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BIBLIOGRAPHIC REVIEW: MRNA PLATFORMS AND LIPID NANOPARTICLES IN FIGHTING THE COVID-19 PANDEMIC

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ABSTRACT

In the case of rapidly emerging viral pandemics such as the novel coronavirus, rapid development and large-scale implementation of vaccines is a critical need that may not be met by the use of conventional technologies alone. Nanostructured materials have proved to be great allies in vaccine design, catalysing their construction and allowing clinical trials to begin at an unprecedented speed. Among the main immunisation techniques benefited by nanoscience, we can highlight RNA vaccines. In this sense, this work proposes to perform a brief literature review of these new mRNA platforms and the role of nanomaterials in their formulations, highlighting their causes, limitations and consequences of their use. These are based on the transport of ribonucleic acid using nanoparticles capable of increasing the stability and delivery capacity of the genetic material. Pfizer/BioNTech's BNT162b2 and Moderna Therapeutics' mRNA-1273 vaccines, which use mRNA technology delivered by lipid nanoparticles, were two of the first five vaccines authorised for use by the WHO to combat the COVID-19 pandemic, associating speed, safety and efficiencies above 90%.

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INTRODUCTION

In December 2019, an outbreak of pneumonia of unknown causes began in Wuhan province, China (Mulligan *et al*., 2020). The following month, a new coronavirus was identified as the etiologic agent. SARS-CoV-2 infections have transcended borders, taking on international proportions, culminating in the current pandemic of COVID-19. As the months went by, the symptoms that were initially thought to be similar to the flu became more and more complex, from fever, fatigue, loss of taste or smell, and respiratory problems to diarrhea, conjunctivitis, and cardiovascular and renal problems (Yuki, Fujiogi & Koutsogiannaki, 2020). By the beginning of September, according to data from the World Health Organization (WHO), the world already had approximately 4.6 million deaths and 222 million confirmed cases (WHO, 2021). Like the coronaviruses that cause SARS and MERS, SARS-CoV-2 is a positive-sense, single-stranded RNA virus with a genome size of approximately 30 kB. Its life cycle within the host has five stages: attachment, penetration, replication, maturation, and release. After binding to host receptors, viruses enter cells, release their genetic material, and hijack the cellular machinery. In this way, the viral mRNA produces the viral proteins that interact to form new viruses that are subsequently released (Yuki *et al*., 2020). (Yuki *et al*., 2020). Coronaviruses are made up of four structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N) (Figure 1a). The spike protein is the leading participant in the infection mechanism. In a mature coronavirus, the spike protein contains three segments: a large ectodomain, a single-pass transmembrane anchor, and a short intracellular tail. The ectodomain consists of two subunits: a three-headed trimer (S1) sitting atop a trimeric rod (S2) (Figure 1b). During the attachment and penetration steps, the S1 subunit binds to the host cell receptor, while the S2 subunit fuses the viral and cell membranes (Verbeke, Lentacker, De Smedt & Dewitte, 2021).

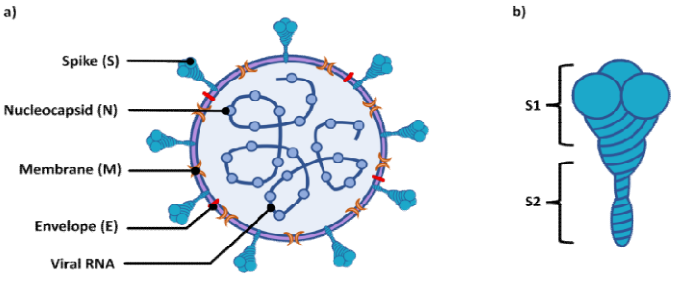

Figure 1. Structure of SARS-CoV-2 (a); Structure of the Spike protein ectodomain (b)

The spike protein contains a receptor -binding domain (RBD) that specifically recognizes angiotensin-converting enzyme 2 (ACE2) as its receptor (Shang *et al*., 2020; Li, 2016). This enzyme can be found in various organs such as the heart, ileum, kidney, bladder, and oesophagus. However, its high expression on the apical side of lung epithelial cells makes them especially vulnerable to SARS-CoV-2 infection (Zou *et al*., 2020). ACE2 is a key regulator in the renin-angiotensin-aldosterone system (RAAS) homeostasis and plays a protective role in several chronic pathologies (Gagliardi *et al*., 2020). The imbalance caused by viral infection is related to lung injury and the progression of COVID-19, especially in those with comorbidities, such as hypertension, diabetes mellitus, and cardiovascular disease (Beyerstedt, Casaro & Rangel, 2021). Several treatments and therapeutic approaches have been tested throughout the pandemic, such as monoclonal antibodies, convalescent plasma, antivirals such as Remdesevir, and antiparasitic agents such as hydroxychloroquine (Tang *et al*., 2021). Research regarding the efficacy and feasibility of available drugs has highlighted the need and urgency for efficient and safe COVID-19 vaccines to limit the spread of the virus and allow for the return of economic and social activities (Izda, Matlock, Jeffries & Sawalha, 2020). Most vaccines applied against other diseases use inactivated or attenuated virus technology to encourage the immune system to produce antibodies (Gebre *et al*., 2021). Although conventional strategies are of great relevance in the fight against endemic diseases, their effectiveness can vary significantly according to the strain to be fought. Besides demanding relatively long periods for production due to the need to produce millions of viruses in the laboratory, isolate, inactivate/weaken, and add other components, only then to arrive at the complete vaccine (Rauch, Jasny, Schmidt & Petsch, 2018). In the last twenty years, great strides have been made in developing new approaches to immunisation, such as viral vector vaccines, protein subunit-based vaccines, virus-like particle vaccines, and nucleic acid vaccines (Callaway, 2020). Nucleic acid platforms based on mRNA stand out for their production speed and high immunogenicity, proving to be a potential approach to combat emerging and rapid viral pandemics such as the novel coronavirus (Rauch *et al*., 2018; Maruggi *et al*., 2019). Unlike many viral vaccines, mRNA cannot integrate into the genome, is not infectious and can be produced without using cells (Kis, Kontoravdi, Dey, Shattock & Shah, 2020). Furthermore, it is an organic molecule with a natural degradation pathway, ensuring its activity is temporary. Although, for a long time, mRNA applications were restricted by the instability of the molecules and inefficient in vivo delivery, the use of lipid nanoparticles (LNPs) as carriers has not only made their use feasible but they have also been presented as enhancers during the immune response generation process (Buschmann *et al*., 2021). The success of the new approach can be seen in two of the first five WHO-authorized pandemic vaccines: BNT162b2 from Pfizer/BioNTech and mRNA-1273 from Moderna Therapeutics (Stamatatos *et al*., 2021). Using LNPs to carry mRNA snippets encoding a version of the ectodomain of the Wuhan-Hu-1 variant-derived protein S, isolated in December 2019, proved efficacy over 94% in preventing COVID-19 in both vaccines (Wu *et al*., 2020; Sahin *et al*., 2021). This paper proposes to conduct a brief literature review on the novel mRNA platforms, BNT162b2 and mRNA-1273, and the role of nanomaterials in their formulations, highlighting the causes, limitations, and consequences of their use.

METHODS

For the literature searches, two databases were consulted: PubMed/MEDLINE and Elsevier/SCOPUS. Two intervals were used to search for publications: 2020 to 2021 for the keywords: mRNA, COVID-19, vaccine, and nanoparticles; 2008 to 2021 for the keywords: mRNA and vaccine.

RESULTS AND DISCUSSION

Design and Mechanism of mRNA platforms: Yellow fever, measles, mumps, and rubella vaccines can be considered early versions of RNA vaccines. These viruses are attenuated and injected subcutaneously to deliver their RNA genome into the host's cells, leading to the viral protein expression. Detecting the viral mRNA and antigens triggers an inflammatory process, allowing an immune response to develop (Pascolo, 2021). The mechanism is very similar to the strategy used by current mRNA vaccines. However, the viral membrane containing lipids/proteins and protecting a complex genetic material has been replaced by a liposome carrying a few simple mRNA strands. The BNT162b2 and mRNA-1273 vaccines are nonreplicating mRNA vaccines, i.e., the active content of the vesicles is limited to the mRNA encoding a specific antigen without the presence of replicase enzymes that amplify the recombinant i.e. Both are administered via intramuscular injection, triggering a localised and transient inflammatory response by recruiting different immune system cells, mainly macrophages and dendritic cells (Verbeke *et al*., 2021). The basic process of generating the immune response by mRNA vaccines can be seen in figure 2. Initially, vesicles containing the mRNA are phagocytosed by antigen-presenting cells (APCs) (1). The LNP membrane interacts with the endosome in the endosomal escape process, releasing the mRNA into the cytosol (2). The mRNA molecules recruit ribosomes and are translated into antigens following three routes: to the proteasome, the membrane surface, or the extracellular medium. The proteasome processes the intracellular antigens, and their fragments are displayed on the cell surface to stimulate CD8+ T cells (cytotoxic T cells) via the Class I Histocompatibility Complex (MHC) (3). The stimulated cytotoxic T cells kill the infected cells through the secretion of molecules such as perforins and granzymes (4). In another pathway, the synthesized viral proteins secreted by host cells can be taken up by other APCs and presented to CD4+ T cells (helper T cells) by MHC class II proteins (5). The helper T cells stimulate B cells to produce specific antibodies against the antigen and activate phagocytes by releasing pro-inflammatory cytokines (Chaudhary, Weissman & Whitehead, (2021); Gao, Yang, Shelling & Wu, (2021)).

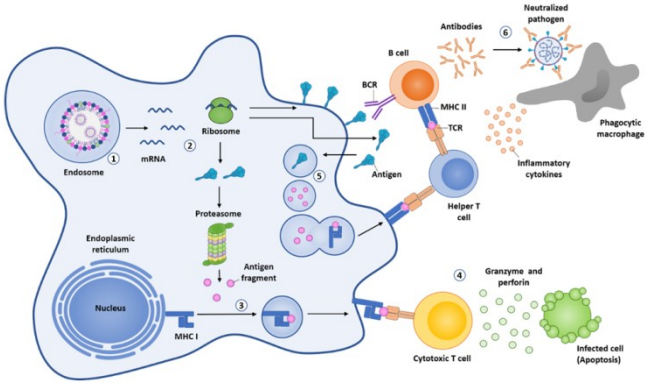

Figure 2. Mechanism of action of mRNA vaccines on APCs. Adapted from Chaudhary, Weissman & Whitehead (2021)

Despite the potential benefits, the development of mRNA vaccines has always faced challenges associated with the intrinsic immunogenicity of the genetic material, susceptibility to enzymatic degradation, and near-negligible levels of cellular uptake (Buschmann *et al*., 2021). To overcome the natural barriers of viral mRNA and optimize the manufacturing process, Pfizer and Moderna's vaccines use in vitro synthesized (IVT) mRNA molecules in a live cell-independent process via bacteriophage mRNA polymerases and a plasmid DNA template (Chaudhary *et al*., 2021). Its structure, illustrated in figure 3, is similar to naturally occurring eukaryotic mRNA, which facilitates the regulation of immunogenic effects. IVT mRNA comprises five regions: a 5' cap structure, the 5' and 3' UTR non-coding regions, the open reading frame (ORF), and the poly-A tail at the 3' end (Figure 3). Each structure is individually optimised to regulate the immunogenic potential of the mRNA and improve the thermodynamic and translation properties. Innate mRNA immunogenicity occurs due to the cellular detection of RNA molecules by pathogen-associated molecular patterning receptors (PAMPs), primarily Toll-like receptors (TLRs) (Devoldere, Dewite, DeSmedt & Remaut, 2016). This detection induces the expression of

type I IFNs (IFN-α and IFN-β) and pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), IL-6, and IL-12. The secreted interferons activate the protein kinase PKR as a general viral defence mechanism. Although the inflammatory response may be of interest in promoting an immune response to vaccine, PKR has an immediate effect of negative translation regulation through phosphorylation of the eukaryotic translation initiation factor eLF2α (Buschmann *et al*., 2021). To avoid identifying the IVT mRNA as an exogenous RNA and the consequent immunogenic response, the U nucleotides are replaced with N1-methyl pseudouridine (Ψ), and thorough purification is performed during the downstream stage to remove residual dsRNAs (Karikó *et al*., 2008; Nelson *et al*., 2020).

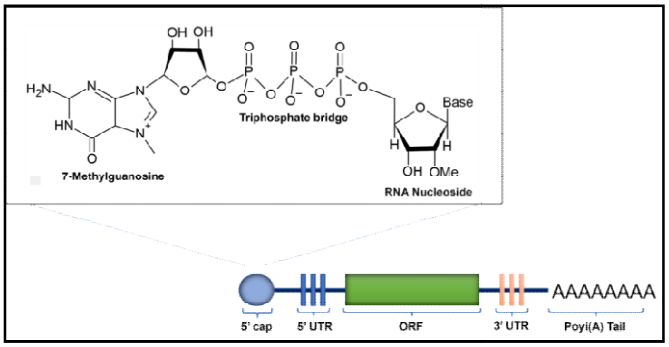

Figure 3. Structure of in vitro transcribed mRNA (IVT). Adapted from Chaudhary *et al*. (2021)

The 5′ cap region prevents RNA degradation by innate immune mechanisms, which interpret uncapped RNA as foreign (e.g., viral) material (Kis, Kontoravdi, Dey, Shattock & Shah, 2020). Its structure is formed by a 7-methylguanosine coupled to the mRNA by a triphosphate bridge and is primarily responsible for the recruitment of the translation initiation factor in eukaryotes (Ramanathan, Robb & Chan, 2016). In BNT162b2 and mRNA-1273 vaccines, cap 5' is the only IVT mRNA region not synthesised directly from the DNA template. Its addition is done later by capping enzymes. The choice between closing the 5' end by CAP0, CAP1, or CAP2 directly influences recognition by the immune system and translation of the mRNA (McCaffrey, 2019). In synergy with the 5' cap, the poly-A tail regulates the stability and efficiency of mRNA translation. Sufficiently long tails must form the complexes responsible for translation and protect the 3' end from enzymatic degradation (Linares-Fernández, Lacroix, Exposito & Verrier, 2020).Because the spike protein is a metastable protein with two leading conformational states, pre-fusion and post-fusion, both Moderna and Pfizer modified the ORF region encoding it by replacing natural residues with two consecutive prolines at amino acid positions K986 and V987 (Baden *et al*., 2021; Sahin *et al*., 2021). The modification allowed the stabilization of the spike protein in its pre-fusion conformation, a strategy used in previous work to potentiate the immune response in vaccines against other coronaviruses, such as MERS-CoV since this protein is the main target of neutralizing antibodies to protect against future infections (Pallensen *et al*.; 2017; Wrapp *et al*., 2020; Graham, 2020; Corbett *et al*., 2020). Although all these modifications potentially improve mRNA stability in vivo, unprotected mRNA alone has limited use due to low cellular penetration. Its high molecular weight (104-106 Daltons) and negative charge prevent diffusion of mRNA molecules across the membrane. Additionally, mRNA is an inherently unstable molecule, highly prone to degradation by 5' exonucleases, 3' exonucleases, and endonucleases (Houseley & Tollervey, 2009). A wide range of approaches has been adopted to overcome these barriers, such as electroporation techniques, ionporation, sonophoresis, and even physical methods such as the use of micro-needles (Pardi, Hogan, Porter & Weissman, 2018; Wadhwa, Aljabbari, Lokras, Foged & Thakur, 2020). Among so many techniques, the use of nanostructured lipid carriers stands out from the rest by associating the protection of genetic material against extracellular enzymes with an increase in cellular uptake and protein expression by up to 1000-fold compared to unprotected mRNA when administered in animal models (Pardi *et al*., 2018; Buschmann *et al*., 2021). This is due to the action of the lipid and polymer components of the vesicles, which can be optimised to regulate circulation time, facilitate endosomal escape and intensify the inflammatory response.

Lipid Nanoparticles: Lipid nanoparticles are nano-sized spherical particles generally formed by four main components: ionisable lipids, auxiliary lipids, pegylated lipids, and cholesterol (Figure 4) (Buschmann *et al*., 2021). To function in vivo, LNPs-mRNA formulations must overcome several extracellular and intracellular barriers. First, the formulation needs to be able to protect mRNA degradation by nucleases in the physiological fluid. Second, the LNPs must avoid the cells of the mononuclear phagocytic system and clearance by renal glomerular filtration long enough for the mRNA load to be delivered. Thirdly, the LNPs must reach the target tissues and be internalised by the target cells. Finally, the mRNA molecules must escape from the endosomes and reach the cytosol, where translation occurs (Hou, Zaks, Langer & Dong, 2021). The structure of LNPs and the main components most commonly used are shown in Figure 4.

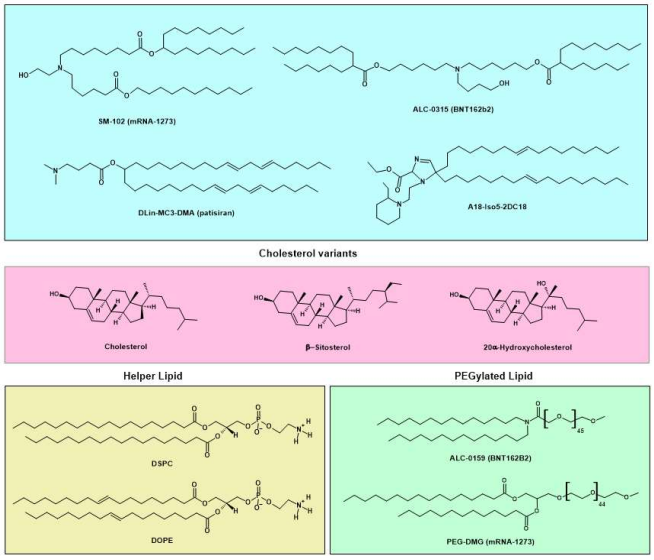

Figure 4. Main lipids used in the formulation of LNPs

Ionisable lipids are by far the most critical components of LNPs. They are neutrally charged at physiological pH and become cationic at low pH. Thus, the synthesis of LNPs occurs in acidic media where these lipids can encapsulate and protect the mRNA molecules due to the negative charge from the phosphate groups. In the physiological environment, the neutral charge reduces the interaction with the anionic cell membranes, improving biocompatibility and prolonging the half-life of circulating LNPs. Within endosomes, where the pH is lower than the extracellular environment, lipids are again protonated, promoting membrane destabilisation and facilitating mRNA release into the cytosol (Buschmann *et al*., 2021). Pegylated lipids are polyethylene glycol conjugated to an anchoring lipid responsible for several structural and pharmacokinetic functions. Changes in PEG molecular weight directly influence the efficacy, circulation time, and uptake by immune cells. Structurally, the presence of polyethylene glycol chains regulates particle size during synthesis by limiting lipid fusion through steric stabilization (Allen & Cullis, 2013). PEG also reduces the interaction of opsonins with ionizable lipids, limiting the action of the complement system and the clearance of LNPs.The larger PEG chains reduce the interaction between the particles and the cell membrane, which prolongs the half-life of LNPs by reducing their uptake into cells. However, the higher PEG content is also responsible for hindering endosomal escape and increasing the production of anti-PEG antibodies, reducing drug efficacy (Reichmuth, Oberli, Jaklenec, Langer & Blankschtein, 2016). Cholesterol, a naturally occurring lipid in the body, and its analogues have mainly structural functions. The association of four hydrocarbon rings linked with a hydroxyl at one end and an aliphatic tail at the other end interacts with the chains of other lipids and considerably

reduces trans-gauche isomerizations. In this way, cholesterol fills the gaps between the membrane lipids of the LNPs, ordering them, regulating their fluidity, and increasing the stability of the nanoparticles. Addition, they also assist in fusion with the endosomal membrane during uptake into the cell, directly impacting the delivery efficacy in vivo (Yang, Kreutzberger, Lee, Kiessling & Tamm, 2016; Patel *et al*., 2020). As the name implies, auxiliary lipids assist the former in their functions by participating in nanoparticle stabilisation and membrane fusion.

For example, dioleoylphosphatidylethanolamine (DOPE) tends to adopt an inverted hexagonal phase that destabilises the endosomal membrane and facilitates the escape of mRNA molecules (Kauffman *et al*., 2015).

Advantages of mRNA platforms: Unlike other vaccines that must adapt their manufacturing processes according to the disease, the development chain for mRNA vaccines is rapid, easily scalable and remains independent of the antigen to be produced. As presented in figure 5, the production manufacturing of mRNA vaccine does not require the growth of the pathogen, only the identification, optimisation, and mRNA expression of the required antigens. Once the optimal composition for LNPs is defined, minor changes are made according to the amount of genetic material to be encapsulated, but the nature of the structure itself is preserved. This is a much more efficient and cheaper process than completely reformulating the vaccine with each strain, as in attenuated/inactivated virus vaccines, or tailoring the components of the delivery vehicle according to the antigen to be carried, as in protein subunit vaccines. This flexibility in manufacturing is exceptionally beneficial for producing mRNA vaccines against rapidly spreading infectious agents (Kim, Eygeris, Gupta & Sahay, 2021). As a comparison, during the 2009 H1N1 pandemic, even with the entire infrastructure to combat other Influenza strains already available, six months elapsed between the start of the epidemic to the application of the first doses, and another two months were needed for the production of the other tens of millions needed (Feldman *et al*., 2019). In contrast, Moderna Therapeutics' vaccine was produced, packaged, and distributed for clinical trials in 42 days from the time the SARS-CoV2 RNA sequence was made available (Gebre *et al*., 2021).

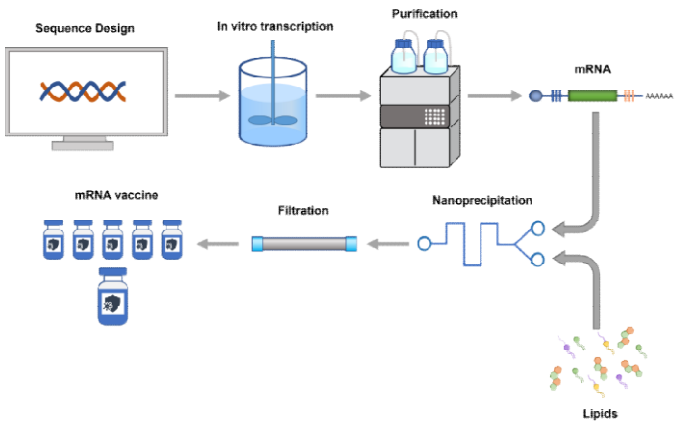

Figure 5. Steps in the development of an mRNA vaccine. Adapted from Chaudhary *et al*., 2021. Once the genetic code of the pathogen is sequenced, the production consists of 6 well-defined steps: (1) genetic engineering to produce the synthetic mRNA; (2) in vitro synthesis of the synthetic mRNA from the plasmid DNA template and bacteriophage enzymes; (3) purification to remove contaminants and reagents; (4) nanoprecipitation: the purified mRNA is mixed with lipids in a microfluidic reactor to form lipid nanoparticles. The rapid mixing causes the lipids to encapsulate the mRNA instantly and precipitate as self-assembled nanoparticles; (5) the LNPs solution is filtered to remove non-aqueous solvents, and any unencapsulated mRNA, and (6) the filtered mRNA vaccine solution receives some excipients and is stored in sterile vials.

The safety profile is another differentiating point of mRNA vaccines. In contrast to attenuated and inactivated vaccines, mRNA vaccines exclude any concerns associated with reversion to virulence and infection. Furthermore, unlike DNA vaccines and viral vector-based vaccines, mRNA vaccines do not pose the risk of genomic integration and insertional mutagenesis, as they do not require nuclear entry for their activity (Sahin, Karikó, & Türeci, 2014). Safety is further enhanced because synthetic antigen expression is limited by the lifetime of the mRNA, which is regulated by innate degradation pathways. The transient nature of mRNA activity is advantageous in providing better temporal control of its activity, avoiding the incessant protein expression. Since the expression of the antigen is ephemeral (about days), the doses do not accumulate in the body, and, if necessary, applications can generate longer-lasting immune responses through booster doses (Kim *et al*., 2021). Finally, the high efficacy of the vaccines can be highlighted due to the specificity of the immune response through genetic engineering of the synthetic antigen. The COVID-19 vaccines, building on prior knowledge about the conformations of the spike protein, developed in record time an mRNA sequence capable of encoding the antigen specifically in the pre-fusion state. As mentioned earlier, the approach guaranteed efficiencies of over 94% in Phase III clinical trials (Wu *et al*., 2020; Sahin *et al*., 2021). Antigen specificity also minimizes adverse effects compared with vaccines from attenuated/inactivated viruses, which carry multiple antigens, or from recombinant viruses, which express several different proteins (Kim *et al*., 2021). However, mRNA vaccines can also be designed to encode more than one antigen of interest, as is the case of Moderna Therapeutics' cytomegalovirus vaccine already in Phase III clinical trials (Yuzhakov *et al*., 2018).

Disadvantages of mRNA platforms: In contrast to Sinovac/Butantan inactivated virus vaccines, which can be stored for 12 months at 2-8 °C and Oxford/AstraZeneca/Fiocruz recombinant viral vector vaccines, which can be stored for six months at 2-8 °C, the Moderna Therapeutics and Pfizer/BioNTech vaccines need to be stored between -15°C to -25 °C and between -60°C and -90 °C, respectively (Meo, Bukhari, Akram, Meo & Klonoff, 2021). Compared to the standard, the need for extremely low temperatures seriously hinders the distribution of mRNA vaccines to low-income countries or in remote areas of the globe, which have their logistics limited by the lack of adequate cold chain structure and distance from major centers. To overcome this problem, vaccine developers are evaluating formulations with greater thermostability or lyophilized formulations that allow storage in milder conditions and for more extended periods. Although the construction of the manufacturing infrastructure is fast and inexpensive compared to conventional vaccines, the availability of raw materials needed for the formulation is also a bottleneck for producing high volumes of vaccines in a pandemic scenario, especially in underdeveloped countries (Kis *et al*., 2020). To date, no research report is available in the public domain on the integrity of the LNPs-mRNA assembly of the vaccines discussed here. In the few studies in which the storage of similar systems is evaluated, the long-term effects have yet to be measured (Zhang *et al*., 2020). However, individual analysis of the system components compared to Onpattro, a siRNA drug loaded on LNPs with a three-year shelf life when kept at 2°C to 8°C, strongly indicates that mRNA instability, rather than LNP instability, determines the storage conditions of the current COVID-19 vaccines (Schoenmaker *et al*., 2021).

Another point to be raised is adverse reactions following vaccine administration, which, although numerically rare, can cause substantial fear and anxiety in the general population contributing to decreased willingness to receive COVID-19 vaccine (Banerji *et al*., 2021). In most cases, vaccine-related-allergic reactions are attributed not to the active ingredients, but to the excipients such as egg, gelatine, formaldehyde, and polysorbate. In the case of mRNA vaccines, which do not contain any of these, suspicions fall on PEG, a polymer widely used in food and cosmetics. Although it is considered safe and biologically inert, it has been studied due to the relationship between its molecular weight and the intensity of allergic reactions (Stone, Rukasin, Beachkofsky & Phillips, 2019). Recently, there have been increasing studies linking severe allergic reactions to the use of

PEG-containing products, and although its mechanism has not yet been elucidated, it has been shown that 70% of patients who have undergone treatment with pegylated therapeutics end up developing anti-PEG IgG antibodies. These antibodies may reduce the drugs' half-life through accelerated blood clearance of subsequent dosages (Yang & Lai, 2015; Yang *et al*., 2016; Cabanillas, Akdis & Novak, 2021). According to the US Centers for Disease Control, cases of anaphylactic reactions associated with mRNA vaccines in the US are between 2.5 and 11 cases per million applications, a value at least five times higher than the average for traditional vaccines (CDC, 2021; Risma *et al*., 2021; Tanno, Castells, Caminati, Senna & Pacal, 2021). Even though the mRNA is inherently pro-inflammatory due to its interaction with PAMPs receptors and, because of its negative charge, can activate the contact system triggered by factor XII, culminating in the production of bradykinin, an oligopeptide associated with anaphylactoid reactions. The mRNA payload is unlikely to be the primary stimulus for allergic reactions, unless the stability of the LNP vesicles has been impaired. Such a phenomenon may occur during freeze-thaw cycles before vaccination (Risma *et al*., 2021; Bender, Weidmann, Rose-John, Renné & Long, 2017).

CONCLUSION

The low mRNA uptake by target cells and its high degradation by extracellular RNAases, even after modifications, endorse the statement that the recent progress of mRNA platforms would not be possible without the action of LNPs, responsible for carrying, delivering, and regulating vaccine immunogenicity. After decades of academic efforts towards enabling technologies based on mRNA delivery, the association of lipid nanoparticles with in vitro-synthesized mRNA has shown its potential in fighting emerging pandemics during the COVID-19 crisis. The developmental manufacturing used in the BNT162b2 and mRNA-1273 vaccines will be a springboard for future nanomedicine applications. The approval of these drugs for emergency use during the pandemic marked the beginning of a new era for vaccinology. The lack of an infrastructure prepared to meet their transportation and storage demands is the main problem to be solved. The search to increase the stability of these formulations and elucidate the mechanisms of interaction between the excipient ingredients and the body will be a challenge to overcome to realise the mRNA platform technology as a key to maintaining public health in the post-pandemic period.

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