

mRNA-lipid Nanoparticle Vaccines: Structure and Delivery

Yiming Cai ^{1,*,†}, Junru Liao ^{2,†}, Haocheng Tan ^{3,†}

¹ School of Environmental and Biological Science, Rutgers University-New Brunswick, New Jersey, USA

² School of Biomedical Sciences and Engineering, South China University of Technology, Guangdong, China

³ School of International Education, Henan Institute of Science and Technology, Henan, China

* Corresponding author: haocheng0509@stu.hist.edu.cn

†These authors contributed equally.

Abstract. As the latest generation of vaccine production technology, mRNA vaccine has achieved vaccine production and clinical application, such as COVID-19 mRNA vaccine. However, a substantial inherent limitation in the development of mRNA vaccines is that mRNA is chemically unstable and susceptible to lysed by lysosome. Therefore, it is necessary to develop suitable vectors for mRNA. Today, the mainly COVID-19 vaccines include mRNA produced by Moderna was approved by the FDA in the world. mRNA-1273 is a nucleic acid vaccine delivered by a lipid nanoparticle (LNP). As mRNA vaccines become widely available for mass vaccination, LNP have emerged as a popular delivery system. In this review, we introduce the history of mRNA after starting from mRNA vaccines, discusses the structure of LNP including ionizable cationic lipids, cholesterol, neutral lipids, PEG-lipids, and the role of each component. It also explains the mechanism of LNP delivery in mRNA vaccine and introduces different types of LNP, and describes several factors affecting particle delivery. It has been shown that the delivery of LNP is affected by particle size, particle surface charge, surface polyethylene glycolization and specific targeting modification. This review serves as a useful provides for insights into the delivery of LNP and the design of new therapeutic vaccines.

Keywords: lipid nanoparticles, mRNA vaccine, COVID-19, ionizable cationic lipid.

1. Introduction

Messenger RNA (mRNA) links DNA information to synthesize proteins. mRNA-based therapeutics have become a promising strategy to manage various diseases, including mRNA vaccines. mRNA vaccines function by transfer RNA to cells for antigen synthesis, leading to an immune response [1]. mRNA vaccine clinical trials focused on cancers such as prostate cancer, melanoma, leukemia, as well as the most recent SARS-CoV-2 pandemic. mRNA vaccine production is less expensive with safer profiles than DNA vaccines. The safety profiles are exerted by avoiding any genomic integration risk. However, a substantial inherent limitation of mRNA vaccines is chemical stability of mRNA is low (mRNA can be easily hydrolysis catalyzed by nucleases), among other risks including allergy, cytokine storms, renal and heart failure [2]. Namely, mRNA delivery into the cell has been an ongoing challenge. It is therefore essential to develop appropriate carriers for mRNA vaccines to reduce degradation and increase immune responses and safety.

Injection of carrier-free mRNA, lipid/polymers/protein derivative-based packaged mRNA have been the current approaches to deliver mRNA [3]. Naked mRNA can strongly stimulate TLR and PKR, leading to substantial RNase degradation. Polymer material-based delivery of mRNA vaccines seems efficient in various preclinical studies, but further improvement in biodegradability and delivery specificity is required for the eventual clinical application. Lipid-derived nanoparticles (LNP) have been widely employed for mRNA vaccine delivery, because LNP-based mRNA vaccines are characterized by enhanced delivery efficacy and reduced infectious complications [4].

2. History of mRNA vaccine

By the late 1980s, investigations on structure and function of mRNA led to the *in vitro*-transcribed mRNA development. In the 1990s, Acsadi and Jiao et al. demonstrated the therapeutic potential of mRNA. The RNA interference (RNAi) development that won the Nobel Prize in 2006 encouraged a bunch of studies on RNA as a therapeutic method, expanding RNA investigations and consequently triggering mRNA vaccine development [5]. Nonreplicating mRNA and self-amplifying mRNA are the major two types of mRNA vaccines. Non-replicating mRNA merely encodes the target antigen, containing 5' and 3' untranslated regions (UTRs) [3]. Self-amplifying mRNA encodes not only the target antigen but also a replicase complex that promotes intracellular amplification of the RNA, which eventually upregulate the protein expression. The mRNA vaccines comprise of a 5' cap, UTRs, an open reading frame (ORF), and a 3' poly(A) tail [3]. More precisely, the formation of LNP-based mRNA vaccine includes: 1) plasmids of antigen gene are retrieved and transformed to *E. coli*. 2) Multiplying *E. coli* are cultured in LB growth medium. 3) The gene sequences of harvested plasmids are confirmed and purified. 4) Linearized DNA is added to nucleoside triphosphates, including ATP, GTP, UTP and CTP, and helicases are used to unravel the DNA template to enable it to be transcribed into mRNA. 5) Posterior to purification, the mRNA is monitored containing the accurate gene sequence. 6) The mRNA is encapsulated into carriers such as liposomes, and then an mRNA vaccine is generated. LNP-mRNA vaccines are reported to trigger immune protection against viral infections, bacterial infections, parasite, as well as sufficiently stimulate chimeric antigen receptor (CAR) T cells [6]. Figure 1 illustrates the scheme of two different mRNA vaccine constructions.

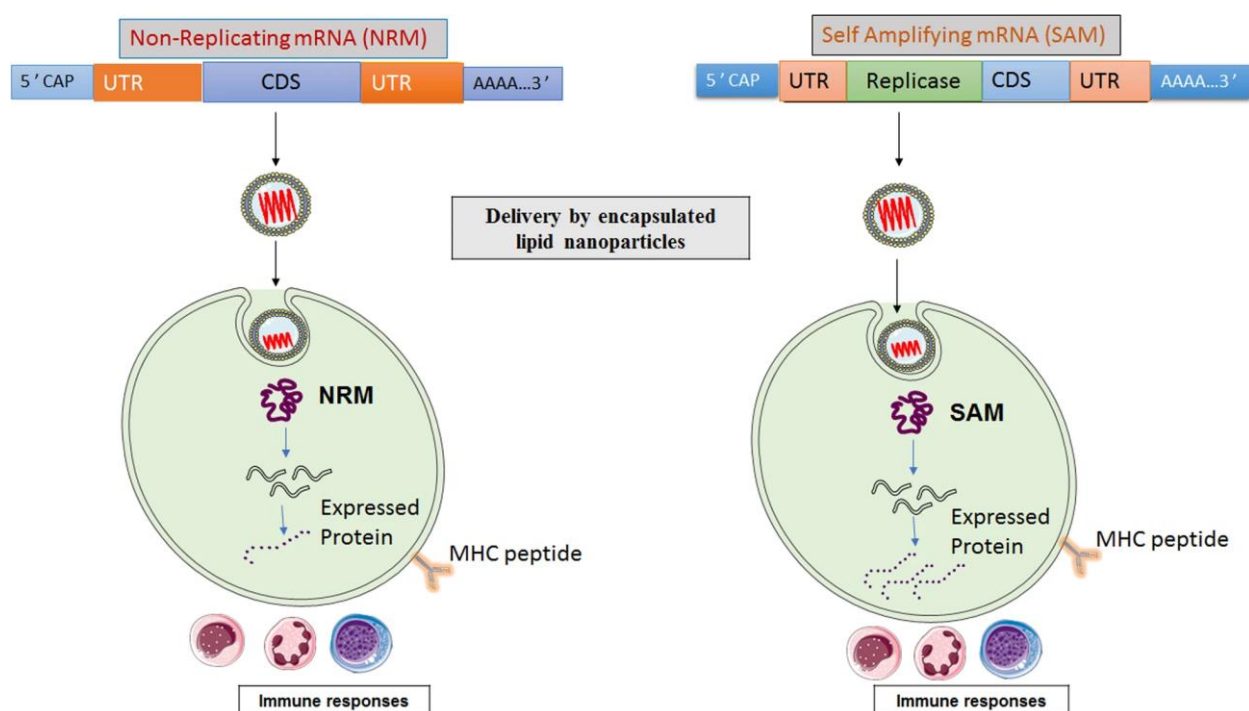


Figure 1. Schematic illustration of non-Replicating mRNA vaccine and Self Amplifying mRNA vaccine constructions [7]

Among various nanoparticles applied for mRNA delivery, LNPs are the most promising strategy to be successfully applied in the clinic for *in vivo* precise mRNA delivery [8]. LNPs are stable particles that consist of a lipid bilayer, inclusive of cholesterol, auxiliary lipids, cationic lipids, and polyethylene glycol encompassing an aqueous core. mRNA encapsulated in lipid nanoparticles induce high levels of germinal center B-cell like (GCB) and T follicular helper (TFH) cells, trigger CD4⁺ T cell response to specifically target antigen. The TFH and GCB cells are associated with antibody generations, providing a long-term protection. Of note, lipid nanoparticles were employed to deliver mRNA-1273 and BNT162b that are the two authorized COVID-19 vaccines [9-11]. Many

other LNP–mRNA generations are in clinical trials for the management of genetic disorders, infection-related diseases and cancers [3].

3. LNP-based delivery in mRNA vaccine

3.1. Structure of LNP

In order to solve the problem that the naked mRNA will be lysed by the lysosome, extensive research has been conducted to develop the protection system which is able to deliver mRNA successfully. Among these, LNP technology has been developed and proven to be efficient in stabilizing mRNA in the cells.

LNP, a lipid nanoparticle, is a lipid vesicle with a homogeneous lipid core. Small molecule and nucleic acid medication delivery are its principal uses. LNP is made up five different kinds of components, and nucleic acid is the main active component among them. The remaining four structural distribution ratios are ionizable cationic lipid (about 50%), cholesterol (about 38.5%), neutral lipid (about 10%), and PEG-lipid (about 1.5%) [12]. The precise structure is displayed in Figure 2.

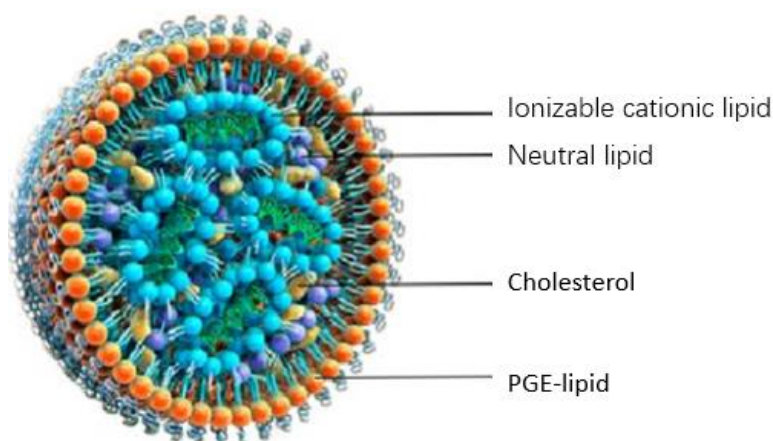


Figure 2. The structure of LNP (From Precision Nano Systems)

These four components have different effects. Neutral lipids (choose Di stearyl Phosphatidylcholine as an example) can accelerate the drug release by accelerating the structural transformation of LNP in cells, it can also stabilize the arrangement of the structure of the lipid bilayer. The cholesterol mainly stabilizes the nanoparticle structure and carries out endocytosis mediated by low-density lipoprotein (LDL) structure receptors, and promotes mRNA intracellular uptake and cytosolic entry [13]. PGE-lipid can improve the overall stability and also be used to control the particle size of nanoparticles and prolong the metabolism time of nanoparticles in blood [14]. And the primary component of LNP is the ionizable cationic lipid. The negative charge, hydrophilicity, difficulty passing through the cell membrane, and fragility of bare nucleic acids make it easier for them to be obliterated by around nuclease in the body. So we need a positively charged lipophilic carrier for delivery. Therefore, the ionizable cationic lipid can be electrostatically adsorbed with nucleic acids, and can directly affect the delivery efficiency of mRNA.

3.2. Mechanisms of LNP delivery in mRNA vaccine

Cationic lipids can take electrostatic complexation with negatively charged mRNA molecules to form complexes and improve the stability of mRNA molecules before entering the cell. After entering the body, LNP is adsorbed on the cell surface and then brings mRNA into the cells through endocytosis. After mRNA enters the cell, the anionic lipid of the cell neutralizes the charge carried by the LNP cationic lipid, so the pH decreases from an acidic environment, which makes the ionizable lipid protonated and destroys the electrostatic interaction between the nucleic acid and the lipid carrier.

Then the mRNA is released after the bilayer structure of LNP is damaged [15]. Figure 3 illustrates the mechanism of action of LNP.

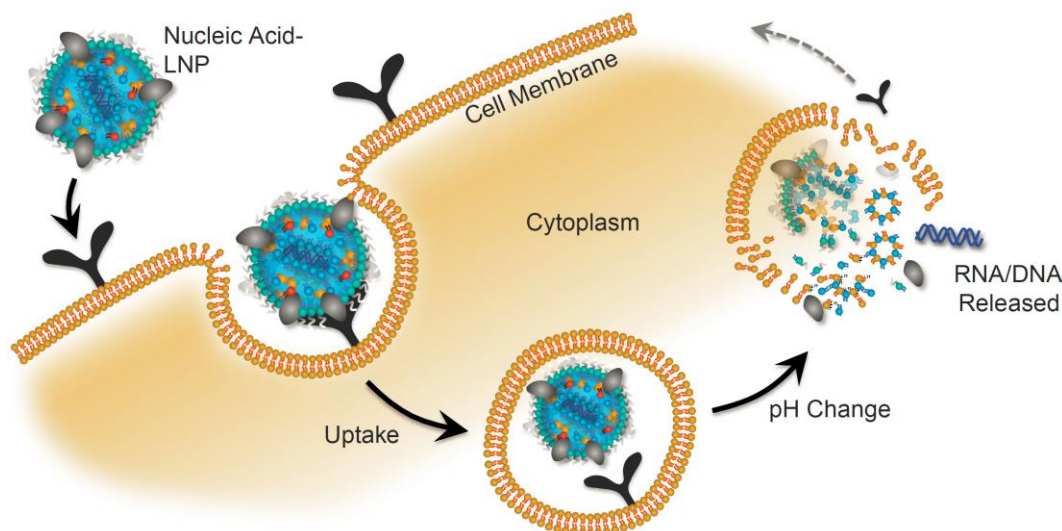


Figure 3. The mechanism of LNP action (From Precision NanoSystems)

The LNP's interaction with the biological milieu and cell membrane is caused by its surface charge. Since LNP has a negative surface charge, it will repel the negatively charged cell membrane and prevent the cell from absorbing it. On the other hand, positively charged LNP may cause cytotoxicity and directly destroy cell membranes. Ionizable lipids are essential in LNP design for the following main reason: In the beginning, LNPs containing ionizable lipids are electrically neutral and avoid any unwanted static interactions, but when the endosomal pH is acidic, they will acquire a positive charge.

Adjusting the ratio of ionized lipids to nucleic acids (changing the N/P ratio), is a common method to adjust the total surface charge of LNP. “N” is an ionizable cationic lipid (nitrogen) and “P” is a nucleotide (phosphate) [14]. At the compound of nucleic acid-lipid nanoparticles, the ratio of negative charge and the positive charge will affect the property of stability, electric potential, and so on in nanoparticles. The negative charge is typically a nucleic acid molecule with phosphate (P), and the positive charge is typically a cationic lipid (N), which can be compounded together by electrostatic adsorption. Unreasonable N/P ratios can result in abnormally large particles, unstable materials, and other flaws. Correctly calculating the positive charge to negative charge ratio (N/P ratio) is crucial. Here are some examples of N/P for different kinds of vaccines (Table 1).

Table 1. Molar N/P ratios [12]

Category	SiRNA	Prizer-BioNTech mRNA vaccine	Moderna mRNA vaccine	Curevac mRNA vaccine candidate
Molar N/P ratios	3	6	6 ^a	6 ^a

a: Estimate.

3.3. Different Types of LNP

Lipid nanoparticles can be broadly classified into five categories, depending on the method of preparation and the different physicochemical properties of their formulation. Their respective names are Liposomes, Niosome, Transfersome, Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs). The different physical and chemical properties of different components make different types of LNPs provide unique and distinct advantages for the delivery of different nanomedicine. In clinical application, different types of LNPs have targeted and wide uses to meet the drug administration needs of different types of drugs.

Liposomes are the most commonly used lipid nanoparticles, spherical in shape and typically 10-1000 nm in size, and the main body of phospholipids and cholesterol gives Liposomes good bio-

affinity and the ability to encapsulate and protect lipophilic drugs. Liposomes are the first nano-drug delivery platforms to be used in the clinic and are also highly reproducible, allowing for mass production in a simple step-by-step process. However, their high cholesterol content makes them more rigid than several other LNPs, making them less penetrating and more susceptible to cuticle restriction in skin delivery. They are also less efficient in encapsulating hydrophilic drugs, which can lead to drug leakage. Today, liposomes have been widely used as delivery vehicles for various vaccines, vaccine adjuvants, and gene editing technologies [16].

Niosomes is an alternative to Liposome. They use a non-ionic surfactant instead of the phospholipid in the original Liposome and has a particle size of 10-1000 nm. This gives them a longer shelf life and better stability. In addition, the Niosomes are electrically neutral compared to the traditional Liposome, which makes them more biocompatible. However, the neutral nature of the body also makes them less efficient for drug encapsulation and tends to lead to particle aggregation in the body. Niosomes are currently used mainly for drug delivery against the blood-brain barrier [17].

Transfersomes, also known as flexible nanoliposomes, have a spherical body but are variable in size, ranging from 30 nm to 300 nm in diameter, and are mostly composed of phospholipids and edge activators. Compared to Liposomes and Niosomes, Transfersomes are highly deformable and can easily produce deformations of 4 - 5 orders of magnitude, resulting in a wide range of targets including skin, muscle, blood and joints. However, they are more difficult to preserve than ordinary liposomes, and Transfersomes in general are more susceptible to exposition or oxidation, which makes them more difficult to purify during manufacture [18].

Both Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs) are made from natural solid lipid nanoparticles and surfactants in the form of spheres, generally with a particle size of 50-1000 nm. Both liposomes have no internal aqueous phase, instead they have a solid organic phase, which makes them very efficient for encapsulation of hydrophobic drugs, and they also perform very well in slow release of drugs. At the same time, both are more complex to prepare and both have lower drug loading capacity. SLNs and NLCs are currently used for the dermal delivery of hydrophobic drugs and large molecules such as peptides and proteins [19].

4. Factors influencing the delivery efficiency of lipid nanoparticles

Structure determines function. The different structures of nanoparticles also determine the different properties of nanoparticles during the drug delivery. These include the efficiency of drug encapsulation, cytotoxicity, half-life in the body and targeting. The timely optimization of the structure of nanoparticles for specific needs is the key to achieve their delivery function. Extensive research has shown that the particle size, surface charge, surface polyethylene glycolisation and specific targeting modifications of LNP are key factors in determining its encapsulation as well as its delivery efficiency.

4.1. Particle Size

The particle size of LNPs is undoubtedly an important parameter that affects its transfer effect. Nanoparticles in different size ranges can contribute to different biological phenomena, including their circulatory half-life in the organism, vascular penetration, propensity for retention in specific tissues, and uptake by phagocytes [15]. When administered intravenously, smaller nanoparticles (diameter < 5 nm) are quickly filtered out by the kidneys. Particles with a diameter of 20 - 150 nm are able to escape kidney filtration effectively and show initial aggregation effects in the liver and spleen. At the same time, particles within this size range can also show a relatively good tendency to aggregate in solid tumors due to the presence of EPR effect (the existence of EPR effect is still controversial [20]). As the particle size increases above 150 nm, more nanoparticles are trapped in the liver, spleen, and lung capillaries [21]. For subcutaneous injections, which are usually used for vaccinations, the smaller size of the lipid nanoparticles facilitates their uptake by the body's lymphatic vessels and capillaries. Particles of around 10 nm can effectively diffuse from the subcutaneous

injection into the capillaries, while particles of around 100 nm can diffuse by convection into the capillary lymphatics. When the particle size increases to the 100-200 nm range, the rapid diffusion of particles decreases significantly. And more specifically, it has been shown that liposomes of 40 nm have a higher lymphatic uptake rate compared to larger particles. After subcutaneous injection, approximately 76% of particles of 40 nm in size are successfully absorbed by the lymph nodes, while the remaining larger particles tend to remain at the subcutaneous injection site [22].

4.2. Particle Surface Charge

The type and amount of charge on the surface of lipid nanoparticles are also key factors in their performance. The different electrical properties affect the ability of the nanoparticles to significantly influence the circulating half-life of the lipid nanoparticles in vivo, as well as their targeting to different organs in vivo and their ability to be specifically taken up by different cells. First of all, compared with neutral liposomes, particles with positive or negative surface charge can interact more effectively with proteins in the body, thus showing better connection and interaction with specific immune cells. For example, Mai et al. found that cationic liposomes could interact with opsonin in the blood, thus being more easily recognized by immune cells. Anion particles attach to the surface of B cells in the microvascular network [23]. This may suggest that charged liposomes are more suitable for use as vaccines or vaccine adjuvants, while neutral nanoparticles could be used for the delivery of chemotherapy drugs. Furthermore, particles with a positive surface charge have stronger cell affinity and cytotoxicity. For example, it has advantages in targeting solid tumors. Studies have shown that liposomes with positive surface charge can be more effectively bound and internalized by vascular endothelial cells associated with solid tumors. And the particles can promote the release of endosomes by causing different biological effects ("proton sponge effect", for example) and prevent their delivery of drugs from being degraded by the endosomes. However, particles with a positive charge are more easily taken up by various cells in the body non-specifically. Meanwhile, neutral or negatively charged particles have been well proved to be able to effectively shield the effect of serum protein in blood vessels, thus effectively extending its circulatory half-life in the body. Compared with positively charged particles, they are less likely to be intercepted by various organs in the body, such as liver, lung and spleen. From the perspective of vaccine, negatively charged LNP can more effectively target lymph nodes [24]. Therefore, the application of ionizable lipids is very ingenious. Reasonable selection of ionizable lipids with different pKa can make the nanoparticles exhibit different electrical properties at different pH values. Therefore, lipid nanoparticles have a long cycle half-life in vivo, but also have strong immunogenicity and cell affinity. Based on this, Hirai designed LNP to produce ionizable lipids consisting of dipalmitoyl phosphatidylcholine (DPPC), cholesterol, and dioleoylglycerophosphate diethylenediamine coupling (dopo-deda). At pH < 6.0, DOP-DEDA-LNPs has positive charge. 6.0, pH < 8.0, it becomes electronegative. In circulation, the particles as a whole are neutral, which can effectively reduce their interaction with plasma proteins. However, after they are swallowed, the particles become trapped in the internal region. Due to the low pH of the environment, their surface will be positively charged, which effectively promotes the release of the contents [25].

4.3. Surface Polyethylene Glycolisation and Specific Targeting Modifications

PEG is a kind of hydrophilic, non-ionic polymer with good biological compatibility. At this stage, they are the most commonly used reagents to prevent the clearance of MPS and prolong the residence time of liposomes in the blood. The PEGylation of LNPs can effectively reduce the amount of LNP and serum proteins through the PEGylation of LNP. In addition, PEG-modified anionic liposomes can better diffuse into the lymphatic system after subcutaneous injection, reducing the retention at the injection site, and better aggregation and retention in the lymph nodes. Furthermore, shorter PEG modified chains allow for better particle retention and retention against lymph [26]. The modification of branched-chain PEG polymer and straight-chain PEG polymer can give the liposomes significantly different targeting and transfection ability [27]. However, recent studies have shown that the human

body produces corresponding antibodies against PEG, which has always been associated with reduced vaccine effectiveness and further adverse reactions. For this reason, efforts are being made to find a better, equally biocompatible polymer to replace the PEG modification. However, some studies have found that adding a specific amount of dexamethasone into liposomes can effectively inhibit the adverse reactions caused by PEG modification [28].

Due to the inherent properties of lipid nanoparticles, most LNP-based vaccines target the liver if they rely on passive targeting by continuous circulation of the particles in the body and cellular uptake. Chen and his colleagues have developed a positively charged PTX-LNP based on paclitaxel (PTX), modified with folic acid-modified bovine serum albumin (FB) to enable dual targeting of tumours [29]. Veiga and his colleagues achieved selective targeting of inflammatory leukocytes in the organism by combining anti-Ly6C antibodies with lipid nanoparticles loaded with siRNA. After inoculation solid tumours tend to show higher levels of p32 expression than non-cancerous tissues. Kuo and his colleagues modified LNP with amphiphilic solid nanoparticles (Ln5-P4-ASLNs) decorated with Ln5-P4 peptide, which can be used to co-deliver nerve growth factor (NGF) and retinoic acid (RA) [30]. Ln5-P4 can $\alpha\beta1$ integrin bind to direct the induction of differentiation of pluripotent cells into neuronal cells.

5. Conclusion

LNP is a highly personalized nucleic acid delivery vector, which shows great potential in mRNA vaccine delivery. Especially in the current international pandemic of COVID-19, the two approved COVID-19 vaccines: mRNA-1273 and BNT162b, use lipid nanoparticles to deliver antigen mRNA. Many other LNP-mRNA formulations are in clinical trials for the treatment of infectious diseases, cancer, and genetic disorders. This article provides an overview of the use of a positively charged lipophilic carrier for the delivery of nucleic acid molecules, addressing the problem that naked mRNA is susceptible to being degraded by the free nuclease in the body. The different types of lipid nanoparticles and the different factors affecting delivery are also enumerated, providing useful insights into different types of drug delivery modalities and the design of new therapeutic vaccines.

However, there are still many challenges associated with the study of LNP, such as the details of the internal structure of LNP to prevent nucleic acid degradation, and how to control the selective binding of LNPs to target cells, which will be the direction of future global health efforts. Regardless, LNP is the main technology of the mRNA vaccine, and the successful development of the mRNA-LNP vaccine marks a phased achievement of nanomedicine. In the future, we believe that LNP has potential value in more fields, such as providing treatment for a variety of diseases such as tumors, so the further development of LNP is highly necessary.

References

- [1] R. Verbeke, M.J. Hogan, K. Lore, N. Pardi, Innate immune mechanisms of mRNA vaccines, *Immunity* 55 (11) (2022) 1993 - 2005.
- [2] S. Rauch, E. Jasny, K.E. Schmidt, B. Petsch, New Vaccine Technologies to Combat Outbreak Situations, *Front Immunol* 9 (2018) 1963.
- [3] X. Hou, T. Zaks, R. Langer, Y. Dong, Lipid nanoparticles for mRNA delivery, *Nat Rev Mater* 6 (12) (2021) 1078 - 1094.
- [4] Nitika, J. Wei, A.M. Hui, The Delivery of mRNA Vaccines for Therapeutics, *Life (Basel)* 12 (8) (2022).
- [5] H. Tabara, M. Sarkissian, W. G. Kelly, J. Fleenor, A. Grishok, L. Timmons, A. Fire, C.C. Mello, The rde-1 gene, RNA interference, and transposon silencing in *C. elegans*, *Cell* 99 (2) (1999) 123 - 32.
- [6] K. Reinhard, B. Rengstl, P. Oehm, K. Michel, A. Billmeier, N. Hayduk, O. Klein, K. Kuna, Y. Ouchan, S. Woll, E. Christ, D. Weber, M. Suchan, T. Bukur, M. Birtel, V. Jahndel, K. Mroz, K. Hobohm, L. Kranz, M. Diken, K. Kuhlcke, O. Tureci, U. Sahin, An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors, *Science* 367 (6476) (2020) 446 - 453.

- [7] M. Bhattacharya, A.R. Sharma, P. Ghosh, P. Patra, B.C. Patra, S.-S. Lee, C. Chakraborty, Bioengineering of Novel Non-Replicating mRNA (NRM) and Self-Amplifying mRNA (SAM) Vaccine Candidates Against SARS-CoV-2 Using Immunoinformatic Approach, *Molecular Biotechnology* 64 (5) (2022) 510-525.
- [8] L. Miao, J. Lin, Y. Huang, L. Li, D. Delcassian, Y. Ge, Y. Shi, D.G. Anderson, Synergistic lipid compositions for albumin receptor mediated delivery of mRNA to the liver, *Nat Commun* 11 (1) (2020) 2424.
- [9] L. R. Baden, H.M. El Sahly, B. Essink, K. Kotloff, S. Frey, R. Novak, D. Diemert, S.A. Spector, N. Rouphael, C.B. Creech, J. McGettigan, S. Khetan, N. Segall, J. Solis, A. Brosz, C. Fierro, H. Schwartz, K. Neuzil, L. Corey, P. Gilbert, H. Janes, D. Follmann, M. Marovich, J. Mascola, L. Polakowski, J. Ledgerwood, B.S. Graham, H. Bennett, R. Pajon, C. Knightly, B. Leav, W. Deng, H. Zhou, S. Han, M. Ivarsson, J. Miller, T. Zaks, C.S. Group, Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine, *N Engl J Med* 384 (5) (2021) 403 - 416.
- [10] E. J. Anderson, N.G. Rouphael, A.T. Widge, L.A. Jackson, P.C. Roberts, M. Makhene, J.D. Chappell, M.R. Denison, L.J. Stevens, A.J. Pruijssers, A.B. McDermott, B. Flach, B.C. Lin, N.A. Doria-Rose, S. O'Dell, S.D. Schmidt, K.S. Corbett, P.A. Swanson, 2nd, M. Padilla, K.M. Neuzil, H. Bennett, B. Leav, M. Makowski, J. Albert, K. Cross, V.V. Edara, K. Floyd, M.S. Suthar, D.R. Martinez, R. Baric, W. Buchanan, C.J. Luke, V.K. Phadke, C.A. Rostad, J.E. Ledgerwood, B.S. Graham, J. H. Beigel, R.N.A.S.G. m, Safety and Immunogenicity of SARS-CoV-2 mRNA-1273 Vaccine in Older Adults, *N Engl J Med* 383 (25) (2020) 2427 - 2438.
- [11] F. P. Polack, S.J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, J.L. Perez, G. Perez Marc, E. D. Moreira, C. Zerbini, R. Bailey, K.A. Swanson, S. Roychoudhury, K. Koury, P. Li, W.V. Kalina, D. Cooper, R.W. Frenck, Jr., L.L. Hammitt, O. Tureci, H. Nell, A. Schaefer, S. Unal, D. B. Tresnan, S. Mather, P. R. Dormitzer, U. Sahin, K. U. Jansen, W.C. Gruber, C.C.T. Group, Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine, *N Engl J Med* 383 (27) (2020) 2603 - 2615.
- [12] L. Schoenmaker, D. Witzigmann, J. A. Kulkarni, R. Verbeke, G. Kersten, W. Jiskoot, D.J. Crommelin, mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability, *International Journal of Pharmaceutics* 601 (2021) 120586.
- [13] J. J. Lu, R. Langer, J. Chen, A novel mechanism is involved in cationic lipid-mediated functional siRNA delivery, *Molecular pharmaceutics* 6 (3) (2009) 763 - 771.
- [14] M. J. Evers, J. A. Kulkarni, R. van der Meel, P. R. Cullis, P. Vader, R.M. Schiffelers, State-of-the-art design and rapid-mixing production techniques of lipid nanoparticles for nucleic acid delivery, *Small Methods* 2 (9) (2018) 1700375.
- [15] C. Chan, S. Du, Y. Dong, X. Cheng, Computational and experimental approaches to investigate lipid nanoparticles as drug and gene delivery systems, *Current topics in medicinal chemistry* 21 (2) (2021) 92 - 114.
- [16] P. Nakhaei, R. Margiana, D.O. Bokov, W.K. Abdelbasset, M.A. Jadidi Kouhbanani, R.S. Varma, F. Marofi, M. Jarahian, N. Beheshtkhoo, Liposomes: Structure, Biomedical Applications, and Stability Parameters with Emphasis on Cholesterol, *Frontiers in Bioengineering and Biotechnology* 9 (2021).
- [17] M. Gharbavi, J. Amani, H. Kheiri-Manjili, H. Danafar, A. Sharafi, Niosome: A Promising Nanocarrier for Natural Drug Delivery through Blood-Brain Barrier, *Advances in Pharmacological Sciences* 2018 (2018) 6847971.
- [18] S. A. T. Opatha, V. Titapiwatanakun, R. Chutoprapat, Transfersomes: A Promising Nanoencapsulation Technique for Transdermal Drug Delivery, *Pharmaceutics* 12 (9) (2020) 855.
- [19] E. B. Souto, I. Baldim, W.P. Oliveira, R. Rao, N. Yadav, F.M. Gama, S. Mahant, SLN and NLC for topical, dermal, and transdermal drug delivery, *Expert Opinion on Drug Delivery* 17 (3) (2020) 357 - 377.
- [20] Challenging paradigms in tumour drug delivery, *Nature Materials* 19 (5) (2020) 477 - 477.
- [21] E. Blanco, H. Shen, M. Ferrari, Principles of nanoparticle design for overcoming biological barriers to drug delivery, *Nature biotechnology* 33 (9) (2015) 941 - 951.
- [22] J. Widmer, C. Thauvin, I. Mottas, F. Delie, E. Allémann, C. Bourquin, Polymer-based nanoparticles loaded with a TLR7 ligand to target the lymph node for immunostimulation, *international journal of pharmaceutics* 535 (1-2) (2018) 444 - 451.

- [23] M.N. Vu, H. G. Kelly, A.K. Wheatley, S. Peng, E.H. Pilkington, N.A. Veldhuis, T.P. Davis, S. J. Kent, N.P. Truong, Cellular interactions of liposomes and PISA nanoparticles during human blood flow in a microvascular network, *Small* 16 (33) (2020) 2002861.
- [24] Y. Sato, H. Hatakeyama, Y. Sakurai, M. Hyodo, H. Akita, H. Harashima, A pH-sensitive cationic lipid facilitates the delivery of liposomal siRNA and gene silencing activity in vitro and in vivo, *Journal of Controlled Release* 163 (3) (2012) 267 - 276.
- [25] Y. Hirai, R. Saeki, F. Song, H. Koide, N. Fukata, K. Tomita, N. Maeda, N. Oku, T. Asai, Charge-reversible lipid derivative: a novel type of pH-responsive lipid for nanoparticle-mediated siRNA delivery, *International Journal of Pharmaceutics* 585 (2020) 119479.
- [26] S. M. Moghimi, the effect of methoxy-PEG chain length and molecular architecture on lymph node targeting of immuno-PEG liposomes, *Biomaterials* 27 (1) (2006) 136 - 144.
- [27] D. Zukancic, E.J. Suys, E.H. Pilkington, A. Algarni, H. Al-Wassiti, N.P. Truong, The importance of poly (Ethylene glycol) and lipid structure in targeted gene delivery to lymph nodes by lipid nanoparticles, *Pharmaceutics* 12 (11) (2020) 1068.
- [28] S. Yonezawa, H. Koide, T. Asai, Recent advances in siRNA delivery mediated by lipid-based nanoparticles, *Advanced drug delivery reviews* 154 (2020) 64 - 78.
- [29] C. Chen, H. Hu, M. Qiao, X. Zhao, Y. Wang, K. Chen, D. Chen, Anti-tumor activity of paclitaxel through dual-targeting lipoprotein-mimicking nanocarrier, *Journal of Drug Targeting* 23 (4) (2015) 311 - 322.
- [30] Y.-C. Kuo, C.-Y. Shih-Huang, R. Rajesh, Enhanced integrin affinity and neural differentiation of induced pluripotent stem cells using Ln5-P4-grafted amphiphilic solid lipid nanoparticles, *Materials Science and Engineering: C* 118 (2021) 111339.