

## Navigating the horizon of mRNA vaccines: Tracing their evolution, ensuring safety, and unveiling therapeutic potential

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### List of abbreviations

**BNT162b1:** Pfizer-BioNTech mRNA vaccine; **CNE:** cationic nanoemulsion; **DCs:** dendritic cells; **Hib:** *Haemophilus influenzae* type B; **HPV:** human Papillomavirus; **ID:** intradermal; **IM:** intramuscular; **IV:** intravenous; **IFN:** interferon; **IVT:** *in-vitro* transcription; **LAVs:** live attenuated vaccines; **LNPs:** lipid nanoparticles; **MHC:** major histocompatibility complex; **MMR:** mumps, measles, and Rubella; **mRNA:** messenger RNA; **mRNA-1273:** Moderna mRNA vaccine; **pDNA:** plasmid DNA; **PEI:** polyethyleneimine; **saRNA:** self-amplifying mRNA; **SARS-CoV-2:** severe acute respiratory syndrome coronavirus 2; **S-protein:** spike protein; **UTRs:** untranslated regions; **VRPs:** virus-like particles.

### Abstract

Vaccines are vital tools in public health as they play critical roles in preventing and controlling infectious diseases. Vaccine technology has advanced from virus-infected lesions to live attenuated, inactivated or killed pathogens, toxoids, and subunits that consist of only specific pathogen parts needed to elicit an immune response. The progression of virus-like particle vaccines, recombinant viral-vectored vaccines, toxoids, protein or polysaccharide-based vaccines designed to conjugate with a distinct carrier protein to enhance immune reaction is a significant milestone. However, some infectious pathogens can avoid the adaptive immune system, while traditional methods may be unsuitable against non-infectious diseases like cancer. Recent studies have demonstrated the potential of messenger RNA (mRNA) vaccines as an alternative to traditional vaccine approaches. mRNA vaccines contain mRNA that encodes the specific antigen and triggers a directed immune response. The two main forms of mRNA used in the study of mRNA vaccines are conventional non-amplifying mRNA and self-amplifying mRNA (saRNA). This article discusses the mRNA vaccine structure, delivery strategies, and protective functions, focusing on mRNA vaccines'

safety and therapeutic potential. Pre-clinical research has demonstrated the broad utility of mRNA vaccines in animal models. Human clinical trials, however, are still under validation. Hence, further studies will need to focus on adapting reliable results of pre-clinical trials to human applications. The evidence to date suggests that mRNA vaccines are promising next-generation vaccines and, in the future, clinical trials would transform basic research into mRNA therapeutics in medical practices.

**Keywords:** COVID-19, mRNA vaccine, safety, therapeutic potential, vaccination

### Introduction

Vaccines serve a critical role in preventing and controlling communicable diseases.<sup>1</sup> The idea of disease prevention emerged in 1798 when Edward Jenner developed the smallpox vaccine. Vaccine technology has advanced from virus-infected lesions to live attenuated, inactivated or killed pathogens, toxoids, and subunits that consist of only specific pathogen parts needed to elicit an immune response. The progression of virus-like particle vaccines, recombinant viral-vectored vaccines, toxoids, protein, or polysaccharide-based vaccines conjugated

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with a carrier protein to enhance immune reaction are significant milestones.<sup>1,3</sup> Despite significant improvements towards vaccine development, some infectious pathogens can still avoid the adaptive immune system. Furthermore, traditional methods may be unsuitable against non-infectious diseases like cancer. Therefore, a more vaccine-related investigation is required to promote a novel vaccine development platform.<sup>2,4</sup> In recent years, nucleic acid therapeutics, especially messenger RNA (mRNA), have emerged as a new replacement for traditional vaccine approaches.<sup>4</sup> Although mRNA was discovered in 1961, the use of mRNA-based therapy only took off in 1989 when researchers showed that mRNA can be successfully expressed in eukaryotic cells.<sup>5</sup> Since then, mRNA technology has been used widely in studies for other diseases, and now this technology has moved to vaccine technology. The rapid advancement of mRNA technology has overcome some of the challenges associated with excessive immunogenicity, mRNA instability, and inefficient mRNA delivery systems.

This article discusses the different types of vaccines, including conventional and nucleic acid vaccine approaches, focusing on mRNA vaccine structures, delivery strategies, protection functions, and mRNA vaccines' safety and therapeutic potential.

### Types of Vaccines

There are many types of vaccines currently available such as killed or inactivated pathogens, inactivated toxins (toxoids), or recombinant proteins of a part of the surface antigen, which can induce active immunity to a specific disease but, at the same time, does not cause any harm to the body (Figure I). Vaccines stimulate the host immune system to make memory cells and antibodies, which means the body will be able to develop immunity without being exposed to the disease first. Once the body develops immunity, infections can be avoided if the body is later exposed to a pathogen. So, clinically, vaccines are administered to prepare the immune system for "battle".

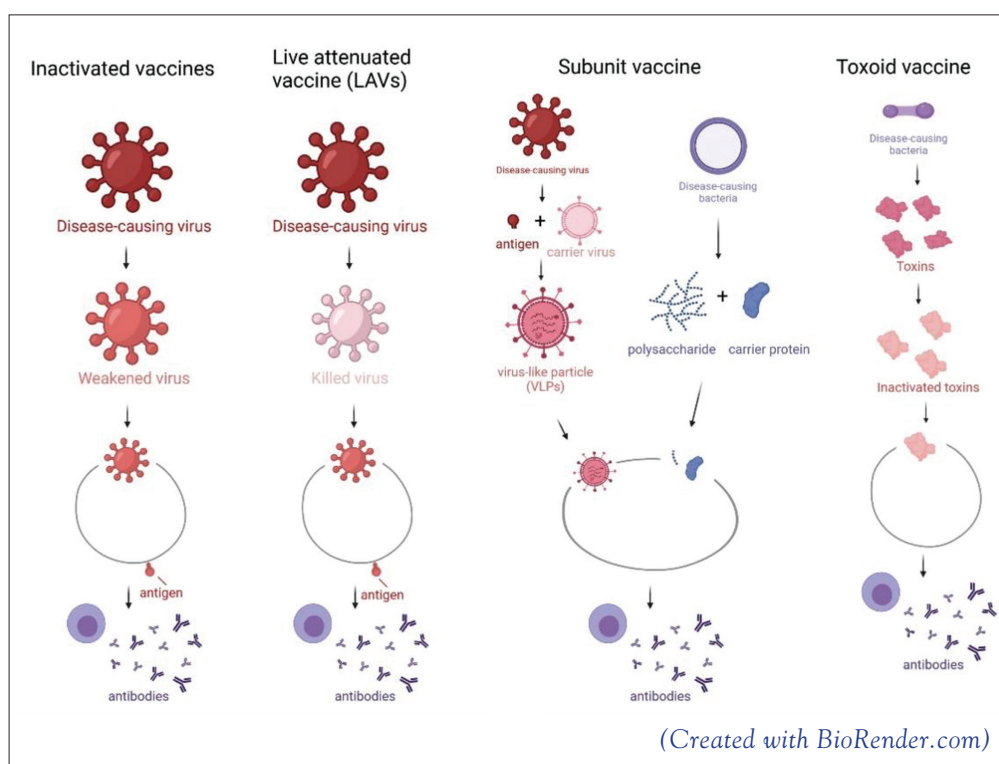


Figure I : Different types of traditional vaccines

### **Traditional vaccines**

Traditional vaccines are categorised based on the process used to produce the vaccines, such as intact killed pathogens (inactivated vaccines) or weakened [live-attenuated vaccines (LAVs)] (Table I). The traditional vaccines can activate the host immune system and develop immunity but do not cause disease as they cannot replicate. Although these vaccines are robust and stable, some safety concerns are associated with the need to use whole pathogens and the lack of defined components of the vaccine. Other traditional vaccines include subunit and toxoid vaccines (Table I). Subunit vaccines use essential parts of the pathogen (proteins or polysaccharides) that can stimulate a specific immune reaction against the specific component. A conjugated vaccine is an improved version of the polysaccharide vaccine where the polysaccharide antigen is covalently attached to a carrier protein, which offers stronger protection.<sup>6,7</sup> Recombinant vaccines are created by inserting antigen-encoding DNA from a pathogen

into a carrier virus or producer cells to produce the recombinant protein vaccine (virus-like particles).<sup>8</sup> Toxoids are chemically inactivated toxins which can provide immunity against the toxins released by bacteria. Regardless of the safety and stability features of subunit and toxoid vaccines, adjuvants are needed to achieve a strong protective immune reaction, as the antigens alone are insufficient to produce long-lasting immunity.<sup>1,6</sup>

### **Modern vaccines**

Nucleic acid vaccines derived from plasmid deoxyribonucleic acid DNA (pDNA) or ribonucleic acid (RNA) have changed the production of the next-generation vaccines. Nucleic acid vaccines contain DNA or RNA-encoding antigens that are developed to link the benefits of live-attenuated and subunit vaccines. These vaccines are known to be safe as there is no risk of pathogenicity and do not require adjuvants.<sup>7</sup> In addition, nucleic acid vaccines can induce B- and T-cell adaptive immune

**Table I : Examples of traditional, DNA, mRNA, and recombinant vaccines**

VACCINE TYPE	EXAMPLES	REFERENCES
<b>Inactivated (killed) vaccine</b>	Polio vaccine (IPV), Hepatitis A, Influenza	8,9
<b>Live-attenuated vaccines (LAVs)</b>	Measles, mumps, and rubella (MMR), Smallpox, Yellow fever, Rotavirus	10
<b>Toxoid vaccines</b>	Diphtheria, Tetanus	6
<b>Subunit vaccines:</b> • Conjugate vaccines	<i>Haemophilus influenza</i> type B (Hib), Pneumococcal, Meningococcal	6,11
<b>DNA vaccines</b>	Influenza, Zika	6,37
<b>Recombinant vaccines</b>	HIV, Influenza, Ebola	15,38
<b>mRNA vaccines</b>	mRNA-1273 (Moderna), BTN162b1 (Pfizer-BioNTech), Influenza, cancer cells	18,19,39

responses specific to the encoded antigen.<sup>12</sup> The pDNA vaccines are plasmids that have the potential to express the targeted gene when injected directly into cells.<sup>13</sup> Another method to deliver pDNA vaccines is to utilise vectors such as adenoviruses to transfer genetic material because of their heightened immunogenicity.<sup>14</sup> Such combinations are known as recombinant vector vaccines. Both pDNA and viral vector vaccines are shown to be immunogenic and safe in clinical studies. A newer approach, messenger RNA (mRNA) vaccines, appears to possess some beneficial features over the pDNA and viral vector vaccines. The mRNA vaccines are reported to be able to overcome some drawbacks of poor immunity observed with some viral vectors and offer the versatility of pDNA vaccines with improved immunogenicity and safety.<sup>16</sup> It should be noted that the mRNA vaccines do not involve the production of infectious particles, and the RNA is not incorporated into the host genome.

Studies on the delivery of mRNA vaccines *in situ* showed that antigen expression occurs without the mRNA crossing the nuclear membrane, and there were no packaging limitations.<sup>12</sup> In addition, mRNA vaccines show effectiveness against various cancerous and infectious diseases, in which traditional vaccines may not succeed in evoking a defense mechanism.<sup>20</sup> Influenza vaccines produced using the mRNA-based approach showed reliable results and elicited strong immunity against homologous and heterosubtypic influenza viruses.<sup>21</sup> The mRNA vaccines have also been used to treat cancers. The targets for cancer mRNA vaccines include tumour-associated antigens that are preferentially expressed in cancer cells and can trigger cell-mediated immune responses. Some of the mRNA vaccines to tumour-associated antigens

that are unique to malignant cells due to somatic mutations are shown in Table I.<sup>22</sup> For the mRNA vaccine approach to be successful, it would require some of the current advanced technologies that permit safer and more reliable mRNA delivery *in vivo*, inexpensive manufacturing process, and rapid, high-quality mRNA production.<sup>17</sup>

## mRNA vaccines

### *The history of mRNA vaccines*

The foundation of mRNA vaccines can be linked to the findings of mRNA in 1961 while investigating the mechanism of protein synthesis within DNA.<sup>23</sup> Two years later, it was determined that mRNA could trigger the production of interferons. Subsequently, in 1975, researches successfully revealed the mRNA cap structure.<sup>24</sup> In 1978, liposomes were utilised as protective carriers to encapsulate and protect the mRNA, facilitating its delivery of mRNA into the cells upon fusion with the cell membrane. Studies confirmed the induction of protein expression in both mouse and human cells through liposomal mRNA transport. Afterwards, the synthesis of mRNA was generated in 1984 using DNA-dependent RNA polymerase enzymes paving the way for *in-vitro* transcription (IVT) with DNA templates.<sup>25-28</sup> Following that, a study by Krieg and Malone in 1989 demonstrated that the transfection efficiency significantly improved when synthetic mRNA was enclosed within cationic liposomes for mRNA delivery. This method was introduced into frog embryos and human cells.<sup>25,29,30</sup> The first description of IVT mRNA in animals was reported in 1990, where reporter gene mRNAs were directly administered into mice intramuscularly, leading to

the expression of the targeted proteins. This addresses prior concerns regarding mRNA stability *in vivo*, as IVT mRNA demonstrated its capability to convey genetic information for the production of specific proteins within living tissue without requiring a virus or non-viral vector.<sup>30-32</sup> Thus, experiments involving the administration of RNA vectors carrying reporter genes (luciferase,  $\beta$ -galactosidase) into murine muscle cells and transfecting vasopressin-encoding mRNA into rats were conducted later as treatments.<sup>29,32</sup> Only in 1993 did mRNA find its application as a vaccine in a preclinical setting, aiming to provoke a targeted immune response against a pathogenic antigen using lipid-mediated delivery (LNPs). This choice was made due to concerns about the potential harmful effects of liposomes in clinical use. These ionizable lipid-based LNPs are recognised for their considerably improved delivery efficiency in hepatocytes following intravenous (i.v) injection or in muscle cells after intramuscular (i.m) injection. In addition, recent discoveries have revealed that LNPs exhibit a strong adjuvant role, providing additional evidence of their advantageous contribution to vaccine development.<sup>26</sup> Martinon and colleagues showcased that an *in vitro*-produced mRNA vaccine encoding the influenza virus nucleoprotein stimulated the creation of virus-specific cytotoxic T lymphocytes in mice. Additionally, Conry *et al.* discovered that *in vivo* mRNA application also generated humoral immunity through B cells. They achieved this by administering a prophylactic vaccine comprised of or containing mRNA encoding a carcinoembryonic antigen, leading to the production of anti-tumoral antibodies.<sup>29,30</sup> However, the first clear demonstration of complete immunity against the influenza virus through mRNA vaccination was

shown in 2012, where Petsch and his team illustrated that when unaltered conventional mRNA, encoding several influenza virus antigens, was administered via intradermal (i.d) injection along with protamine-complexed RNA adjuvant, elicited an immune response in mice. This response was comparable to the immune protection offered by a licensed inactivated virus vaccine.<sup>33</sup> Following multiple publications in 2015, influenza vaccines based on mRNA packaged in LNPs or cationic nanoemulsions (CNE) have proven to stimulate comprehensive immunogenicity in both T and B cells.<sup>34</sup> Therefore, mRNA has gained recognition as a potent vaccine platform and has found extensive use in the production of cancer therapeutic vaccines, as well as preventative vaccines for diseases.<sup>27</sup> In June 2020, the SARS-CoV-2 mRNA vaccine had entered the clinical trial stage, just weeks after the virus sequence was made available. Finally, in December 2020, mRNA vaccines for COVID-19 made history as the first FDA approved mRNA treatments for human use.<sup>31,35</sup>

#### ***The characteristics of mRNA vaccines/ the functioning or the operation of mRNA vaccines***

The mRNA vaccines contain a piece of mRNA that encodes a specific antigen which can trigger the host immune response.<sup>2</sup> The two main forms of RNA used to develop the mRNA vaccines include the conventional non-amplifying mRNA and self-amplifying mRNA (saRNA). The conventional non-amplifying mRNA vaccines consist of a coding region surrounded by 5' and 3' untranslated regions (UTRs), which encode the desired antigen. In contrast, the saRNA vaccines consist of a coding region surrounded by 5' and 3' untranslated regions (UTRs). The saRNA

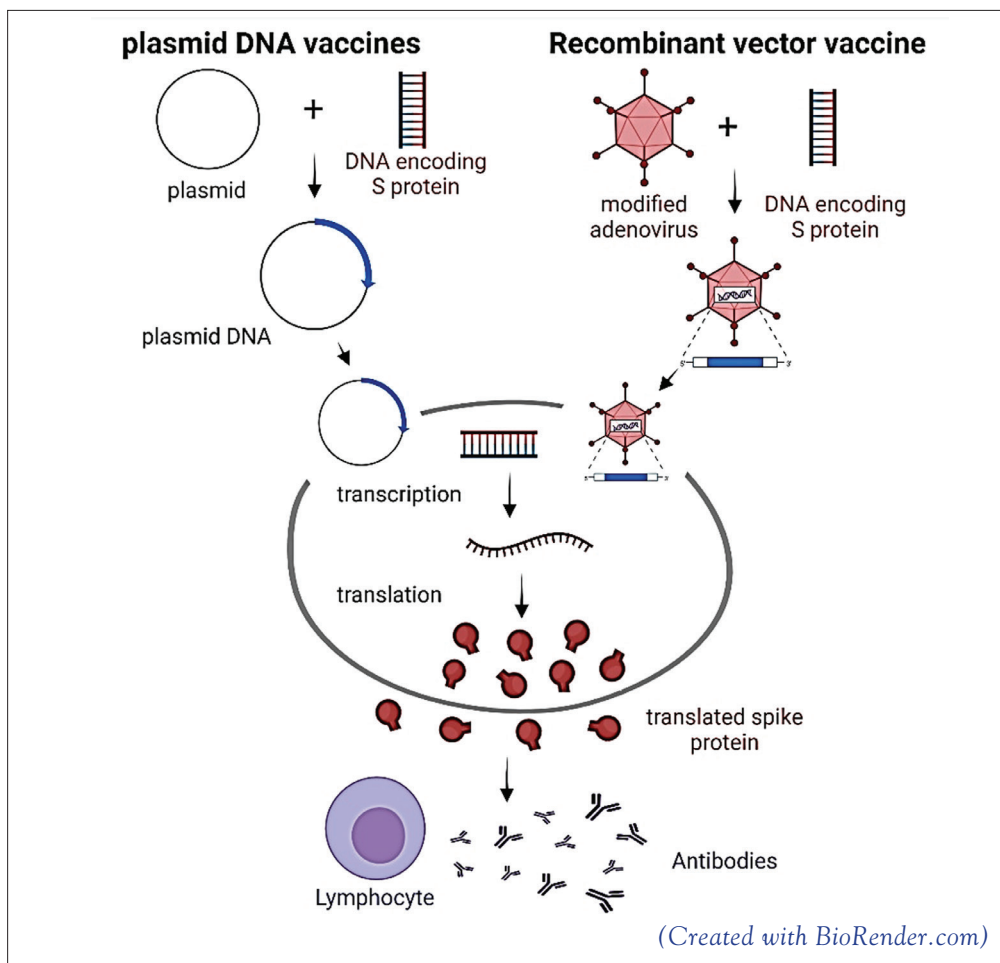


vaccines encode the antigen of interest and contain an additional mRNA coding for the viral replicase enzyme, which amplifies the production of antigen by producing multiple copies of the antigen-coding mRNA.<sup>4,36</sup>

**Understanding the mechanisms of mRNA vaccines**

The mRNA vaccines introduce a fragment of mRNA that typically codes for a small part of the protein located on the virus’s outer surface. The cells then use this mRNA instruction to produce the viral protein, which the immune system recognises and responds to, thereby building immunity (Figure II). Currently, mRNA vaccines against the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

are the only mRNA vaccines authorised by the World Health Organization (WHO) to be used to combat the COVID-19 pandemic. The mRNA COVID-19 vaccines carry a small part of the mRNA that encodes for the spike protein (S-protein) found on the SARS-CoV-2 virus. The S-protein is the vital surface protein situated on the coronavirus virion and serves as the main objective for neutralising antibodies. Upon administration, the mRNA will be internalised by target cells, and the COVID-19 mRNA vaccine will instruct the target cells to generate copies of the S-protein. The ribosomes will translate S-protein mRNA into S-protein in the cell cytoplasm, and this protein will then be expressed on the host cell’s surface (Figure II).<sup>40,41</sup>



**Figure II : DNA and recombinant vaccines**

Detection of the S-protein by the innate arm of the immune system will cause the activation of the adaptive immune system, which will ensure a quick response for future encounters.<sup>42</sup> Pre-clinical studies proved that SARS-CoV-2 attacks relevant replication sites and mimics the characteristics of COVID-19-like infection in animal models, such as short-term viral replication in the respiratory system and light infections. Administration of a low dose of mRNA vaccine (mRNA-1273) in a mouse model induced a robust SARS-CoV-2 neutralising activity and high-level protective effects in the upper and lower airways without any pathologic changes in the lungs of non-human primates. This assessment of immunogenicity and protection of mRNA vaccines in a pre-clinical animal model helped identify clinically relevant doses of this vaccine.<sup>43,44</sup> Table II shows some of the advantages and disadvantages of mRNA vaccines.

### Safety and therapeutic potential of mRNA Vaccines

#### *The safety of mRNA vaccines*

The mRNA vaccine uses a manufacturing process based on an *in vitro* cell-free transcription reaction. Hence, there were safety concerns about using

mRNA vaccines on healthy individuals, which means that the development of mRNA vaccines limits the risks correlated with other vaccine approaches. In addition, the risk of contamination can be lowered due to the short manufacturing time, reducing the chance of the invasion of contaminants.<sup>4</sup> Compared with DNA vaccines, mRNA vaccines do not need to enter the cell nucleus to be translated into proteins. These will be rapidly degraded using cellular processes upon expression of the antigens. Also, the probability of genome integration is minimal because the virus gene sequences are used instead of the virus strains.<sup>1,2</sup> This means that the non-integrative feature and the short-term expressing mechanism inside the cell provide a protective profile of mRNA vaccines.<sup>4</sup> For these reasons, mRNA vaccines are thought to be a relatively safe vaccine format.

The remarkable achievement of the SARS-CoV-2 mRNA vaccine provides significant motivation for the field, yet numerous challenges persist in its future development as the distinctive properties of mRNA molecules require special strategies to ensure the efficacy, stability, and the safety of mRNA vaccines.<sup>45</sup>

**Table II : Advantages and disadvantages of mRNA vaccines**

ADVANTAGE	DISADVANTAGE
<ul style="list-style-type: none"> <li>• Rapid research development and simpler manufacturing process</li> <li>• Nuclear localisation signals and <i>in vivo</i> transcription are not required</li> <li>• No risk of pathogenicity</li> <li>• Higher efficacy than inactivated vaccines</li> </ul>	<ul style="list-style-type: none"> <li>• Unstable and degrades easily</li> <li>• Strong immunogenicity stimulating an unnecessary immune reaction</li> <li>• Safety is lower than inactivated vaccines</li> <li>• Lower efficacy than DNA vaccines</li> </ul>

*adapted from* <sup>56</sup>

To start, mRNA molecules are intrinsically unstable and susceptible to degradation primarily attributed to the widespread presence of RNAses.<sup>46</sup> Research suggests that most naturally occurring mRNA is rapidly broken down within 15 minutes.<sup>29</sup> In addition, both the mRNA and its delivery system displayed a notable level of immunogenicity, triggering innate immune responses while simultaneously reducing mRNA translation.<sup>46</sup> The inherent adjuvant properties of mRNA are associated with the activation of interferon (IFN) type I.<sup>26</sup> These properties can either enhance or inhibit the immune response, depending on the context and timing of IFN type I signaling. They regulate dendritic cells and cytotoxic T cells, affecting their maturation, survival, and translation. However, strong IFN signals may also trigger cytotoxic T cell apoptosis.<sup>27</sup> In addition to this, the delivery systems also possess adjuvant qualities either through their inherent characteristics or when they encapsulate other immune-stimulating agents. Adjuvants can induce local inflammation, which could potentially lead to rare allergic reactions.<sup>46</sup> Anaphylaxis, although rare, has been observed as a side effect of mRNA COVID-19 vaccines. While mRNA may not be the direct cause of allergies, trace impurities in mRNA vaccines may lead to delayed immunological reactions. It is suggested that the presence of PEGylated lipid in LNPs might be a potential allergen for anaphylaxis.<sup>45,47,48</sup> The most commonly reported adverse effects include localised injection pain and local or systemic reactions, such as fever and malaise.<sup>49</sup> Besides, other severe adverse events, including myocarditis, pericarditis, cerebral venous thrombosis, and cytokine release syndrome, have been documented after mRNA vaccination.<sup>50</sup> Current mRNA is modified to enhance stability

and reduce possible immunogenicity. The methods employed to attain these objectives include modifying the 5' cap, extending the poly(A) tail, adjusting the untranslated regions (UTRs), and introducing modified nucleotides. These approaches commonly involve altering sequences and structures to extend mRNA stability and improve translation to optimise mRNA vaccine effectiveness.<sup>51,52</sup> Another significant strategy in mRNA vaccine design involves the effective delivery of mRNA using nanoparticles, which will be discussed further below. In terms of thermal stability, the vaccine efficacy is strongly impacted by temperature sensitivity, making it crucial to maintain appropriate temperature conditions during storage and transportation throughout the entire process. While cold chain is typically essential for preserving vaccines (2–8°C), the demand for mRNA vaccines goes beyond standard cold storage requirements. Examples include anti-Covid vaccines (BNT162b2, mRNA-1273) require temperatures of -80°C and -20°C. However, recent studies reported that mRNA-1273 has shown thermostability for up to 12 hours at room temperature and up to one month at +5°C, while BNT162b2 remains stable for two weeks when stored at +5°C.<sup>31,45</sup> The necessity for extremely low temperature storage presents a limitation for mRNA vaccines due to the instability of the LNP-mRNA system, particularly in resource-constrained countries, where maintaining the ideal temperature range for vaccine storage and transportation can be problematic.<sup>45,46</sup> Furthermore, rapidly mutating antigens pose formidable challenges in developing effective vaccines, causing breakthrough infections and immune evasion in cancers. The emergence of variants, such as in the COVID-19 pandemic, exhibit varying mutation rates during transmission,



ultimately reducing vaccine effectiveness even after a third vaccination. Various strategies including mixed mRNA vaccination and adaptive T-cell based immunity, are explored to address these challenges. In cancer vaccines, complexities arise from antigen loss, mutation, and immunosuppressive tumour environments. Combining mRNA vaccines with agents to reverse immunosuppression is more effective, but immune escape remains a concern.<sup>45,53</sup> Balancing antigen production, adjuvant effects, and side effects in current RNA vaccines presents a significant challenge, and this necessitates further investigation into the interactions between LNPs, mRNA, and the innate immune system.<sup>45</sup>

#### The therapeutic potential of mRNA vaccines

Advances in the structure of mRNA production and intracellular delivery systems have enabled clinical applications of mRNA-based therapeutics.<sup>22</sup> The

mRNA must enter the cytoplasm to be translated into specific antigens. Hence, mRNA vaccines are delivered together with a carrier molecule to overcome the difficulties of transporting mRNA across the cell membrane owing to its negative charge, relatively larger size, and easily degradable by extracellular nucleases.<sup>17</sup> Carrier molecules include lipid, polymer, and peptide-based carriers, virus-like replicon particles (VRPs), and cationic nanoemulsion delivery (CNE). Nonetheless, other strategies of mRNA delivery include direct injection of naked mRNA vaccines and through transfection of dendritic cells (DCs) (Figure III).<sup>1</sup>

In the case of carrier-based vaccines, lipid nanoparticles (LNPs) are considered suitable mRNA vaccine vectors, whereby positive outcomes were seen in LNP-based mRNA vaccines for influenza and Zika virus.<sup>1</sup> Advantages of LNPs include protecting

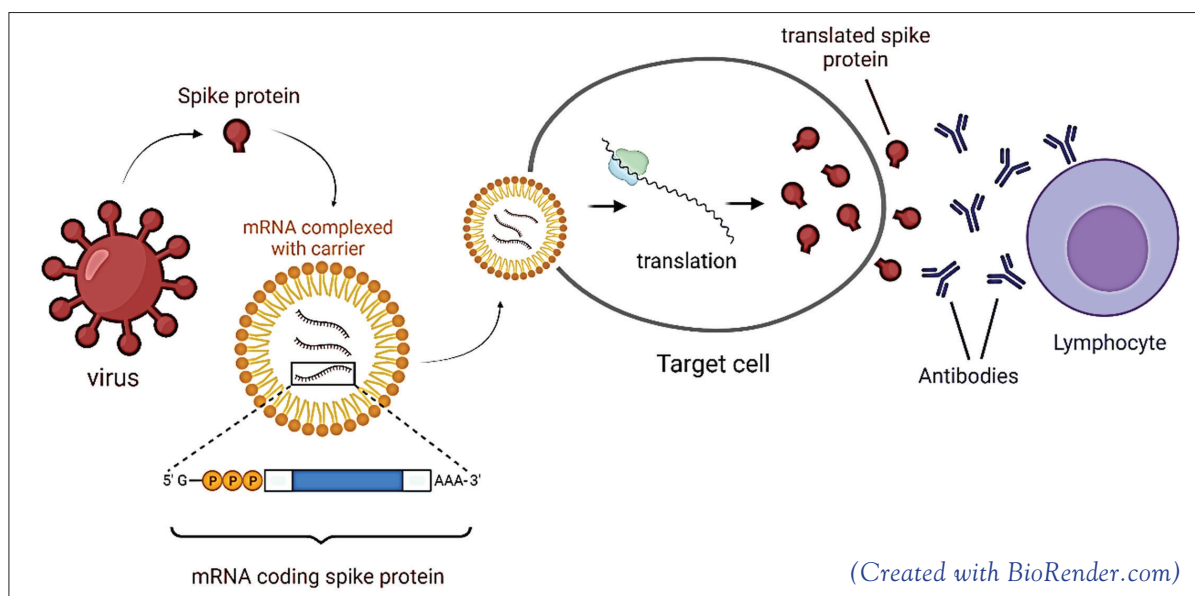


Figure III : Mechanism through which mRNA vaccines stimulate immune responses

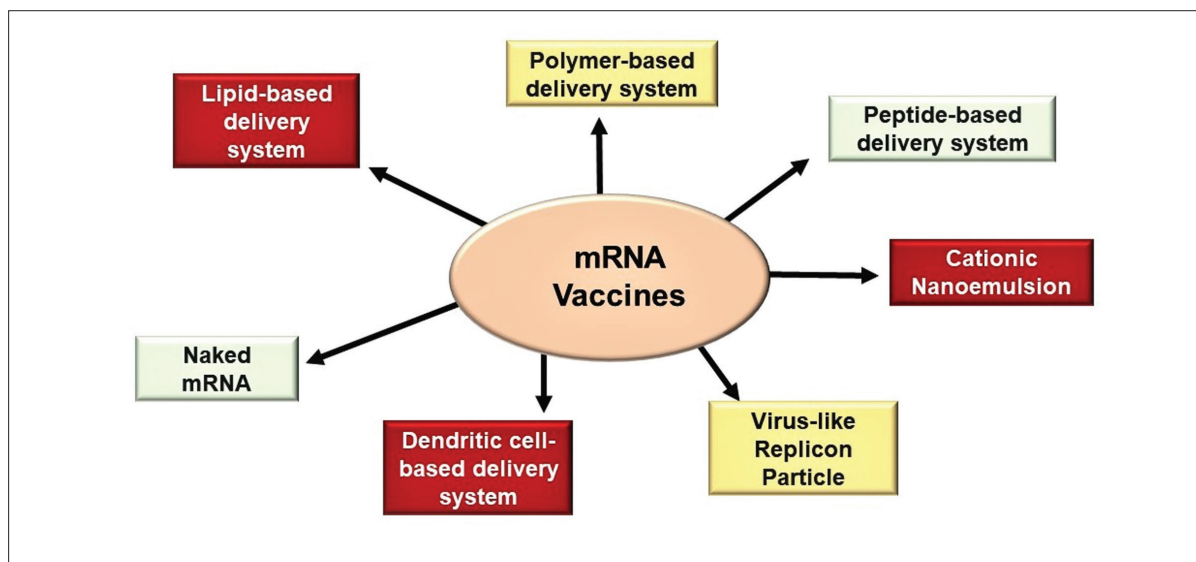


Figure IV : Major delivery methods for mRNA vaccines <sup>1,2</sup>

the breakdown of mRNA by endosomal enzymes, which ensures high encapsulation efficiency and having excellent biocompatibility to deliver mRNAs for expression. Polymer-based delivery systems such as polyethyleneimine (PEI) function like LNPs but to a lesser extent because of their polydispersity and the removal of large molecules, making them less clinically explored (Figure IV). This modification of polymer delivery materials with lipid chains is done to enhance the therapeutic effect.<sup>54,55</sup>

Protamines are cationic peptides delivering mRNA materials.<sup>2,16</sup> Protamine defends mRNA from degradation by serum RNases, while protamine-complexed mRNA results in strong immune responses from the leucocytes (monocytes, neutrophils), indicating that protamine can also be an immune activator.<sup>4,5</sup> The virus-like particles (VRPs) act similarly to a virus-infecting method, encapsulating the desired antigen-encoding saRNA to be sent into the cytoplasm.<sup>2</sup> A good example is the alphavirus-derived replicon RNA encoding SARS-CoV-2 S-protein inducing anti-SARS-CoV-2 neutralising antibody when tested in mice and primates.<sup>57</sup>

Cationic nanoemulsion (CNE) is a non-viral delivery method that binds to saRNAs to increase the effect of vaccines.<sup>58</sup> The CNE-based vaccine vector elicits

more robust cellular immunity than the VRP-based delivery vectors.<sup>2,59</sup> Naked mRNA through direct injection is frequently used to regulate modified mRNA vaccines with other delivery methods.<sup>5</sup> DCs were meant to be the ideal vaccine design considering that they internalise, process, and display antigens to immune cells. An effective adaptive immunity comes from the upregulation of major histocompatibility complex (MHC) molecules for joining antigens, co-stimulatory molecules to give off secondary signals, cytokines for T cell proliferation, and the release of chemokines for T cell recruitment.<sup>2</sup>

#### Future Directions

To date, pre-clinical research has demonstrated the broad utility of mRNA vaccines in animal models. Human clinical trials, however, are still under validation.<sup>4</sup> Preliminary clinical targets were on cancer therapeutics vaccines, and the immunogenicity and safety profile was achieved. Additionally, preventive vaccines against infectious diseases have been tested in humans while other fields of mRNA therapeutics, including the treatment for cardiovascular disease, rare diseases, and personalised medicine, are also explored.<sup>60</sup> Since mRNA vaccines are new, different RNA platforms have insufficient clinical data for comparison and evaluation. Hence, further studies

will need to focus on adapting reliable results of pre-clinical trials to human applications to determine how humans react to components of mRNA vaccine, signs of inflammation, and the most effective immune signalling passage.<sup>61</sup> Future improvements are addressed in mRNA formulations with various expressional and immunostimulatory profiles to gain high efficacy *in vivo* delivery.<sup>62</sup>

### Conclusion

Following the initial publications on mRNA delivery in animal models and the development in pre-clinical and clinical phases in humans, this area has advanced rapidly. It is regarded as one of the vital and promising next-generation vaccines. The concern about this

new type of vaccine arises from its rapid development capacity, safety, and flexibility compared to conventional approaches.<sup>1</sup> Therefore, advancing new mRNA vaccines as the technology improves will be critical. More in-depth studies concentrate on various delivery methods. LNPs, polymers, and peptides have made mRNA delivery more robust, while VLP and CNE also increase the delivery efficacy and widen the range of delivery strategies. Furthermore, antigen-encoding mRNAs delivered by DCs elicit antigen-specific immune reactions. The potential for mRNA vaccines is significant, and in the future, clinical trials will transform basic research into mRNA therapeutics in medical practices.<sup>2</sup>

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