantage is also obvious that it is extremely susceptible to RNase degradations and is not able to as accurate as other kind of vaccines.

2.2. Dendritic Cell-Based mRNA

Dendritic cell-based mRNA vaccines involve loading autologous dendritic cells with mRNA and then re-infusing them into the body to stimulate an immune response. Dendritic cells are professional antigen presenting cell (APC), playing pivotal role in triggering adaptive immune response. It has high expression of both MHC (major histocompatibility complex) I, II, and cytokines, and move to lymph nodes when antigen presented to further elicit response, what's more, it is highly amenable to modification, which all contribute to its ability serving as a mRNA vaccine.

The primary methods for delivering mRNA-loaded dendritic cells into the body are through ID, SC, IV, and IN injections. Different routes of administrations have effects on different parts of body, for instance, injections through IV spread mRNA to spleen, liver and bone marrow, but totally missing local lymph nodes. Thus, a combination of IV and ID administration are chosen for clinical trials. After injections, two methods are deployed: electroporation and lipid-derived carrier. Electroporation uses electric shock to disrupt plasma membrane and allow dendritic to goes in. Related components, including voltage and resistance, should be adjusted to maximize efficacy. Lipid-derived carrier also provides route through encapsulation and endocytosis. A recent trial on mice shows prophylactic anticancer efficacy with usage of DC mRNA and ionized lipid based LNP, indicating potential of DC-based mRNA to treat cancer.

Dendritic cell-based mRNA is relatively mature type of mRNA vaccine and it has distinctive characteristics of modified capacity. Though due to its complicated manufacturing process, it could hardly be rapidly produced to be a commercial product, dendritic cell-based vaccines offer a highly customizable approach for treating specific conditions, such as cancer. This personalized aspect of the vaccines holds significant promise for targeted therapies.

2.3. Polymer-Based Delivery

Polymer-based delivery, in essence, is like lipid-based carrier which protect mRNA from RNase degradation and enhance intercellular transmission through encapsulation of mRNA. Instead of using lipid, it uses different polymer to deliver mRNA. Most polymers used are cationic polymers because positive charge polymer could bind to negative charge RNA. Two kinds of cationic polymers are mostly used: polyethylenimine (PEI) and polyamidoamine (PAMAM).

Both have strong efficacy. In preclinical mouse trial, PEI successfully delivers mRNA that encodes HIV gp120 proteins, which elicits immune responses after IN administration, and PAMAM dendrimer successfully protect mice from Ebola and H1N1 influenza after IM administration. Both PEI and PAMAM use microfluidic mixing method to form vaccine. Microfluidic mixing is the mixing of mRNA and polymer in micro scale. Another cationic polymer used is chitosan, however, it is second abundant natural polysaccharides instead of synthetic polymers.

Apart from cationic polymers, anionic polymers are also employed in delivery. Because anionic polymers cannot encapsulate negatively charged mRNA, cationic lipid is added to create lipid-polymer hybrid. PLGA (poly (lactic-co- glycolic acid)) is typical type of anionic polymer and has excellent properties of biocompatibility.

Although the instances above have showed high capabilities of polymer-based delivery, it has severe limitations that it is cytotoxic. Due to high cationic charge density and their disruption of cell membrane, which could lead to lysis of cells, it is a common concern for their safety. Second, relatively complexity of their manufacturing processes also limits their use. Furthermore, biocompatibility and biodegradability of all polymers are crucial problems that must be solved (although some type of polymers like PLGA also has recognized biocompatibility)

2.4. Cationic Nanoemulsion(CNE)

Cationic nanoemulsion uses the particle formed by combination of nanoemulsion and cationic lipids to deliver mRNA to body, along with proper adjuvants to trigger immune response boosting efficacy. Nano emulsion utilizes both hydrophilic and hydrophobic surfactants to form a particle with core of lipids and surrounding of aqueous substances. MF59 is a typical oil-in-water nanoemulsion adjuvant, previously used as part of Flu vaccine. MF59 consists of squalene, which occurs naturally and has properties of good biocompatibility and biodegradability, sorbitan trioleate, polyoxymethylene, sorbitan monooleate and citrate buffer. As an adjuvant, it enhances efficacy by MyD88-mediated release, which cytokines and chemokines, recruiting immune cells and without triggering TLRs. Adjuvants like MF59, incorporate with cationic lipids, then could combine with negatively charged mRNA. Cationic lipids, with typical one DOTAP (1, 2-dioleoyl-3-tri-methylammonium propane), also serves function of preventing degradation of RNase. According to results from preclinical trials on mice and rabbits, through IM administration, CNE induced high antigen-specific IgG titer and efficient leukocytes infiltration.

2.5. Peptide-based Delivery

Peptide serve similar functions as polymer used in mRNA delivery. Cationic peptides, such as lysine and arginine are mostly used as they can form complex with negatively charged mRNA through electrostatic interactions, thus, forming complex. The ratio of proportions of peptide and mRNA determine several properties of complex, including particle size and encapsulation efficiency.

Several different peptide delivery mechanisms are developed, with protamine the most outstanding one. Protamine is a arginine rich basic protein, naturally occurring when packaging DNA in sperm cells. Protamine functions well in protecting mRNA and serving as adjuvant. Protamine protects mRNA from RNase degradation and could withstand under harsh conditions of high temperatures. Protamine also serves as an adjuvant, largely due to its similar structure virus, it is immunogenic activating TLR7. According to study, when beta-galactosidase mRNA was complexed with protamine and injected into glioblastoma tumor, coming out with strong anti-tumor effects. However, weakness of protamine complex is that it has mRNA poorly translated. Therefore, further study of potential use of protamine complex like the combination of protamine complex along with naked mRNA is still needed.

RALA peptide is an amphipathic arginine-rich CPP (cell-penetrating proteins). After ID injection to mice, it condenses mRNA into modified dendritic cells, which then trigger cytotoxic T cells. The advantage of this kind of delivery, depending on its properties of amphipathic nature, is the easily achieved endosomal escape because RALA would disrupt endosome membrane.

Anionic peptides are also a medium of delivery, however, given the fact that anionic peptides are negatively charged, they can’t bind to mRNA directly. Therefore, it requires the combination with positively charged polymers. An example of anionic peptide is the OVA-mRNA first copolymer pHDPA (HPMA-DMAE-co-PDTEMA-co-AzEMAm). Then, conjugating with anionic peptide GALA. The result particle shows similar efficiency with other methods of delivery, but with lower cytotoxicity.

3. LNPs and Application for COVID-19

3.1. Lipid Nanoparticle(LNP) Delivery

LNP is the most mature and widely used method of mRNA delivery. Two types of most recognized mRNA treating COVID-19, Pfizer-BioNTech's BNT162b2 and Moderna's mRNA-1273, both use LNP as delivery method. LNP encapsulates mRNA protecting them from being degraded by RNase. Then, it enters the cell through a series of endocytosis process. This endocytosis process transported the mRNA-loaded LNPs into cell membrane-bound vesicles including endosome and lysosome. LNPs eventually send mRNA escape from endosome and mRNA could starts to encode proteins of interest in cytosol. More detailly, LNP interacts with anionic lipid inside endosome, forming special structure which disrupt endosome's membrane from functioning properly, eventually allowing mRNA leaves endosome to cytosol.

A functional LNP consists of four components: Ionizable lipid, phospholipid, Polyethylene glycol (PEG), and cholesterol. Among these four, the most important component is ionizable, or cationic lipid. Ionizable lipid plays significant roles of encapsulation of mRNA, pH value adjustment, escape of endosome, and stability. Ionizable lipid should be positively charged because it needs to encapsulate negative charge mRNA, in which process is done by electrostatic interaction, and have a suitable pH value otherwise it accumulates toxicity. The earliest case of LNP used is combination of DOTAP (1, 2-Dioleoyl-3-Trimethylammonium-Propane) and DOPE (Dioleoyl phosphatidyl ethanolamine), which is adopted from DNA transfection. Though this combination has high efficacy, it cannot sustain for long time in systemic circulation, and could accumulate a high level of toxicity. Then, SPLP (stabilized plasmid–lipid particle) is developed by combining DOPE with ionizable lipid DODAC (Dioctadecyl dimethylammonium chloride). This is a remarkable progress because DOPE ions could interact with endosome's phospholipid to facilitate escape. However, first approved cationic lipid by FDA (Food and Drug Administration) is not used in mRNA, but in siRNA, which is DLin-MC3-DMA developed by Moderna, and it is also the predecessor of SM-102, the ionizable lipid used in COVID-19 vaccine. Other typical ionizable lipid examples are TT3(N1, N3, N5-tris (3-(didodecylamino) propyl) benzene-1, 3, 5-tricarboxamide) and DOTMA (1, 2-di-O-octadecenyl-3-trimethylammonium propane).

Apart from cationic lipid, other three parts: phospholipid, PEG, and cholesterol also play important role in keeping LNP functional. Phospholipid have functions of supporting LNP structure and promoting fusion with endosomal membranes. There diferent types of phospholipids deployed for different properties needed. Saturated phospholipid DSPC (1, 2-Dioctadecanoyl-sn-glycero-3-phophocholine) is suitable for short section of RNA like siRNA, and unsaturated DOPE is suitable for longer section of nuclear acids. DPPC is also used as phospholipids, although it has been widely used in pulmonary drugs. PEG has mainly two functions: provide colloidal stability and improve storage ability. Colloidal stability is achieved through process called opsonization, inhibiting binding of protein with nanoparticle, which is done by a hydrophilic layer made by PEG separating them. PEG improve storage ability by preventing physical aggregation of LNPs in solution, which is done through several methods, including forming steric barrier, creating repulsive force between water and reduce attraction. Cholesterol inserts in gaps, adjusting LNP's thickness, fluidity and water penetration ability, like its functions in plasma membranes. To obtain a functional LNP, it is crucial to keep a balanced molar ratio among ionizable lipid: phospholipid: cholesterol: PEG. This ratio could be slightly different depending on diferent developer. A typical ratio is 50: 10: 38.5: 1.5 from Imperial College London's result of self- amplifying mRNA.

LNP injections have several diferent routes, including IM, ID, SC, IN, and IV. Local injections, using IM, ID, SC, and IN administrations could deliver LNP to local immune cells, triggering strong and long-lasting immune responses. Systemic injection, using IV administration, guides LNP to organs like liver and spleen. It leads to accumulation of mRNA and proteins encoded so it is used when high concentration of antibodies is needed.

Though there are many methods forming liposome, there are only two types that showing fundamental differences: thin-film hydration and continuous-flow microfluidic device. Thin-film hydration is simplest and oldest method of manufacturing liposome. Lipid dissolves in organic solvents and a thin layer of lipid is yielded when it is dried. Then the lipids are hydrated to form a liposome. Lipid film's diameter, extent of hydration, and extrusion cycles are factors that could affect the final product. Another method is forcing a stream of lipid to intersect, sheath, with aqueous solution, mostly water, together. Reciprocal diffusion of alcohol and water causes lipid precipitates to self-assemble to liposome. Most other techniques of liposome formation are variants of there two types.

3.2. mRNA Vaccines' Application

Under severe stress from global health crisis brought by COVID-19, mRNA vaccine first has its chance to become widely used vaccines. Currently, both unmodified and modified mRNA vaccines prove feasible for defense against COVID. Notably modified mRNA vaccines decrease mRNA sensor like TLR cells, enabling high translational efficiency of mRNA. It also has efect of bolster resistance against RNase degradation. It is possible to see this technology been deployed in vaccines treating other diseases, such as Zika virus. More detailed, BNT162b2 from Pfizer BioNTech and mRNA-1273 from Moderna are two typical, and most recognized vaccines used.

BioNTech, in collaboration with Pfizer, initially developed five COVID-19 mRNA vaccine candidates, including both nucleoside-modified and non-modified versions. Among these, BNT162b2 emerged as a leading candidate due to its effective induction of neutralizing antibodies and lower reactogenicity observed in clinical trials. BNT162b2, which encodes a full-length spike protein optimized for pre-fusion conformation, received Emergency Use Authorization (EUA) from the US and Conditional Marketing Authorization (CMA) from the EU, followed by approvals for use in various age groups, becoming leading member of the global health crisis.

Moderna's mRNA-1273 vaccine utilizes a nucleoside-modified mRNA that encodes a transmembrane-anchored, spike protein, delivered within a LNP like the MC3 prototype but incorporating ionizable lipid SM-102 mentioned before. Studies have demonstrated that this vaccine formulation induces robust neutralizing antibodies and a balanced Th1/Th2 T cell response in animal models, effectively reducing viral titers in the lungs and nasal passages with appropriate dosing. Clinical trials in humans have shown that a 100 μg dose generates high binding and neutralizing antibody titers, with a Th1-biased CD4 T cell response, and is generally well-tolerated, though some adverse events were reported.

These findings, coupled with a 94.5% efficacy rate observed in a phase 3 trial, led to the selection of the 100 μg dose for broader use in combating COVID-19, despite higher instances of mild to moderate adverse events compared to some other vaccines.

4. Conclusion

The development of mRNA technology over several decades, coupled with its significant advantages, positions it as a promising alternative to traditional vaccines. The Emergence of Covid-19 not only facilitates the development of mRNA vaccines, but it also examines its capabilities in facing pandemic, from which result turns out to be positive. Many different methods of mRNA delivery are introduced in this passage. Some seems too out of date like naked mRNA, DC and Polymer based mRNA have potential to become strong vaccines solving problems that can't be, LNP already emerges as most advanced vaccine standing out to play pivotal role in fight against COVID. Improvements are still needed for both efficacy and safety reasons. Furthermore, several factors influencing mRNA delivery and vaccine performance remain to be fully explored and optimized for maximal effectiveness.

References

[1] Chunxi Zeng, Chengxiang Zhang, Patrick G. Walker, and Yizhou Dong. Formulation and Delivery Technologies for mRNA Vaccines (2020).

[2] Michael D. Buschmann, Manuel J. Carrasco, Suman Alishetty, Mikell Paige, Mohamad Gabriel Alameh, and and Drew Weissman. Nanomaterial Delivery Systems for mRNA Vaccines (2021).

[3] Norbert Pardi, Michael J. Hogan, Frederick W. Porter and Drew Weissman. mRNA Vaccines—A New Era in Vaccinology (2018).

[4] Enyue Fang, Xiaohui Liu, Miao Li, Zelun Zhang, Lifang Song, Baiyu Zhu, Xiaohong Wu, Jingjing Liu, Danhua Zhao, Yuhua Li. Advances in COVID-19 mRNA vaccine development (2022).

[5] Rumiana Tenchov, Robert Bird, Allison E. Curtze, and Qiongqiong Zhou. Lipid Nanoparticles─From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement (2021).

[6] Sivakumar Ramachandran, Soumya Ranjan Satapathy, Tathagata Dutta. Delivery Strategies for mRNA Vaccines (202).