The mRNA Vaccine of COVID-19

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Abstract: mRNA vaccine is one of the most widely discussed topic, especially in a nowadays COVID-19 world. It has a lot of benefit from the traditional vaccines and can help build sound public health system. Some renowned biotech companies are now conducting research about their most recently study, which help providing a prospective of how this modern technology is going to develop in the near future.

1. Introduction

Since the outbreak of corona virus 2019 (COVID-19) has created a global health crisis that profoundly affected the way we view our planet and our daily lives, the pandemic of COVID-19 has caused more than 211 million confirmed cases and over 4.43 million deaths all over the world till 23 August, 2021 (WHO, 2021, https://covid19.who.int), there has been many recent research conducted to decrease possible future harms to the globe. The COVID-19 virus is particularly deadly because it is an RNA virus, which means it mutates more quickly. Although lots of countries have already managed to contain the epidemic of SARS-CoV-2 through rigorous public health interventions such as the use of face mask, develop efficient vaccines and build herd immunity are the key to end the pandemic. The mRNA (messenger RNA) is a kind of RNA that involved and instruct the progress of protein synthesis. Based on the understanding of mRNA, scientists developed a new kind of vaccine called the mRNA vaccine.

The mRNA vaccine is very different from the traditional vaccine. Although lots of articles have already discussed these topics before, but at the time when the epidemic is raging, the mRNA vaccine is fast becoming a key instrument in containing the epidemic. In this essay, I will review the existing research and provides new insights into the mRNA vaccine in the now a time of severe COVID-19 outbreak world. Specifically, I will state the function of the mRNA, what is the COVID-19 mRNA vaccine and how it works, as well as its differences and advantages from traditional vaccines, comparisons between mRNA vaccines, and what can be improved about it will be shown.

2. The mRNA

The mRNA is the basis to understand mRNA vaccine. Messenger RNAs, also known as mRNA, are one of the types of RNA that are found in the cell. This particular one, like most RNAs, are made in the nucleus and then exported to the cytoplasm where the translation machinery, the machinery that actually makes proteins, binds to these mRNA molecules and reads the code on the mRNA to make a specific protein. So, in general, one gene, the DNA for one gene, can be transcribed into an mRNA molecule that will end up making one specific protein. [2]

Generally, the mRNA is involved in the transcription and translation of genetic information. Just like what the picture showed, in the transcription process, RNA is synthesized from a strand of DNA in the nucleus, using RNA polymerase as a template. When the cell starts to synthesize a kind of protein, RNA polymerase binds to a piece of DNA that encodes this protein, making the double strand of DNA untie, so the basis can get exposed. The free ribonucleotides in the cell complement each other with bases on the DNA template strand, which, in turn, are linked by RNA polymerase to form an

mRNA molecule. The newly bound ribonucleotides bind to the mRNA molecule being synthesized, which is liberated from the DNA strand. The DNA double strand then recovers.

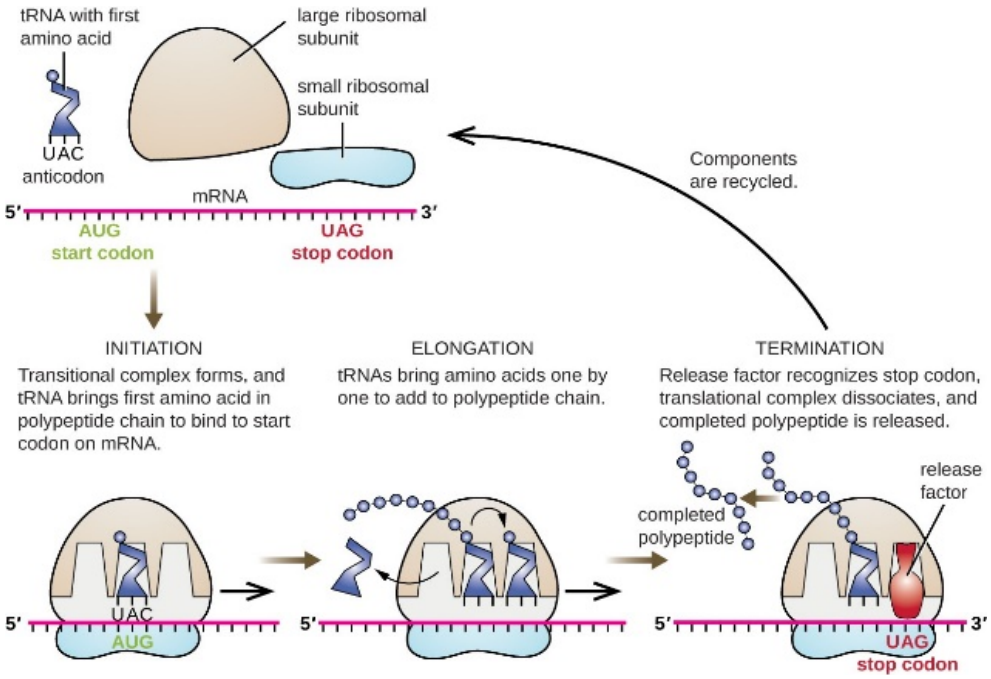

Figure 1. How the mRNA function in the process of protein synthesis [1]

After the mRNA is synthesized, it enters the nucleus through the nuclear pore. Various amino acids in the cytoplasm are used as templates to synthesize proteins with a certain sequence of amino acids. This process is called translation. The picture shown above can demonstrate the process more clearly. During translation, the mRNA first enters the cytoplasm and binds to the ribosome. The ribosome moves along the mRNA in order to read the next codon. As the ribosome moves, the peptide chain is synthesized until the termination codon for an mRNA of the ribosome is known. Once cells finish making a protein, they quickly break down the mRNA.

From knowing how mRNA involves the process proteins are made, we can be able to learn that mRNA is qualified to do most of the job of guiding protein synthesis.

3. The mRNA vaccine

mRNA vaccines work by introducing a piece of mRNA that corresponds to a viral protein, usually a small piece of a protein found on the virus's outer membrane. So, this ensure that individuals who get an mRNA vaccine are not exposed to the virus, nor can they become infected by the vaccine. Using this mRNA blueprint, cells produce the viral protein. As part of a normal immune response, the immune system recognizes that the protein is foreign and produces specialized proteins called antibodies. Antibodies help protect the body against infection by recognizing individual viruses or other pathogens, attaching to them, and marking the pathogens for destruction. Once produced, antibodies remain in the body, even after the body has rid itself of the pathogen, so that the immune system can quickly respond if exposed again. If a person is exposed to a virus after receiving mRNA vaccination for it, antibodies can quickly recognize it, attach to it, and mark it for destruction before it can cause serious illness.[3]

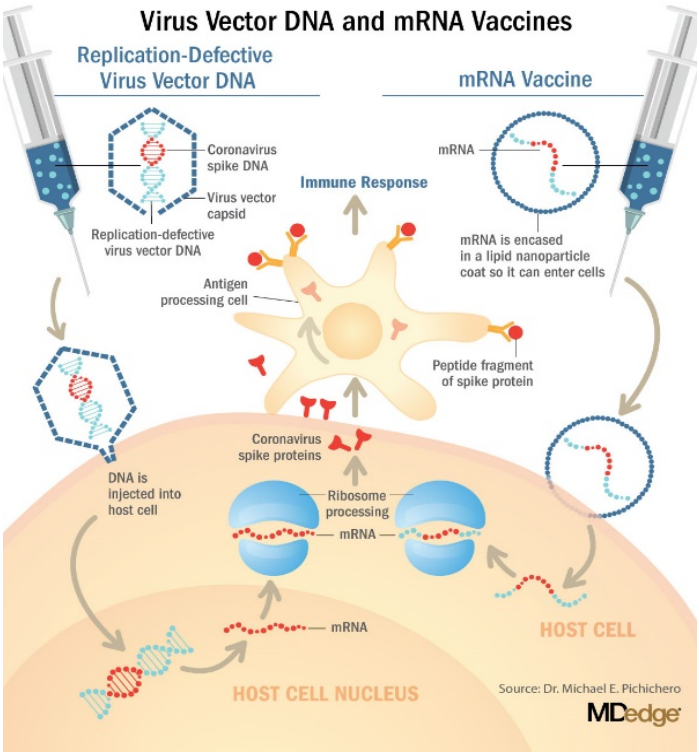

Figure 2. The differences of virus vector DNA an mRNA vaccine [6]

The figure above compares the differences of traditional vaccine (virus vector DNA vaccine) and the mRNA vaccine. We can see that in the traditional vaccine, there is a whole DNA of the virus, while in the mRNA vaccine there is only a mRNA, which means there is less opportunity for the mRNA vaccine to cause uncomforting feelings after injected. After the vaccine is injected, the traditional one has to pass the host cell's nucleus to produce the produce the coronavirus spike proteins and cause immune response, while the mRNA vaccine can have the same effect without getting into the nucleus. I will provide some examples of different kinds of mRNA vaccine and state the advantages as well as the principles of them each.

3.1 Pfizer's mRNA vaccine

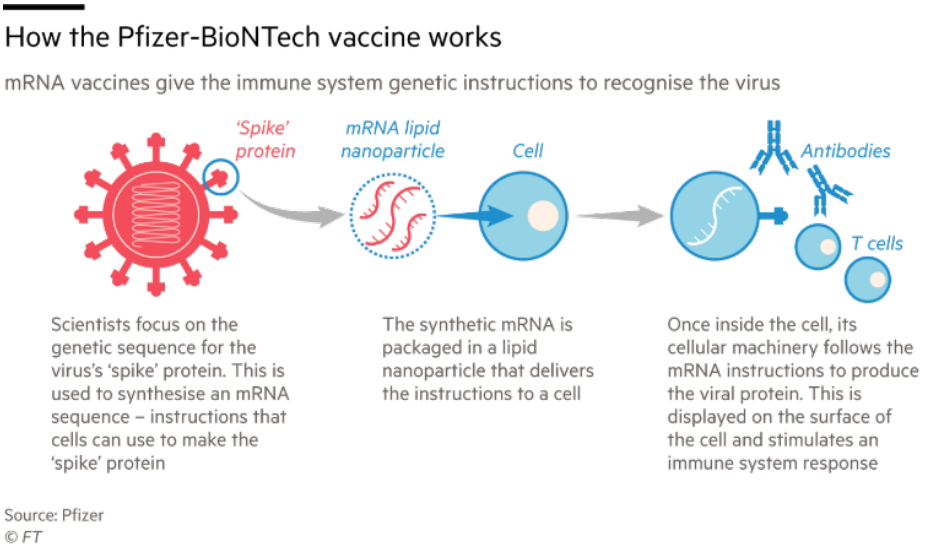

Figure 3. The Pfizer's mRNA vaccine [7]

Like the figure above demonstrates. After the COVID-19 vaccine come into our body, first, the COVID-19 mRNA vaccine is given intramuscularly through the upper arm. The mRNA enters the muscle cell and instructs the cellular machinery to produce harmless fragments of the spike protein.

Spike proteins are found on the surface of the COVID-19 virus. After the protein fragment is made, our cells break down the mRNA and then clear it. Our cells then show spike proteins on their surface. Our immune system recognizes that the protein doesn't belong to us, which triggers our immune system to produce antibodies and activate other immune cells to fight what it thinks is an infection. If you get sick from COVID-19, this is how your body fights off the infection. By the time this process is over, our bodies have learned how to protect against future infection with the COVID-19 virus. As with all vaccines, the benefit of the COVID-19 mRNA vaccine is that the person receiving the vaccine does not face the risk of potentially serious consequences from falling ill with COVID-19. Any temporary discomfort after vaccination is a natural part of the process and shows that the vaccine is effective. [4]

3.2 Abogen's mRNA vaccine

Above is the explanation from the first ever company that made the COVID-19 mRNA vaccine. However, recently, a Chinese company also had invented the mRNA vaccine of COVID-19. Although the function of it is quite similar to the Pfizer's vaccine, they had done some improvements.

The new vaccine is called the ARCoVaX (ARCoV). This Phase I clinical trial aims to evaluate the initial safety, tolerability and immunogenicity of ARCoV, an mRNA vaccine encoding the receptor binding domain (RBD) of novel Coronavirus spike protein (S protein). The test results showed that ARCoV was safe and well tolerated at 5 different doses (5ug, 10ug, 15ug, 20ug and 25ug), and could induce strong humoral and cellular immune responses. Among them, the neutralizing antibody titer induced by the 15ug test group was the highest, which was about twice that of the patients who recovered from COVID-19. These results support further large-scale clinical testing of ARCoV.

It is worth mentioning that the mRNA vaccine ARCoV developed by Abogen has excellent stability and can remain stable at normal refrigerator temperature (2-8℃) for a long period of time (at least 6 months), which provides great convenience for the use of mRNA vaccine to the public. In contrast, Moderna's mRNA vaccine needs to be stored and shipped at -20°C (stable at 2-8°C for 30 days) and BioNTech at -70°C (stable at 2-8°C for 5 days).

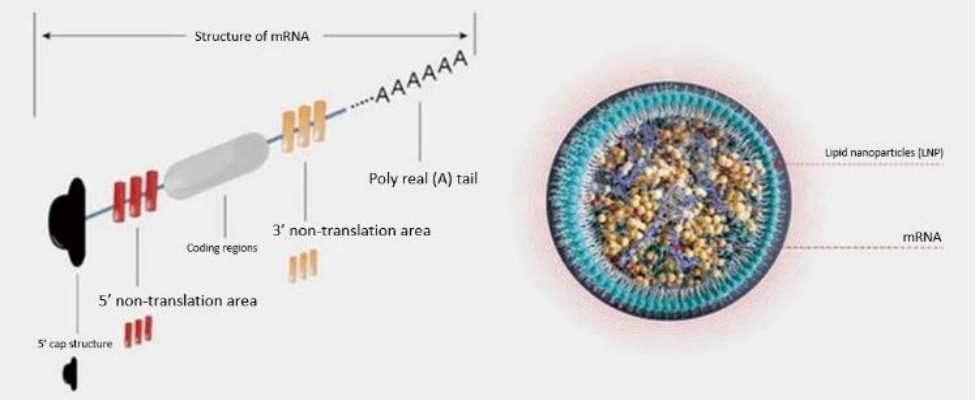

Figure 4. The structure of Abogen's mRNA vaccine.

This vaccine is particularly stable. Stability is one of the major challenges in the development of mRNA vaccines, which is mainly determined by mRNA itself and LNP delivery system. Fig 4 shows that the Abogen's mRNA vaccine's structure, which the mRNA inside the Lipid nanoparticles (LNP) have 5 structures, the 5' cap structure, the 5' non-translation area, the coding region, the 3' non-translation area, and the poly(A) tail at the end. This structure of the mRNA vaccine makes it more stable.

It is also improved in the LNP delivery system. Moderna's mRNA vaccine (mRNA-1273) needs to be stored and transported at -20℃, while BioNTech's mRNA vaccine (BNT162b2) needs to be stored and transported at -70℃, which increases the difficulty and cost of vaccine preservation and transportation. It is not good for the vaccine to be used in more areas. Moderna claims that its mRNA-1237 vaccine can be stored for 30 days at 2-8 ℃, while BioNTech's BNT162b2 vaccine can be stored for up to five days. Details of stability at 2-8°C for both vaccines have not been released.

According to InBev, the long-term stability of Abogen's mRNA vaccine ARCoV can be preserved at 2-8 degrees Celsius. Firstly, the mRNA of ARCoV vaccine encodes the sequentially optimized RBD region of COVID-19 S protein, and the mRNA length is only about 1100nt. This is much shorter than the mRNA sequence of the full-length S protein used by Moderna and BioNTech. The mRNA sequence and structure as well as specific modifications are critical to mRNA stability. In addition, Abogen used LNP, which has its own intellectual property rights, to improve and optimize its process. Detailed stability of ARCoV at 2-8℃ has been published in Cell and Signal Transduction and Targeted Therapy journals. Below is a table that shows the data.

Table 1. Comparison of preservation of different mRNA vaccines

	mRNA-1273	BNT162b2	ARCoV
Transport temperature	-20℃	-70℃	
Preserve temperature	2-8℃		2-8℃
Preserve time	30 days	5 days	6 months

3.3 Circular mRNA vaccine

The circRNA-RBD vaccine elicits an effective neutralizing antibody and T cell response, providing effective protection against Delta and Omicron mutants. Unlike linear mRNA vaccines in use today, the circRNAs are highly stable and do not require nucleotide modification, and can be stored at room temperature for up to two weeks without effect. This shows that circRNAs vaccine has a very good application prospect in fighting the novel coronavirus variant virus.

CircRNAs are ubiquitous in fungi, plants, insects, fish and mammals in nature, and even the genomes of some viruses are circRNAs themselves, such as hepatitis D virus and plant viroids. Unlike linear mRNAs, circRNAs are highly stable because their covalently closed ring structure protects them from exonuclease - mediated degradation. So far, only a few endogenous circRNAs have been shown to serve as templates for protein translation. Although circRNAs lack the necessary components to translate into proteins, they can achieve protein translation through either the internal ribosomal entry site (IRES) or m6A modifications in its 5'UTR region. As what the figure 5 shown, Wei's team designed a circRNA vaccine against novel Coronavirus and its variant strains, and the Wei Laboratory is the first in the world to use circRNAs in vaccine development. The research team used self-splicing type I intron ribozyme to produce circRNA-RBD, which encodes the SARS-COV-2-RBD antigen. To enhance the immunogenicity of RBD antigens, they fused the phage T4 plasminin trimer motif to its C-terminal, thereby mimicking the natural conformation of the s-protein trimer of COVID-19.

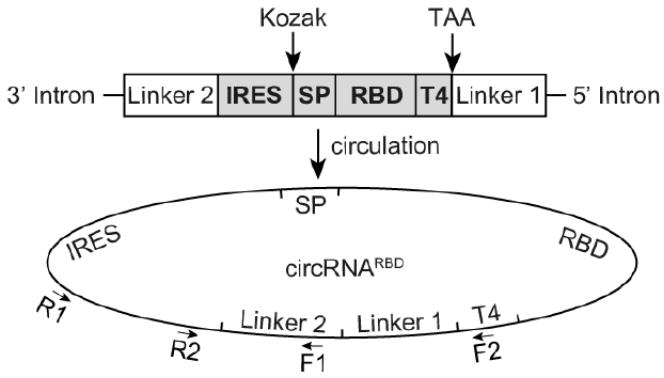

Figure 5. The structure of circular mRNA vaccine.

Cell experiments showed that circRNA-RBD could significantly express novel Coronavirus RBD antigen in human and mouse cells, with significantly higher expression levels than linear mRNA-RBD, and could effectively block the cells infected by COVID-19 pseudovirus. In mice, circRNA-RBD delivered by LNP can effectively neutralize COVID-19 pseudomaviruses and generate a strong T cell immune response in the spleen of mice. This suggests that circRNA-RBD vaccine did induce a durable humoral immune response and a strong T-cell immune response in mice.

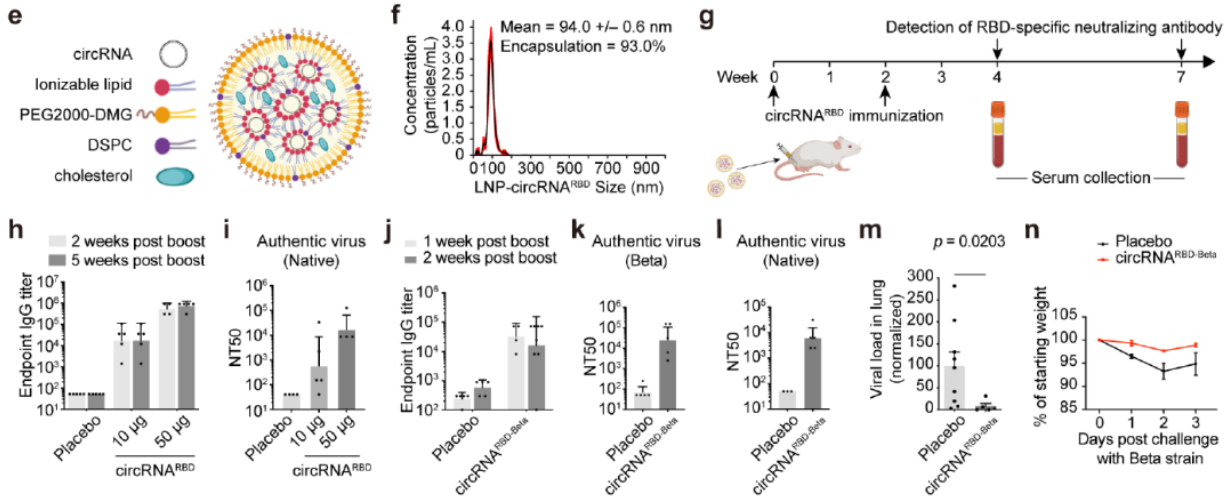

Figure 6. CircRNA-RBD vaccine induced persistent humoral and strong T cell immune responses in mice

The team also designed a circRNA-RBD vaccine against the Delta mutant, which was shown to produce high levels of neutralizing antibodies against Delta and Omicron mutants. This time, the team tested the circRNA vaccine in monkeys and showed that it was effective in protecting rhesus monkeys.

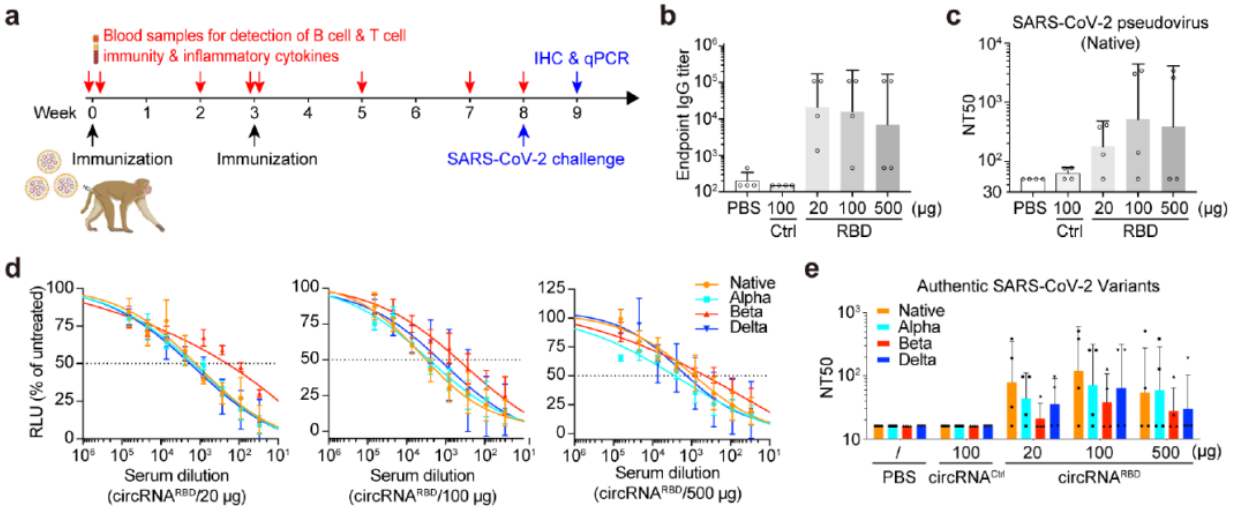

Figure 7. The experiment results of circRNA vaccine protecting the rhesus monkeys.

LNP delivered circrnas vaccines induce effective neutralizing antibody and T cell responses, producing stronger and longer-lasting effects than linear modified mRNA vaccines. Importantly, the study found that the circrnas vaccine against Omicron only induced high levels of neutralizing antibodies against Omicron, whereas the circrnas vaccine against Delta induced high levels of neutralizing antibodies against both Delta and Omicron. This suggests that a circrNA vaccine targeting Delta is a strong vaccine option that could provide extensive protection against the current dominant COVID-19 pandemic strain.

Overall, circular RNA vaccine has been confirmed to have has good thermal stability, high encoding antigen expression quantity and extensive applicability, etc, and design the corresponding circular RNA vaccine to fight will be coronavirus infection and its mutant strains, shows that circular RNA in COVID - 19 pandemic vaccine can be treated as a kind of new vaccines and platform.

3.4 Oral capsule mRNA vaccine

Like most vaccines, the mRNA vaccine needs to be injected using a syringe, which can be a barrier for people who are afraid of needles. If the mRNA vaccine could be delivered orally, it would certainly make it easier for people to accept it. The oral capsule mRNA vaccine's structure is quite similar to other traditional mRNA vaccines. The biggest difference if that it has a special capsule.

The nucleic acids are easy to degrade, and RNA is especially easy to degrade, not to mention in the digestive tract. If the problem of nucleic acid degradation in the digestive tract can be overcome, it can be used to deliver RNA or DNA drugs directly to the digestive tract in addition to oral mRNA vaccines, thus making it easier to treat gastrointestinal diseases.

The research team Professors Robert Langer and Giovanni Traverso of the Massachusetts Institute of Technology (MIT) led developed a new method to deliver mRNA vaccine via oral capsules instead of needles. The team tested the capsules on pigs, and the capsules delivered up to 150 micrograms of mRNA into pigs' stomachs, which is larger than the amount of mRNA used for COVID-19 vaccine. The team believes this oral delivery of mRNA could facilitate the rapid deployment of intermittent interventions, such as vaccines, and support long-term treatment.

They developed a blueberry-sized capsule that can be delivered orally to the stomach and inject insulin into the stomach wall to lower blood sugar. Importantly, the capsule is self-correcting, ensuring that the drug is injected accurately into the stomach wall. Inside the capsule, the needle is attached to a compression spring that is held in place by a disc made of sugar. When swallowing the capsule, the water in the stomach dissolves the sugar disk, releasing the spring and injecting the needle into the stomach wall. There are no pain receptors in the stomach wall, so patients can't feel pain from the injection. To ensure that the drug is injected into the stomach wall, the researchers designed capsules that can be used in the stomach, where the needle may be in contact with the lining. The process of how it alters in the stomache is shown in the Fig 8.

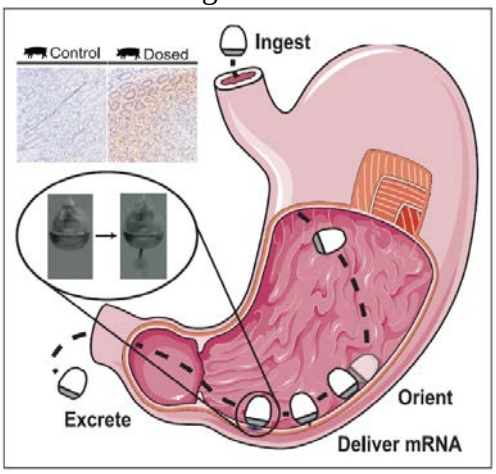

Figure 8. How the oral mRNA delivers the mRNA in the stomach.

Professor L Robert Langer and Professor Giovanni Traverso recently developed a novel polymer nanoparticle capable of efficiently delivering RNA. The nanoparticles are made of poly (β-amino esters), and they found that the branched form of the polymer protects nucleic acids and allows them to enter the cell better than the linear form.

The research team tested the effect of this branched poly (β-amino ester) nanoparticles, and the experimental results showed that after injection, the mRNA carried by the nanoparticles was delivered to the stomach, liver and other organs, and was effectively expressed.

They freeze-dried the mRNA-nanoparticle complex and packaged it into capsules. In collaboration with scientists at Novo Nordisk, they loaded about 50 micrograms of mRNA in each capsule and delivered 3 capsules to the pig's stomach at a time in oral form.This delivery exceeds the current use of the mRNA vaccine, which is about 30-100 micrograms of mRNA at a time.

4. Further development of mRNA vaccine

Although we already achieved some milestones in the development of mRNA, we still have a lot to do. Right now, there are some mRNA vaccine giants challenge common latent viruses. On January 6, 2022, Pfizer /BioNTech announced the start of research and development of an mRNA vaccine for herpes zoster caused by varicella zoster virus (VZV) infection. Clinical trials will officially begin in

the second half of this year. At the same time, Moderna announced the completion of the first volunteer injection of its mRNA vaccine for epstein-Barr virus (EBV) induced mononucleosis. mRNA vaccines, which stimulate an immune response by delivering mRNA containing the genetic code for key parts of a pathogen into human cells, could be a game changer for many diseases. Compared with traditional vaccines, mRNA vaccines have many advantages: they can be developed in a shorter time and can be deployed quickly; mRNAs are not integrated into the genome, avoiding worries about insertion mutations; mRNA vaccines can be manufactured in a cell-free manner, enabling rapid, economical and efficient production. A single mRNA vaccine can encode multiple antigens, targeting multiple microbial or viral variants at once.

Some companies also automated LNP enhances mRNA delivery efficiency and facilitates mRNA therapy development. Vaccines of mRNA have many advantages, such as instantaneous expression of protein in the cytoplasm. mRNA can also be used in tumor immunotherapy, protein replacement, gene editing and in vitro cell therapy. However, the disadvantages of mRNA, such as immunogenicity, sensitivity to RNA and short half-life, seriously limit the application of mRNA. Therefore, it is of great significance to construct new vectors to optimize mRNA delivery strategies, thereby reducing mRNA immunogenicity and increasing delivery stability. Astrazeneca developed an automated high-throughput platform to screen ionizable lipid nanoparticles (LNPS) for enhancing the intracellular functional delivery of mRNA and revealed the mechanism by which LNPS enhance the delivery of mRNA to the cytoplasm. Compared with LNP prepared by traditional techniques, the mRNA delivery efficiency of LNP generated by automated techniques was 4.5 times higher. In this study, ionizable liposomes DLIN-MC3-DMA (MC3) were used as reference lipids, dynamic light scattering (DLS), cryogenic transmission electron microscopy (cryoTEM), Neutron small Angle scattering (SANS) and small Angle X-ray scattering (SAXS) were used to characterize the morphology and structure of mRNA LNP prepared by automated formulation and standard microfluidic techniques. It was found that the fluid dynamics size and polydispersion index (PDI) of automatic mRNA LNP were larger than that of standard mRNA LNP. The shell volume of the automated mRNA LNP is 1.6 times that of the standard mRNA LNP, and the surface is more hydrophobic. Ph-sensitive behavior is critical for LNP endosomal escape, and the team investigated the ph-sensitive behavior of two types of mRNA LNP by studying protonation and hemolysis behavior under acidic buffer conditions. Among them, the hemolysis rate induced by the automatic mRNA LNP was much faster than that of the standard mRNA LNP. Therefore, the automatic mRNA LNP had a better ability to escape from the endosome and enter the cytoplasm than that of the standard mRNA LNP. Finally, the team explored the potential mechanisms by which automated LNP enhances functional mRNA delivery in vivo. Larger LNP particles can accommodate more mRNA, have more hydrophobic surfaces, are more hemolytic, bind to larger protein corona, and tend to accumulate more in macropinocytosomes, thus facilitating the delivery of mRNA to the cytoplasm in quantity.

Moreover, companies are developing high-throughput nanoparticle screening platform enhances whole-body delivery of mRNA. Gene therapy based on mRNAs is promising because, compared with plasmid DNA, mRNAs carry little risk of integration into the host genome, thus providing a safer and more controlled way of gene expression. However, mRNA molecules cannot cross the membrane barrier actively, which seriously hinders the efficiency of mRNA cytoplasmic delivery. In order to achieve effective mRNA gene therapy, the mRNA delivery system must be designed reasonably to overcome the obstacles such as cell internalization and endosomal escape. At present, several methods have been reported to quantify the nanoparticle delivery carrier's ability to overcome endobody interception, such as transmission electron microscopy, high dynamic range confocal microscopy or super-resolution random optical reconstruction microscopy, but they cannot achieve the high-throughput screening function of the nanoparticle delivery carrier. Therefore, the design and development of quantitative and high-throughput nanoparticle screening platform with high predictive ability is of great significance for non-viral gene delivery and optimization of new materials for genetic medicine. Researchers at Johns Hopkins University and Astrazeneca quantified nanoparticle induced endosomal destruction and cell internalization based on mRuby spots and different fluorophore labeled mRNAs generated by gal8-MRuby aggregation on the damaged endosomal membrane. The structure-

function relationship of PBAE quaternary polymer was evaluated, which proved the excellent predictive ability of the screening platform for mRNA delivery. The fig 9 demonstrates these ways explicitly. This study shows that image-based quantitative, high-throughput in vitro screening platform can effectively predict the in vivo delivery capacity of mRNA, opening a new way for the whole-body delivery of mRNA. [5]

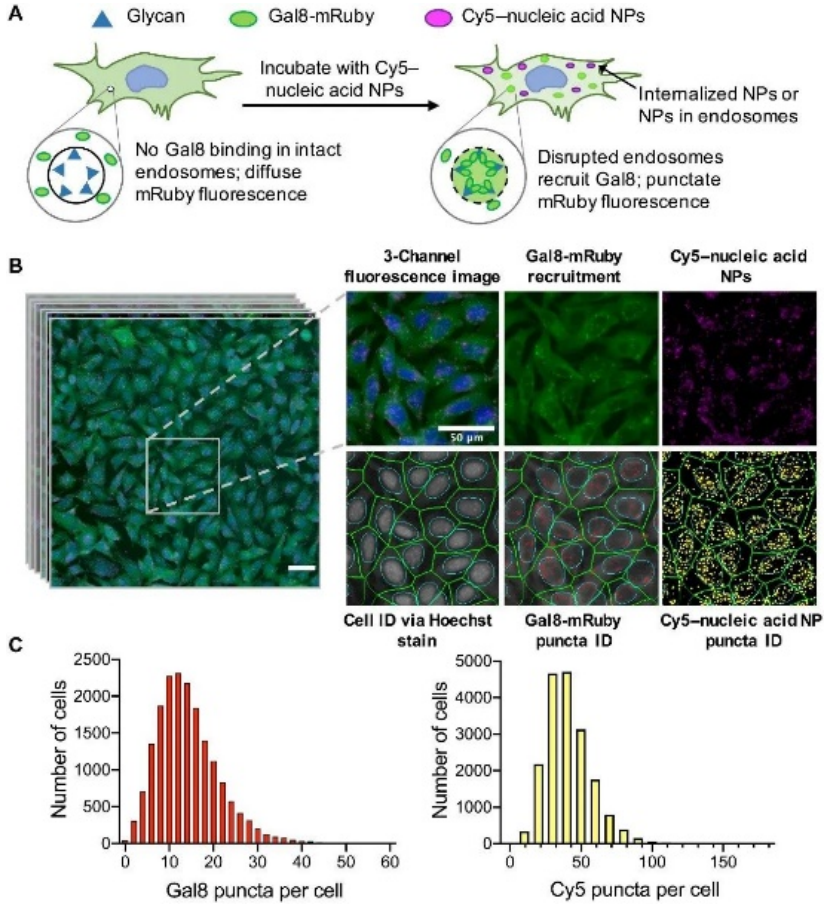

Figure 9. 4 ways to improve the mRNA delivery

5. Conclusion

Conclusively, I discussed about the function of mRNA and mRNA vaccines, compared the different kinds of COVID-19 mRNA vaccines, and provided one idea of how the mRNA vaccines are going to develop in the near future. From what has been discussed above, we can see that Pfizer's mRNA vaccine is effective and as the first company that invented the mRNA vaccine, it is quite impressive. The Abogen's mRNA vaccine is more stable, the circRNA is more effective, and the oral capsule mRNA vaccine is more convenient for people to take. In the future, it may challenge common latent viruses, enhances whole-body delivery, and enhances delivery efficiency in vivo. It can then help the public to achieve herd immunity and therefore promote the public health.

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mRNA vaccine 2.0! Wei Wensheng's team developed a circular RNA vaccine, effective for Delta and Omicron;
Farewell needle injection: oral capsule mRNA vaccine is coming, inspired by turtles;
Into new fields, mRNA vaccine giant challenges common latent viruses;
Science sub-journal: high-throughput nanoparticle screening platform, enhance the whole-body delivery of mRNA, automate LNP, enhance mRNA in vivo delivery efficiency, help mRNA therapy research and development;
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