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REVIEW

Lipid carriers for mRNA delivery

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Abstract Messenger RNA (mRNA) is the template for protein biosynthesis and is emerging as an essential active molecule to combat various diseases, including viral infection and cancer. Especially, mRNA-based vaccines, as a new type of vaccine, have played a leading role in fighting against the current global pandemic of COVID-19. However, the inherent drawbacks, including large size, negative charge, and instability, hinder its use as a therapeutic agent. Lipid carriers are distinguishable and promising vehicles for mRNA delivery, owning the capacity to encapsulate and deliver negatively charged drugs to the targeted tissues and release cargoes at the desired time. Here, we first summarized the structure and properties of different lipid carriers, such as liposomes, liposome-like nanoparticles, solid lipid nanoparticles, lipid-polymer hybrid nanoparticles, nanoemulsions, exosomes and lipoprotein particles, and their applications in delivering mRNA. Then, the development of lipid-based formulations as vaccine delivery systems was discussed and highlighted. Recent advancements in the mRNA vaccine of COVID-19 were emphasized. Finally, we described our future vision and perspectives in this field.

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1. Introduction

Messenger RNA (mRNA) is the template for protein biosynthesis. Natural mRNA is composed of a 5'untranslated region (5' UTR), open reading frame (ORF) and 3'untranslated region (3'UTR) (as shown in Fig. 1)[1]. mRNA guides protein biosynthesis on ribosomes through the codon in the ORF. *In vitro* transcribed mRNA (IVT mRNA) shares a similar structure[2]. Delivering exogenous mRNA into cells can generate proteins instantaneously and accurately, offering an alternative to gene therapy[3]. Compared with other nucleic acid-based therapies, mRNA-based therapies do not need to enter the nucleus to function[4]. Once the mRNA reaches the cytoplasm, it translates immediately[5]. Furthermore, insertional mutagenesis is not risky because mRNA does not integrate into the genome[6]. IVT mRNA has become a promising candidate therapy for tumors, infectious diseases and protein replacement due to its safe and effective protein expression profile[7]. Regarding tumors and related infectious diseases, IVT mRNA can translate the corresponding antigens and activate or strengthen specific immunity, preventing infectious diseases[8] or strengthening tumor immunity[9]. Current mRNA-based protein replacement therapies have focused on inherited metabolic diseases. Protein levels in patients can be restored using mRNA to express proteins lost due to genetic mutations[10,11]. Especially, rare genetic diseases, referring to a group of diseases caused by specific gene mutations, are one of the problems that modern medicine urgently needs to overcome[12]. Targeted delivery of mRNA has shown great potential in this field, including methylmalonic academia, ornithine transcarboxylasess deficiency and Fabry disease[13,14]. Now, most mRNA-based protein therapies are in preclinical studies. AZD8601, launched by AstraZeneca and Moderna, is the fastest trial and has completed patient enrollment in phase II, a low-dose cohort[15,16]. The therapeutic strategy of AZD8601 is to improve cardiac function and reduce heart damage by locally inducing VEGF-A protein expression to promote new angiogenesis in areas of the myocardia that undersupply blood[10]. In phase I clinical trials, AZD8601 showed good safety, and its intradermal injection temporarily increased skin blood flow while increasing local VEGF-A protein expression level, indicating the potential of promoting angiogenesis[10].

However, mRNA has inherent drawbacks, such as large size, negative charge, instability, hydrophilicity, etc[17]. The mRNA stability could be improved by structural modification[18]. Nevertheless, the membrane diffusion of mRNA is extremely poor due to its negative charges, high molecular weight and hydrophilicity. Consequently, intracellular delivery of mRNA is the critical factor limiting the application of mRNA-based therapies. Three challenges involve intracellular mRNA delivery: extracellular barrier, endosome escape and intracellular immunity[19]. In detail, mRNA is easily degraded by ribonuclease (RNase) in the body, so it is necessary to use an appropriate carrier to wrap mRNA to avoid degradation when delivering mRNA. Furthermore, the mononuclear phagocytic system (MPS) can systematically identify and eliminate foreign nanoparticles (NPs), resulting in NPs accumulating mainly in the liver and spleen and poor delivery to other tissues[20]. Then, when reaching the targeted cells, most mRNA carriers enter the cells by endocytosis[21,22]. Endocytic uptake localizes the cargo in early endosomes, which mature *via* late endosomes into endolysosomes[23]. Both cargo and carrier are degraded due to the acidic pH and lysosomal hydrolases in endolysosomes[24]. Therefore, mRNA requires release from endosomal vesicles before binding to ribosomes[6]. Finally, when foreign mRNA reaches the cytoplasm, it is recognized by Toll-like receptors 3 (TLR3) and TLR7/8 or other receptors to activate the natural immune system[25]. Although foreign mRNA can promote cellular or humoral immunity after mRNA immunization, this immunoreaction also compromises the effect of mRNA. For example, pattern recognition receptors (PRRs) in the TLR family can specifically recognize double-stranded or single-stranded mRNA and degrade it before it functions[19]. As a result, the efficient delivery of mRNA is fundamental to disease treatment.

In general, mRNA can be delivered using three main strategies: physical methods (disrupting the membrane barrier temporarily), viral vectors (utilizing biological modes of uptake) and non-viral vectors (reasonable design of nanocarriers)[3,18,26]. Physical methods are effective to some extent, especially electroporation[27]. Nevertheless, the physical methods are usually harmful to cells, therefore, unsuitable for use *in vivo*. Viral vectors are considered effective carriers to deliver mRNA; however, they are not extensively used due to limitations, such as small packing size, immunogenicity, cytotoxicity, carcinogenesis and production difficulties[28–31]. Compared with viral vectors, non-viral vectors have lower immunogenicity and higher payload-capability and are easier to synthesize, making them the preferred vectors for mRNA delivery[32–34]. The most common non-viral carriers include lipids, polymers, and inorganic nanoparticles[35–37]. Here, we mainly discuss the lipid carrier-based mRNA delivery.

Lipid carriers can protect the mRNA from enzymatic degradation with a high encapsulation efficiency. Incredibly, some lipids are positively charged in the physiological environment and can be electrostatically coated with negatively charged mRNA, allowing the delivery system to associate well with the targeted cell membrane[19]. Furthermore, the NPs assembled from lipids can

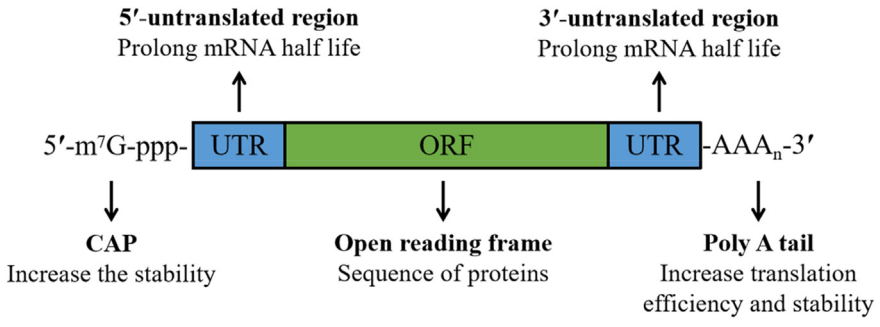

Figure 1 Schematic illustration of mRNA.

fuse with the endosomal membrane through a phospholipid bilayer, facilitating endosomal escape[38]. Here, this review summarizes the structure and properties of different lipid carriers and the mechanisms of mRNA delivery. At the same time, the status and clinical research of mRNA-based vaccines were introduced first, and then the development of mRNA vaccine in COVID-19 was emphasized. Eventually, we afford perspectives for lipid carrier-based mRNA delivery.

2. Lipid carriers for mRNA delivery

Lipid carriers possess a development history of more than 60 years[39], aiming to improve drug delivery and reduce side effects[40]. Lipid carriers for mRNA delivery can be divided into several types, including liposomes (LPs), liposome-like nanoparticles (LLPs), solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), lipid-polymer hybrid nanoparticles (LPNs), nanoemulsions, exosomes and lipoprotein particles (LPTs)[41]. The characteristics of lipid carriers and their use for mRNA delivery are summarized in this section and Table 3 [42,43]. Meanwhile, the structures of different lipid carriers are shown in Fig. 2.

2.1. LPs

LPs, a closed phospholipid bilayer spherical vesicle system composed of amphiphilic molecules, are able to carry hydrophobic/hydrophilic molecules[41,44,45]. Since this closed bilayer phospholipid structure was first observed in 1961[46], LPs, as an attractive lipid carrier, are widely used for drug delivery to combat various diseases, such as tumor, fungal and bacterial infection[47]. So far, over 20 liposome products have been approved for the clinic, for example, Doxil®, Vyxeos®, Lipusu®, Ambisome®, Arikayce®, DepoDur™, etc[48]. Doxil®, doxorubicin liposomes, is the first U.S. Food and Drug Administration (FDA)-approved nanomedicine to treat the recurrent ovarian tumor and the human immunodeficiency virus (HIV) related Kaposi's Sarcoma[49–51]. The liposomes can prolong blood circulation time, effectively load doxorubicin and passively target tumors. Accurately, modern mRNA delivery systems derive from these traditional liposomal formulations for small-molecule therapeutics. Now LPs are a effective delivery vector for mRNA due to their low toxicity and high biocompatibility advantage, as LPs are analogs of biological membranes, which can be composed of natural and synthetic phospholipid preparation[52]. In addition, mRNA encapsulated in LPs is not easily degraded by RNase. Commonly used lipid materials for mRNA delivery are shown in Supporting Information Table S1 [3,53,54]. Martinon et al.[55] encapsulated the mRNA encoding the influenza virus nucleoprotein into negative LPs comprised of cholesterol (chol), dipalmitoylphosphatidylcholine (DPPC), and phosphatidylserine (PS). Despite the low encapsulation efficiency, the LPs induced the proliferation of anti-influenza cytotoxic T lymphocytes *in vivo*.

In order to improve the interaction with LPs, mRNA can be formulated with cationic LPs to form lipoplexes (LPPs). The complexation of negatively charged mRNA with positively charged cationic LPs increases the encapsulation efficiency of mRNA. Moreover, cationic LPs could bind to the cell membrane, assisting mRNA entering the cell[56]. Cationic lipids, the main components of cationic LPs, share a similar structure with natural lipids, except for the presence of a cationic head group[57]. Historically, *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA) was the first synthetic cationic lipid used to deliver mRNA[58]. DOTMA combined with 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) was commercialized as Lipofectin[59]. 1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP), as a DOTMA derivative, is a cationic lipid-containing monounsaturated fatty acid with low preparation cost and high mRNA delivery efficiency[60]. Therefore, it has become a widely studied cationic lipid material. DOTMA and DOTAP have been used for mRNA delivery alone or in combination with other materials. For example, spleen-targeted DOTMA-mRNA LPPs has been designed as mRNA vaccine to treat autoimmune encephalomyelitis[61]. This vaccine induced the proliferation of antigen-specific $CD4^+$ T cells. The results indicated that immunosuppression was enhanced, and mice's pathological symptoms were relieved. This method provided ideas for the clinical translation of antigen-based therapy for autoimmune diseases. For another, most researchers modify LPs to improve the delivery capacity of the carrier. In order to increase the carrier concentration on the cell surface and thus enhance the uptake and transfection potency of mRNA, Zohra et al.[62] used carbonate apatite, an inorganic crystal with pH sensitivity and strong affinity for mRNA, to form a complex between the LPs formed by DOTAP. The results showed that the mRNA-DOTAP-carbonate apatite complex could significantly elevate mRNA expression in mitotic cells compared with the control group. Wherein carbonate apatite could reinforce the transport of cationic LPs and mRNA due to a simple physical phenomenon: the mRNA-DOTAP-carbonate apatite complex precipitate on the cell surface by gravity. Zhang et al.[63] prepared DOTAP LPs decorated with chol-modified cationic hydrophilic peptide DP7-C, aiming to increase the delivery efficiency of mRNA encoding individualized neoantigens to dendritic cells (DCs) and enhance the activation of DCs. The ternary complex consisting of cationic LPs-cationic peptide-mRNA was called lipopolyplexes (LPR)[1]. DP7-C in the complex has low cytotoxicity and potential immunomodulatory effects and could effectively load antigen into cells through the caveolin and clathrin-dependent pathway. In addition, DP7-C can stimulate DCs maturation using the TLR2-MyD88-IKK-IκB−NF−κB signaling pathway and boost the immune response to the neoantigen-loaded DCs vaccine[64]. The results displayed that the LPR promoted DC maturation and cytokine secretion, enhancing antigen-specific lymphocyte response and anti-tumor effect. The complex had dual carrier and immune adjuvant functions, potentially improving the intracellular mRNA delivery and immune response. Protamine is a basic protein in the nucleus of mature sperm of fish, which can condense mRNA into nano-complex to raise mRNA's stability and encapsulation efficiency. Zhang et al.[65] used protamine-DOTAP cationic LPs to deliver mRNA encoding the Survivin-T34A gene, demonstrating a significant therapeutic effect on various C26 colon cancer cell models *in vitro* and *vivo*. Protamine in the complex was employed to concentrate the mRNA into nanosized particle and protect it from nuclease degradation. Whereas cationic lipids have significant toxicity, affecting clinical application[66,67]. New materials are urgently needed to solve this issue.

2.2. LLPs

LLPs, always termed lipid nanoparticles, are sphere-shaped and nanosized vesicles commonly composed of ionizable lipids, phospholipids, chol and polyethylene glycol (PEG)−lipid conjugate[68]. In 2018, the first small interfering RNA (siRNA) drug, patisiran (trade name: Onpattro®), encapsulated by LLPs, was approved by the FDA to treat hereditary transthyretin-mediated

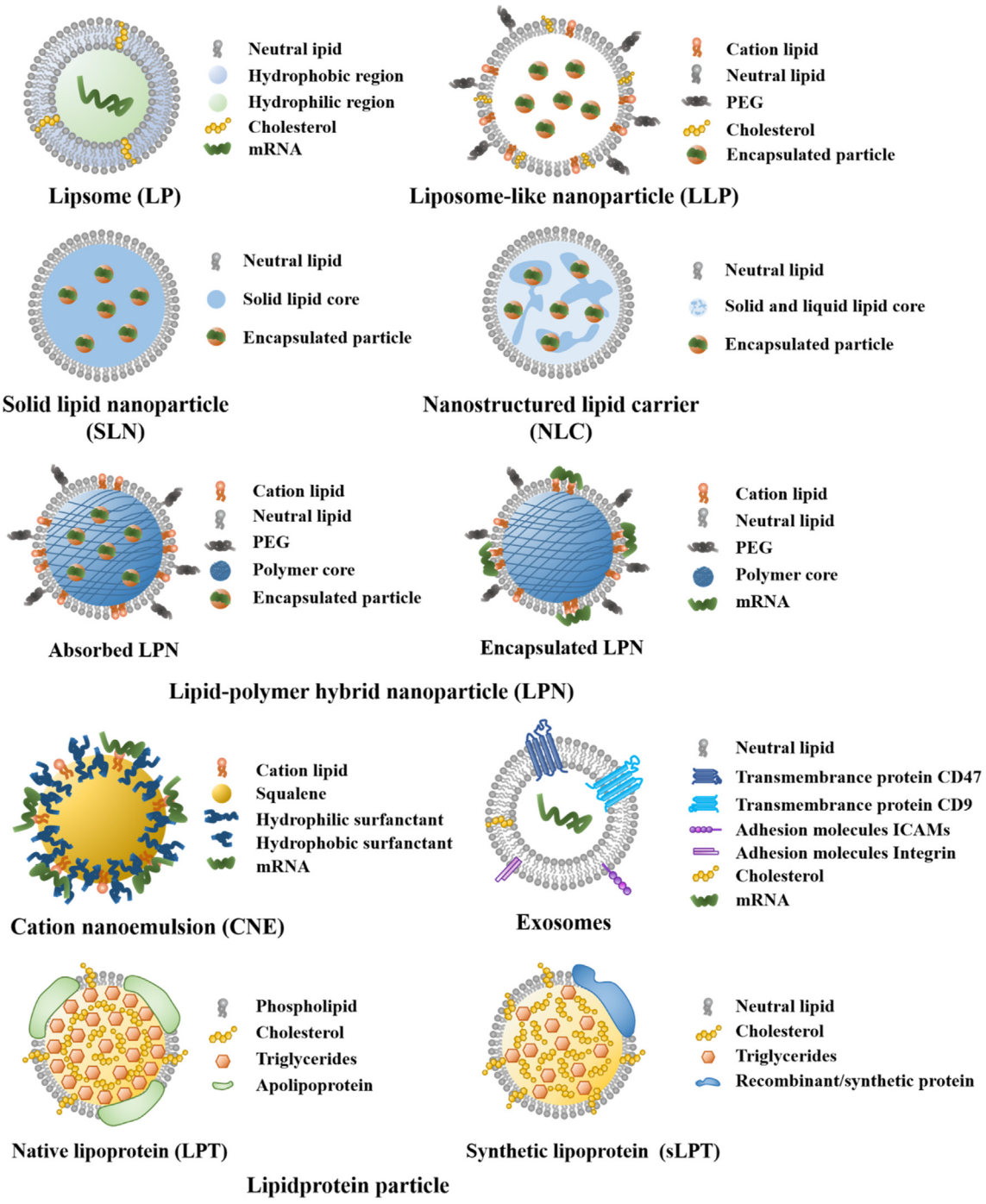

Figure 2 Schematic illustration of different lipid carriers.

amyloidosis[2,69]. It is considered a milestone for the LLPs delivery system. After that, LLPs have been increasingly applied for mRNA delivery.

Generally, each lipid in LLPs performs a unique function. Lipids containing tertiary amine groups, such as Dlin-MC3-DMA, C12-200, etc., are ionizable lipids that favor mRNA encapsulation and endosomal escape[57,70]. The early-use cationic lipids are positively charged under physiological conditions, readily interacting with other negatively-charged molecules and easily captured by immune cells in the physiological environment[71]. By contrast, ionizable lipids with pH sensitivity are less toxic than nonionizable cationic lipids because as the pH changes *in vivo*, these lipids are only positively charged in the cell and not charged in the bloodstream[72]. Meanwhile, when the pH value is lower than the apparent p*K*a value of the ionizable lipids, ionizable lipids become positively charged and enable efficient encapsulation of mRNA in LLPs through electrostatic interactions with the negatively charged mRNA. During the endosomal escape, as endosomes mature, pH drops below the apparent p*K*a of ionizable lipids; consequently, most ionizable lipids are protonated[73]. Ionizable lipids interact with anionic lipids on the endosomal membrane to form destructive non-bilayer structures that

eventually release the encapsulated mRNA into the cytoplasm[19,74]. In mRNA vaccines, ionizable lipids may act as adjuvant agents. Recently, a study investigated the adjuvant effect of ionizable lipids by administering mRNA-LLPs to mice: the ionizable lipids produced the auxiliary effect by activating interleukin-6[75]. Another study screened 30 LLPs that differed only in ionizable lipids structure to encapsulate mRNA encoding H10N8 influenza hemagglutinin antigen. The immunogenicity produced in mice varies, indicating that mRNA-LLPs' immunogenicity depends highly on ionizable lipid structure[76]. Interestingly, an optimal lipid p*K*a for intramuscular injection (im) immunogenicity is between 6.6 and 6.9[77]. This differs from the optimal lipid p*K*a range (6.2−6.6) for intravenous injection (iv) immunogenicity[77]. Indeed, the relationship between ionizable lipids and LLPs-induced immunogenicity is still ambiguous.

Over the past few decades, the design of ionizable lipids has undergone significant improvements. When the first ionizable lipid, 1,2-dioleoyloxy-3-(dimethylamino)propane (DODAP), was applied for nucleic acid delivery, the transfection efficacy was modest[78]. Subsequently, new ionizable lipids were developed, including DLinDMA, DLin-KC2-DMA, DLin-MC3-DMA (used in Onpattro®), SM102, and ALC-0315[79,80]. Two ionizable lipids, SM-102 and ALC-0315, have been used in two COVID-19 vaccines: mRNA-1273 and BNT162b2. Traditional PEGylated lipids, the lipid component with the smallest mole percentage in LLPs, affect the size and dispersion of LLPs and improve the bilayer stability and nonspecific cellular uptake[60,71,81]. Traditionally, ionizable lipids are mainly screened by synthesizing numerous lipids and testing their effectiveness *in vivo*, which requires high cost, time and materials[82]. Wang et al.[83] attempted to apply computational methods to accelerate the development of ionizable lipids and then developed the first machine learning prediction model for LLP-based mRNA vaccines. This model can be applied for virtual screening of LLP preparations in the future. PEG chains form a hydrophilic steric barrier on the surface of LLPs, promoting LLPs' self-assembly and preventing aggregation to ultimately improve stability[84,85]. Lokugamage et al.[68] designed LLPs for lung delivery of mRNA *via* nebulization. The results demonstrated that lacking PEG-lipids produced unstable LLPs with elevated polydisperse index. Whereas LLPs containing PEG-lipid improved mRNA delivery to the lung. Furthermore, PEG-lipids affect the mRNA encapsulation efficiency, circulation time and immune response[86,87]. PEGylation is a widely used method to prevent rapid clearance and increase the circulation time of NPs[86]. For instance, within the anticancer drug Doxil®, distearoyl phosphoethanolamine-PEG (DSPE-PEG) is included to limit protein binding and clearance by the MPS, thus prolonging circulation time, thereby increasing tumor accumulation of doxorubicin and reducing cardiotoxicity[88]. However, an accelerated blood clearance (ABC) phenomenon has been observed in PEGylated NPs, demonstrating rapid clearance *in vivo* after repeated injections[41,89,90]. Besin et al.[91] found that LLPs also had the ABC phenomenon by producing anti-phosphatidylcholine and anti-PEG antibodies. Fortunately, the ABC phenomenon has not seriously disturbed the clinical use of PEG anticancer drugs[92]. In addition to DSPE-PEG, dimyristoyl phosphatidylethanolamine-PEG (DMPE-PEG) and 1,2-dimyristoyl-*rac*-glycero-3-methoxy polyethylene glycol (DMG-PEG) are also commonly utilized. As the skeleton of LLPs, phospholipids [distearoyl phosphatidylcholine (DSPC) and DPPC] contribute to the formation of the lipid bilayer and the transition from lamellar to hexagonal phase during the endosome, promoting the cytosol release of mRNA[60]. However, due to its well-known stability, only DSPC is included in the commercial LLPs. Chol plays a role in stabilizing the lipid bilayer and reducing the phase transition temperature, facilitating the stratified transition to hexagonal phase[60]. In LLPs formulations, chol can significantly decrease the amount of surface binding proteins, prolonging the circulation half-life[93]. In addition, chol facilitates mRNA encapsulation. Increasing chol leads to increased membrane rigidity, reducing drug leakage from the liposomal core.

The structure of LLPs-mRNA is unlike that of LPs-mRNA. For LLPs-mRNA, mRNA is complexed with ionizable cationic lipids in acidic circumstances and loaded in the aqueous cores of LLPs, while mRNA is encapsulated directly in the hydrophilic core of LPs[94]. Small-angle scattering determination demonstrated that LLPs have a disordered inverse hexagonal internal structure in the presence of mRNA[70]. Furthermore, test by contrast variation small-angle neutron scattering indicated that DSPC, PEG-lipids, as well as part of ionizable lipids and chol, are localized mainly on the surface of LLPs. Major parts of ionizable lipids, chol, water and mRNA reside inside the core. The results implied that the mRNA is partially exposed to the water inside the LLPs, causing instability under non-freezing conditions[70]. Interestingly, Sebastiani et al.[95] found that the outer shell of mRNA-LLPs is a monolayer, while other researchers proposed that the outer shell consists of one or multiple bilayers[96]. These findings suggest that mRNA-LLPs possess several structures, likely depending on lipid properties and the preparation procedure.

For the intracellular delivery, the entrapment of acidic endosomes allows LLPs to have a positive charge, facilitates the association with the endosome membrane and disrupts the bilayer structure, enabling an increased release of mRNA into the cytoplasm compared to LPs[97]. Meanwhile, during the endosomal escape, the ionizable lipids are prone to form an inverted hexagonal phase structure and destroy the endosomal membrane[53]. Therefore, LLPs are more effective for intracellular delivery than LPs. Overall, LLPs have become a relatively mature mRNA carrier because of the strengths of high encapsulation rate, low toxicity, enhanced cell uptake, high stability and prolonged circulation time. Even so, LLPs' stability and storage conditions are still challenging.

2.3. *SLNs and NLCs*

Unlike LPs, SLNs and NLCs have been developed with more complex internal architectures and higher physical stability[57,98]. SLNs are tiny spherical vesicles with a solid lipid core surrounded by a layer of surfactants as stabilizers[42]. Whereas NLCs have a solid and liquid lipid core[42]. The SLN core is coated by triglycerides, steroids and fatty acids, remaining solid at room temperature[99,100]. SLNs have more robust mRNA protection and better ability to prevent leakage because their solid core allows only a little water to be loaded[101]. Meanwhile, SLNs prepared with several techniques (*i.e.*, high-pressure homogenization) implemented in the pharmaceutical industry show long-term stability and the possibility of being subjected to commercial sterilization and lyophilization procedure[102]. Gomez-Aguado et al.[103] employed a combination of cationic and ionizable lipids to construct SLNs by the solvent emulsification-evaporation for delivering mRNA, aiming to evaluate the influence of formulation on the mRNA delivery efficiency by studying the transfection efficiency, protein expression and intracellular disposition of mRNA in human retinal pigment epithelial cells (ARPE-19) and human embryonic kidney cells (HEK-293). The formulation that involved SLNs, mRNA, the cationic peptide protamine and a

polysaccharide showed the highest delivery efficacy. And SLNs with DOTAP exhibited good long-term stability.

Over time, the maturation of the crystalline structure of SLNs gives rise to the expulsion of the incorporated mRNA[104,105]. Moreover, many studies have shown that numerous drugs may attach or strand on the outside layer surface rather than the solid core, raising questions about the drug delivery efficacy and stability of SLNs[43,106]. NLCs are considered an advanced version of SLNs[107]. The addition of liquid lipids to NLCs tolerates structural rearrangement of the matrix[108]. Liquid lipids reduce the crystalline degree of lipid core, thereby avoiding drug leakage and increasing drug loading and physicochemical stability[109]. Due to the presence of solid and liquid lipids, NLCs can encapsulate components in both solid and liquid phases and control drug release rates[110]. Gerhardt et al.[111] demonstrated the thermal stability and adaptability of the NLCs-mRNA delivery system. They found only liquid NLCs were stable at refrigerated temperature for more than 1 year. NLCs complexed with mRNA could be lyophilized and stored at room temperature for more than 8 months. This finding potentially addresses the restriction that LLPs need to be stored at −20 °C or −70 °C, improving formulations' transport and storage capacity.

2.4. *LPNs*

Polymers (polymer NPs, micelles and dendrimers) and lipid carriers (LPs, LLPs, etc.) can effectively deliver drugs. Polymer NPs are prepared from natural polymers such as chitosan or synthetic biodegradable and biocompatible polymers such as polylactic-glycolic acid (PLGA)[112,113]. The core−shell structure of polymeric NPs enables them to encapsulate water-insoluble drugs, reducing the biofouling of NP, extending the circulating half-life and controlling the release of the cargo. However, polymer NPs still have some disadvantages, including the risk of particle aggregation and toxicity[114,115]. Compared with polymer NPs, LPs have been considered more promising drug delivery vehicles because of their excellent biocompatibility[52]. However, the conventional LPs are quickly cleared by the reticuloendothelial system (RES), resulting in their poor bioavailability; and the LPs are prone to leakage of their contents during drug delivery[116]. In order to solve the limitations of polymer NPs and LPs, a new generation of therapeutic agent delivery carriers called LPNs has been developed. LPNs are a core−shell nanoparticle structure composed of polymer core and lipid shell and integrate the physicochemical properties and biocompatibility of lipids and polymers[117]. The polymer core can effectively load the therapeutic drug; the lipid layer wraps the polymer core, confers biocompatibility, and act as a molecular fence to minimize leakage of the encapsulated contents during LPNs preparation[118]. In addition, the lipid layer slows the polymer degradation rate of the LPNs by limiting inward water diffusion, enabling sustained drug release kinetics. The outer layer lipids can be coupled with PEG to extend the blood circulation time of LPNs[117].

The research of LPNs mainly focused on polymer designing to enhance RNA delivery. Zhao et al.[119] synthesized anN^1,N^3,N^5-tris (2-aminoethyl) benzo-1,3,5-tricarboxamide (TT)-derived lipid-like nanomaterial (TT3-LLNs) for delivering multiple types of mRNA. The LPNs obtained by co-preparation of PLGA4 (MW 24,000−38,000 g/mol) with TT3-LLNs significantly increased the mRNA delivery efficiency, approximately 2-fold over that of using TT3-LLNs alone.

Compared with PLGA polymers, incorporating cationic or ionizable lipids can significantly enhance the loading efficiency of mRNA. Meyer et al.[120] prepared ionizable LPNs using PLGA, cationic lipid DOTAP and ionizable lipids D-Lin-MC3-DMA through a microfluidic mixing strategy. These LPNs could bind mRNA efficiently. The positive surface charge of LPNs increased as the percentage of cationic lipids increased, while the charge decreased with increasing mRNA dose. The loading efficiency of mRNA in LPNs prepared with DOTAP is high, ranging from 80% to 100%[120]. LPNs prepared with DOTAP/MC3 mixture showed 80%−100% mRNA loading efficiency at high DOTAP concentrations. In contrast, at high MC3 concentrations, the loading efficiency decreased to 30%−50%, likely owing to the transition of MC3 from positively charged to neutral after increasing pH, reducing the ability to bind mRNA. Yasar et al.[121] prepared LPNs coated with cationic lipid DOTMA by optimizing NPs composed of PLGA. The LPNs significantly improved transfection compared to polymer/mRNA particles, an elevation that approached 80%, while the transfection effectiveness of chitosan-PLGA NPs was only 5%[121]. LPNs can not only encapsulate mRNA in the polymer core but also are able to load mRNA on the surface of LPNs through an adsorption strategy. For instance, Su et al.[122] prepared LPNs with a pH-sensitive poly (β-amino ester) (PBAE) core coated by the phospholipids of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), DOTAP and DSPE-PEG and then loaded mRNA onto the surface of the LPNs by electrostatic adsorption. *In vitro* experiments demonstrated that the LPNs/mRNA complex was readily taken up by DCs and could deliver mRNA into the cytoplasm, with 30% translation efficacy. The two approaches to load mRNA in LPNs have their advantages. The adsorption strategy showed higher mRNA expression levels in tissues *in vivo*, whereas the encapsulation approach could protect the mRNA and have a controlled release effect[120]. In practical applications, the strategy of mRNA loading in LPNs should be amended according to therapeutic purposes.

Various combinations of lipids and polymers have been extensively studied to construct LPNs for siRNA delivery, but little is known about the effect of different polymers on lipid-mediated mRNA delivery. Whether incorporating polymeric materials as cores brings unwanted side effects compared to pure lipid materials has not been reported in detail. Therefore, although LPNs have shown the potential to deliver mRNA, further research is urgently required.

2.5. *Nanoemulsions*

Nanoemulsions comprise a heterogeneous colloidal dispersion of nanodroplets dispersed in another liquid phase[123,124]. Nanoemulsions are mainly applied to load active molecules in the lipid core, protecting drugs from degradation and prolonging the drug's half-life in plasma[125,126]. An emulsifier is added to the formulation for the droplet dispersion in the aqueous phase. Emulsifiers are compounds with an amphiphilic character being able to reduce the interfacial tension between two immiscible phases[125]. Lipid nanoemulsions are distinguished from traditional lipid particles. Nanoemulsions are divided into oil-in-water (O/W) nanoemulsion and water-in-oil (W/O) nanoemulsion[127]. O/W lipid nanoemulsions consist of an inner oil core and a monolayer of phospholipids and emulsifiers[128]. Insoluble drugs are dissolved in the inner liquid oil phase, and phospholipids wrap the outer layer; therefore, the nanoemulsion can encapsulate lipophilic drugs in an aqueous solution and improve the drug stability[129]. W/O nanoemulsion consists of an internal phase of water droplets and an external oil phase[130]. Similar to LPs, the components in

nanoemulsions are highly biocompatible and induce less toxicity or irritation *in vivo*[42]. Therefore, nanoemulsions are effective drug delivery vehicles.

The most utilized nanoemulsion for gene delivery is cationic nanoemulsions (CNEs) and are prepared by different methods, such as high-pressure homogenization, microfluidization, and sonication[131]. The technology used in the manufacturing process, lipid type selection, and formulation parameters significantly affect CNE properties, including droplet size, surface charge, and decomplexation kinetics of nucleic acids[131,132].

For gene delivery, CNEs typically contain cationic components complexed with nucleic acids and a hydrophobic oily core surrounded by phospholipids and other surfactants. Significant progress has been made in the preclinical and clinical development of CNEs to deliver pDNA[133,134], miRNA[135], siRNA[136] and mRNA[137]. For immune response enhancement, emulsion adjuvants could be formulated in CNEs, such as MF59 and AS03. Recently, several researchers summarized the emulsion adjuvants approved and under development (Table 1)[138,139]. For mRNA delivery, the drug is bound to the CNE surface[140]. Brito et al.[137] developed a CNE formulation to deliver a 9 kb self-amplifying mRNA vaccine (saRNA). The CNEs contain the cationic lipid DOTAP and are emulsified with a safe emulsion adjuvant, MF59. The prepared CNE could protect the mRNA from degradation by RNase *in vivo*[137]. The results demonstrated that the CNE vaccine had comparable protein expression efficacy to a typical viral vector and had well tolerated and immunogenic properties in several animal types, including rabbits, rats, mice and nonhuman primates[137]. Compared with traditional mRNA vaccines, saRNA vaccines can self-amplify in the body and elevate antigen expression. Therefore, robust immune responses were displayed at low saRNA doses (saRNA dose was typically 100 times lower than traditional mRNA vaccines)[141]. HIV saRNA vaccine technology delivery using CNEs was proven safe and immunogenic in a rhesus monkey animal model, eliciting both humoral and cellular immune responses[142]. In addition, a novel rabies self-amplifying mRNA vaccine was established using CNEs, stimulating potent neutralizing antibody responses in rats. Using the vaccine delivered by CNEs, the injected saRNA is expected to enter the host cell and express the target antigen locally. A cationic nanoemulsion (NCT04062669) is currently in clinical trials for rabies virus[143].

2.6. *Exosomes*

Extracellular vesicles (EVs) secreted by all cell types in the organism can transmit biologically active molecules, including proteins and nucleic acids, and regulate the communication between cells[144–146]. According to biogenesis, EVs can be generally divided into three populations: exosomes, microvesicles and apoptotic bodies[147,148]. Exosomes are 30–150 nm vesicles originating from the fusion of multivesicular endosomes with the plasma membrane[3], and the 30–100-nm exosomes were used in most studies[144]. Exosome membranes are enriched in lipids and proteins, qualifying them as a drug delivery system for mRNA[149]. Firstly, exosomes can intrinsically carry and protect nucleic acids[150,151]. Valadi et al.[152] confirmed 1,300 different mRNAs in the exosomes isolated from a mast-cell line. Secondly, specific proteins on the surface of exosomes, such as transmembrane protein CD47, are “don’t eat me” signals, avoiding the MPS and decreasing the clearance from the circulation[153,154]. A study indicated that intravenously-injected exosomes had similar clearance with LPs composed of phosphatidylcholine and chol[155]. Thirdly, lipid components and protein components enable exosomes to cross cellular barriers and then release mRNA to the cytosol by endogenous mechanisms. Several mechanisms have been hypothesized to describe the interactions of exosomes and recipient cells[152], *i.e.*, ligand–receptor

Table 1 Emulsion adjuvants approved and under development.

Name	Time of approval	Clinical development stage	Formulation	Mechanism or receptor	Immune profile	Adverse reaction
MF59	1997	Licensed for seasonal and pandemic flu vaccine	Squalene oil, span 85, Polysorbate 80	Immune cell recruitment and antigen uptake	Antibody, Th1, Th2	Local reactions and inflammatory arthritis
AS03	2009	Licensed for pandemic flu vaccine, phase III for COVID-19	Squalene oil, α-tocopherol, polysorbate 80	Immune cell recruitment, antigen uptake	Antibody, Th1, Th2	Injection-site pain, fatigue, headache, and muscle aches
AF03	2010	Licensed for pandemic flu	Squalene oil, span80, eumulgin B1, mannitol	Immune cell recruitment and antigen uptake	Antibody, Th1, Th2	Fever and erythema
CoVaccine HT	2022	Phase III for COVID-19	Squalane oil, polysorbate 80, sucrose fatty acid sulfate esters	Little is known	Antibody, Th1	Produce pro-inflammatory cytokine, exacerbates immunopathology
GLA-SE/ SLA-SE	–	Phase I – phase III for tuberculosis, schistosomiasis, leishmaniasis	Squalene oil, poloxamer 188 and synthetic phosphatidylcholines	TLR4	Antibody, Th1	Increased levels of pro-inflammatory cytokines
Sepivac SWE	–	Preclinical, phase I for COVID-19	Squalene oil, span85 and polysorbate 80	Immune cell recruitment and antigen uptake	Antibody, Th1, Th2	Local reactions

GLA-SE, glucopyranosyl lipid adjuvant-stable emulsion; SE, stable emulsion; SLA-SE, synthetic lipid A stable emulsion; SWE, squalene-in-water emulsion.
–, not applicable.

interactions[156], fusion with target-cell membrane[157] and endocytosis[158]. However, the mechanism by which exosomes release the cargo into the cytoplasm and the fate of exosomal components in recipient cells are still unknown.

mRNA can be loaded into exosomes by direct loading and indirect loading[3,159]. For direct loading, pDNA coding the desired mRNA is used to transfect cells and then mRNA-loaded exosomes are isolated. For indirect loading, exosomes are co-cultivated with cells transfected by pDNA, and then bulk electroporation (BEP) is employed to create transient pores in the membrane of exosomes to facilitate the migration of mRNA from cells to exosomes[160−162]. Exosomes prepared in these two manners could be stored at −80 °C. Compared with indirect loading, direct loading seems to be easier to operate with a lower cost.

Nonetheless, unmodified exosomes delivered systemically prefer to accumulate in the liver, kidney, and spleen, causing a low concentration of mRNA in targeted tissues[163,164]. For the improvement of mRNA delivery, exosomes could be bioengineered by inserting tissue-special coding sequences or combining natural exosomes with peptides. Tissue-specific miRNAs could regulate mRNA at the translational level, allowing the mRNA only to work in the targeted tissues[165]. Sun et al.[166] encapsulated tissue-specific miRNA-controlled mRNA into exosomes to increase targeted delivery efficiency. To obtain engineered mRNA, they transfected HEK293T cells with a plasmid coding PGC1α possessing two miR-148 binding sites, in which PGC1α is an essential transcription factor for fat browning[167] and miR-148 is adipose tissue-specific miRNA[168]. *In vitro* study showed that engineered mRNA was successfully encapsulated into exosomes and was translationally activated by corresponding miRNAs in the recipient cells. *In vivo* experiment, significantly increased PGC1α protein expression was found in the adipose tissue of mice treated with exosomes loaded with miR-148-PGC1α, which was much lower in the lungs and other tissues[166]. Besides tissue-specific expression of the delivered genes, the modified exosomes hold promise for increasing delivery to targeted cells. Yang et al.[169] cloned glioma-targeting peptides (a CDX peptide for U87 targeting and a CREKA peptide for GL261 targeting) into the N-terminal of CD47 to produce targeted exosomes (Exo-T). The uptake of Exo-T in U87 and GL261 cells was dramatically increased as well as the translation of mRNA. Compared to non-targeted exosomes, Exo-T could cross the blood−brain barriers and deliver mRNA efficiently to U87 cells, inhibiting tumor growth and prolonging survival. Moreover, immunohistochemical staining results confirmed that Exo-T treatment had little effect on other tissues examined.

In summary, exosomes exhibit appealing properties for mRNA delivery, including biocompatibility, stability in the circulation and biological barrier permeability[170,171]. Bioengineered exosomes show more significant potential in disease treatment than natural exosomes due to improved performance *in vivo*. So far, few clinical applications of nanocarriers based on exosomes have been reported. One possible reason is that preparing engineered or modified exosomes is time-consuming with high costs. With a deeper understanding of exosomes, several physical approaches, such as ultrasound, could assist the exosome delivery of mRNA[166]. Finally, given that natural exosomes derived from different cells have different lipids and proteins, the selected components of natural exosomes, such as CD47, could be formulated into other lipid carriers to enhance stability, targeting, and uptake[144].

2.7. *LPTs*

LPTs, heterogeneous NPs present in biological fluids, mainly composed of lipids (triglycerides and chol) and proteins (apolipoprotein), play an essential role in the metabolism of lipids[172]. Due to different ratios of lipids and proteins, LPTs have an extensive range of size and density[173]. LPTs can be divided into five subclasses: chylomicrons, very low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), intermediate-density lipoproteins (IDLs), and high-density lipoproteins (HDLs) (Table 2), according to the origin, size and density[174]. Similar to EVs, the native origin of LPTs ensure many benefits for drug delivery, such as biocompatibility, non-immunogenicity and stability in circulation[175]. LPTs can transport lipids from intestines to other tissues for storage and move lipids from other tissues to the liver for metabolism, implying their intrinsic targeting ability *in vivo*[174]. For example, during the transport of lipids, LDLs and HDLs can bind to receptors on specific cells, such as the LDL receptor (LDL-R), scavenger receptor class B type 1 (SRB1) and adenosine triphosphate-binding cassette transporter subfamily G member 1 (ABCG1)[90,175]. Significantly, some of these receptors are overexpressed on tumor cells and atherosclerotic plaques, allowing LDLs and HDLs a promising vehicle for small molecule drugs to treat cancers and cardiovascular diseases[176−178].

Yet, there are various other particles in biological fluids, such as EVs, possessing a similar size and density to LPTs, leading to difficulty separating LPTs from other components[179,180]. Besides, the purification of LPTs is time-consuming and poorly scalable, hindering their use in drug delivery[181]. Synthetic lipoproteins (sLPTs) are composed of proteins (recombinant or synthetic origin) and synthetic lipids or other hydrophobic compounds[175]. The strategies for sLPT synthesis include vortex[182] and microfluidic technology[183], followed by purification using dialysis or ultracentrifugation[184,185]. sLPTs demonstrate similar properties, such as size, density and shape, to natural LPTs, maintaining the advantages of biocompatibility, stability and targeting ability[184]. So far, synthetic HDLs (sHDLs) and synthetic LDLs (sLDLs) have been applied to deliver RNAs. After modification with hydrophobic components, such as chol or cationic lipids, RNAs could co-incubate with sLPTs for targeted delivery[186,187]. Nakayama et al.[186] prepared sHDLs and sLDLs to deliver chol-conjugated siRNAs (chol-siRNA) for atherosclerosis therapy. sHDLs were formulated from recombinant apolipoprotein A1 (apoA) and palmitoyloleoylphosphatidylcholine (POPC), called

Table 2 Classification of lipoproteins.

Lipoprotein subclass	Origin	Density (g/cm^3)	Size (nm)	Ref.
Chylomicrons	Intestines	<0.950	80−1000	[195]
VLDLs	Liver, intestines	0.930−1.006	30−80	[196]
IDLs	Catabolism of VLDLs	1.006−1.019	~25	[196]
LDLs	Catabolism of IDLs	1.019−1.063	~20	[197]
HDLs	Liver, intestines	1.063−1.210	5−10	[174]

IDLs, intermediate-density lipoproteins; LDLs, low-density lipoproteins; HDLs, synthetic HDLs; VLDLs, very low-density lipoproteins.

Table 3 The comparison of lipid carriers for mRNA delivery.

Carrier type	Characteristic	Advantage	Disadvantage
LPs	Spherical vesicles with a lipid bilayer.	Low toxicity, high biocompatibility, protection of envelope.	Low EE.
LPPs	Spherical vesicles with a cationic lipid bilayer.	Higher EE.	Significant toxicity.
LLPs	Sphere-shaped, multi/monolayer nanosized vesicle.	Less toxic, higher EE, improved endosome escape.	Instability under non-freezing conditions.
SLNs	Spherical vesicles with a core consisting of a solid matrix.	Higher physical stability, more robust mRNA protection, and higher delivery efficacy.	Expulsion of the incorporated mRNA.
NLCs	Spherical vesicles with a core consisting of both solid and liquid lipids.	Higher physicochemical stability, higher drug-loading capacity, less drug leakage, and controlled drug release.	In the preparation (high-pressure homogenization) process, the high temperature may promote the degradation of drug and the carriers.
LPNs	Core-shell nanoparticle structure composed of polymer core and lipid shell.	Less biofouling, prolonged half-life, sustained drug release.	Polymeric materials may bring unwanted side effects.
Nanoemulsions	A heterogeneous colloidal dispersion of nanodroplets dispersed in another liquid phase.	Prolonged half-life, less irritation.	Emulsifier incorporation may generate safety risks.
Exosomes	Nanosized lipid vesicles secreted from living cells, ranging from 30 to 150 nm.	Extremely high biocompatibility and stability, biological barrier permeability, and extended circulation time.	Modification is required to obtain high target site affinity. The preparation of exosomes is time-consuming with high costs.
LPTs	Heterogeneous NPs present in biological fluids, mainly composed of lipids and proteins.	Extremely high biocompatibility, non-immunogenicity and stability, intrinsic targeting ability.	The purification of LPTs is time-consuming and poorly scalable.

These lipid carriers have lower toxicity and higher inclusion protection than non-lipid vectors. The advantages and disadvantages of the carriers were demonstrated by comparing with LPs.
EE, encapsulation efficiency; LPs, liposomes; LPNs, lipid-polymer hybrid nanoparticles; LLPs, liposome-like nanoparticles; LPPs, lipoplexes; LPTs, lipidprotein-particles; NLCs, nanostructured lipid carriers; SLNs, solid lipid nanoparticles.

A-sLPTs, while sLDLs were composed of apolipoprotein E3 (apoE) and dimyristoylphosphatidylcholine (DMPC), named E-sLPTs[186]. *In vivo* studies showed that silencing apolipoprotein B (apoB) mRNA was enhanced by administering chol-siRNA/sLPTs complex. E-sLPTs could deliver chol-siRNA to mouse liver with higher efficacy than A-sLPTs because E-sLPTs were not significantly affected by high endogenous levels of LDLs in plasma, indicating the potential as carriers for lipophilic conjugates such as chol-siRNA[186].

At present, sHDLs and sLDLs with a diameter of less than 20 nm have been applied to deliver microRNA[188,189] and siRNA[190,191] rather than mRNA, probably owing to the difference in the payload size (siRNA is 20−30 nucleotides *vs.* mRNA is at least 1,000 or more nucleotides) or the flexibility (siRNA is a rigid duplex *vs.* mRNA is a flexible single strand)[192], resulting in poorly loading mRNA for sLPTs. Moreover, mRNA might form local secondary structures depending on the nucleotide sequence and compromise the complexation with sLPTs[192–194].

3. Lipid carriers for mRNA vaccine delivery

3.1. *Application of lipid carriers for mRNA vaccine delivery*

mRNA was initially proposed in 1989 as a drug to treat diseases, demonstrating that mRNA could be directly delivered and expressed in eukaryotic cells under the packaging of the cationic lipid DOTMA[198]. Subsequently, mRNA was injected into mouse skeletal muscle, and effective expression was observed *in vivo*, offering the preliminary foundation for mRNA as a vaccine platform and proving the feasibility of mRNA vaccine development[199]. mRNA vaccines are divided into non-replicating mRNA vaccines and self-amplifying mRNA vaccines[200]. Non-replicating mRNA vaccines are composed of conventional or base-modified non-amplifying mRNA molecules. Self-amplifying mRNA vaccines are constituted of saRNA, also known as replicon able to self-amplify[201]. Compared with non-replicating mRNA vaccines, this sequence-optimized "next-generation" vaccine allows cells to synthesize proteins similar to the viral replication process[201]. Each mRNA sequence can be replicated multiple times in the cell before it is translated into protein, increasing the protein expression and strengthening the vaccination[202].

The carrier-based mRNA vaccines work by delivering the mRNA-expressing antigen target into the body, translating and expressing the protein *in vivo*, and stimulating the body to produce a specific immune response and immune protection (Fig. 3)[203]. *E.g.*, mRNA lipid NPs are usually internalized by somatic cells (such as muscle cells and epidermal cells) after injection[204,205]. Once escaping from the lysosome to the cytosol, the mRNA is sensed by Toll-like receptors (TLRs), promoting the release of type I interferon (IFN) into the extracellular matrix and creating an environment that favors Th1 responses, a pro-inflammatory response that leads to specific immunity[7,206]. Then, the released mRNA is translated into antigen proteins by ribosomes[207]. Antigen proteins translated from exogenous mRNA are extracellularly expressed in the secreted form and then taken up by antigen-

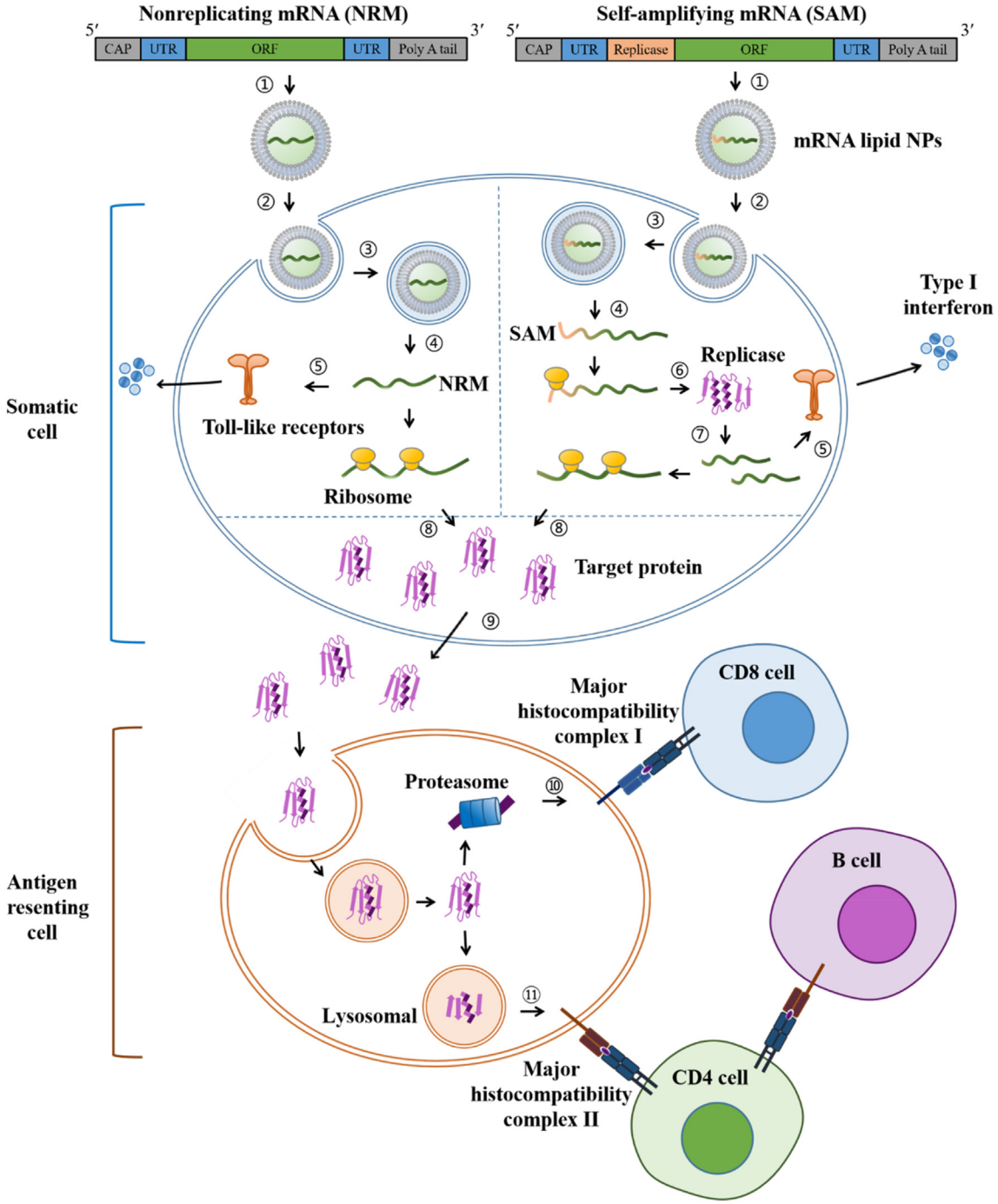

Figure 3 mRNA vaccinization *in vivo*. (1) Construction of mRNA lipid NPs, (2) endocytosis, (3) entry into lysosome, (4) escape from lysosome, (5) recognization by (TLRs, (6) translation of replicase, (7) self-amplification of mRNA, (8) translation of antigen protein, (9) secretion of antigen protein, (10) MHC-I presentation, (11) MHC-II presentation.

presenting cells (APCs)[192]. Antigen proteins are degraded into fragments by the proteasome in APCs, followed by MHC-I presentation, activating CD8 T-cells, or undergo lysosomal degradation *via* various mechanisms such as autophagy and signal peptide, followed by MHC-II presentation, activating CD4 T-cells and leading to the maturation of B cells[192,208].

In addition to the characteristics of the mRNA vaccine, the injection routine and site also affect its effectiveness. Administration routes are essential to induce protein expression, frequency, and side effects[209]. For instance, 1) intraveous injection (iv): the acting time is short. After iv administration, the preparation is first ingested by reticuloendothelial-rich tissues (liver, spleen), then phagocytosed by mononuclear macrophages and, to a lesser extent, uptaken by lung and marrow[210]. The blood clearance rate is related to the sizes and charges of carriers. Larger nanoparticles are removed by the blood faster[211]. Moreover, iv administration would cause more side effects if toxic solvent remains in preparation. Also, the patient compliance with iv administration is poor

due to the injection pain. 2) Subcutaneous injection (sc): This sc administration can directly transfect mRNA into DCs in lymph nodes[212]. In addition, when the particle size is > 150 nm, NPs are more likely to be phagocytosed by immune cells and reach lymph nodes. Nonetheless, sc is administered with limited doses (less than 2 mL). 3) intramuscular injection (im): the im could be dosed with a large dose of 2−5 mL, but it has special requirements on particle size and charge[137]. The particles with smaller particle sizes (<50 nm) and less surface charge could deliver mRNA to DCs with high efficacy. 4) Nasal administration: It has the advantages of convenient administration, high bioavailability and fast access to local lymphatic tissues[213], but the dose is limited. 5) Intradermal injection (id): id can effectively induce a Th1 immune response, but it is also impossible to administer with a high dose[5]. Accordingly, we should select the appropriate administration route to improve mRNA delivery. Furthermore, direct injection of mRNA into secondary lymphoid tissue facilitates targeted antigen delivery to APCs without needing DCs migration. mRNA vaccines have many specific advantages over traditional vaccines: 1) mRNA theoretically meets all the genetic information requirements for encoding and expressing various proteins. The vaccine's protective efficacy can be optimized by modifying the mRNA sequence, providing a more convenient method of vaccine upgrading than other types of vaccines[214,215]; 2) the development of mRNA vaccines takes a short time, has a low cost, and is easy to realize industrialization. mRNA vaccines encoding different antigens generally posess similar production and purification process. Development costs could effectively be reduced by establishing a standardized operation process[214,216]; 3) mRNA has autoimmune properties able to activate adaptive immune responses through cytokines secreted by immune cells, while traditional vaccines require additional immune adjuvants to enhance immunity[217]; 4) mRNA is translated *in vivo* to produce therapeutic proteins with the potential for less immunogenicity and complete functionality, preventing contamination by foreign proteins and viruses[214].

However, the development of mRNA vaccines is also limited due to mRNA instability, potential immunogenicity, and high *in vivo* instability[199]. The emergence of lipid carriers provides an effective solution for the *in vivo* delivery of mRNA vaccines. Currently, LLPs are the most widely and maturely used platform with the best clinical results. The possible reasons for LLPs to stand out from numerous lipid carriers are as follows: 1) Based on the preparation and production technology of LPs, as the developed version of LPs, LLPs have a more mature production and preparation process; 2) the materials of LLPs are relatively easy to get and are safe[218]. The addition of ionizable lipids enhances the encapsulation of mRNA and endosomal escape. The lipid carriers are predominantly used in preventative mRNA vaccines against infectious diseases and therapeutic mRNA vaccines against cancer[219]. Compared with traditional vaccines, mRNA vaccines can be developed in a shorter time to combat pandemic infectious diseases. In addition, mRNA opens up a new line for several infectious diseases where traditional vaccination approaches have failed. The mRNA vaccine offers a choice to fight against vaccine-preventable infectious diseases. So far, several mRNA vaccines against infectious diseases are under clinical trial. Moderna's cytomegalovirus vaccine has entered phase II, while Zika, influenza, and respiratory syncytial virus vaccines are in phase I[220]. There are also many mRNA tumor vaccines under clinical trial. Moderna's mRNA-4157 and BioNTech's BNT122 activate the immune system by expressing patient-specific tumor neoantigens[221]. Currently, tumor-associated antigens and tumor-specific neo-epitopes are the core targets of mRNA cancer vaccines[7,198,222]. Jabulowsky et al.[223] prepared an RNA-lipoplex vaccine against melanoma. The vaccine allowed APC-targeting, efficient uptake and expression of encoded antigens. The vaccine is under a human Phase I/II dose-escalation Lipo-MERIT trial (NCT02410733) to evaluate the safety, tolerability and biological efficacy of RNA (LIP) immunotherapy in patients with stage IIIB/C and iv melanoma. In addition, various hybrid vectors have also been utilized to deliver mRNA vaccines, including LPNs, lipid nanoemulsions, etc. Regarding the formulation, cationic or ionizable lipids are essential components to improve the delivery efficiency of mRNA vaccines. mRNA vaccines are now available for various diseases such as infectious diseases and cancer. The clinical trial of LLPs for mRNA vaccine delivery is shown in Table 4[6,224].

3.2. *COVID-19 mRNA vaccine based on lipid carriers*

With the global epidemic of COVID-19, mRNA vaccines, as a new type of vaccine, have played a leading role in this battle. Here, we compared the two marked COVID-19 mRNA vaccines (mRNA-1273 from Moderna[225] and BNT162b2 from BioNTech[226]) in antigen choice and LLPs design[227].

By understanding the structure of SARS-CoV-2, the full-length spike glycoprotein (S protein) on the surface of SARS-CoV-2 and the receptor-binding domain (RBD) located at the C-terminal of the S1 subdomain, the subunit of S protein induces highly potent neutralizing antibodies and T cell-mediated immunity[200,228,229]. Therefore, mRNA encoding S protein or RBD was selected as an active ingredient for coronavirus vaccine development[230,231]. Both mRNA vaccines selected mRNA encoding the S protein, in which the mRNA sequence is slightly changed by two proline substitutions, enhancing the stability of the prefusion conformation of the glycoprotein[232]. In addition to antigen choice, the LLP design of the two mRNA vaccines is similar, containing ionizable lipid (enabling mRNA complexation and escape from lysosome), PEG-lipid (conferring more prolonged systemic circulation), DSPC and chol (helping pack the cargo into the LLPs) (Table 5)[57]. The molar ratios of the ionizable lipid: PEG-lipid: DSPC: chol are 50: 1.5: 10: 38.5 for the mRNA-1273 vaccine and 46.3: 1.6: 9.4: 42.7 for the BNT162b1 vaccine[57]. The ionizable lipids in the two vaccines, SM-102 (mRNA-1273) and ALC-0315 (BNT162b2), are chemically comparable. Both have branched hydrocarbon chains, optimizing the formation of nonlamellar phases and enhancing the efficiency of mRNA delivery[233]. Furthermore, compared to conventional ionizable lipids, ester-linkages are introduced to SM-102 and ALC-0315, improving their biodegradability[218]. Besides, the two vaccines include similar PEG-lipids (PEG2000-DMG and ALC-0159) and identical helper lipids (DSPC and chol)[218].

In summary, the two mRNA vaccines share many similarities. Both choose LLPs to deliver mRNA encoding the S protein with the same mRNA-to-lipid ratio, 0.05 (wt/wt)[218]. According to some review materials authorized by the FDA, the effectiveness of the two vaccines is comparable (mRNA-1273 is 94.1% and BNT162b2 is 95.0%). However, the variance in composition causes differences in administration and storage conditions. Regarding administration, BNT162b2 needs thawing followed by dilution with saline, while mRNA-1273 can be injected after thawing[192]. In terms of storage conditions, BNT162b2 is stored at −60 to −80 °C, whereas mRNA-1273 can be stored at −15 to −25 °C[192]. Compared with other vaccines just stored at 2−8 °C,

Table 4 Clinical trials for mRNA vaccine delivery (COVID-19 vaccines are not included).

Drug	Disease	Administration route	Lipid-based carriers	Trial phase	Ref.
Core-g28v2 60mer and eOD-GT8 60mer	HIV	im	LLPs	I (NCT05001373)	https://clinicaltrials.gov/ct2/show/NCT05001373
CV7202	Rabies	im	LLPs	I (NCT03713086)	https://clinicaltrials.gov/ct2/show/NCT03713086
GSK 692342	Tuberculosis	im	LLPs	II (NCT01669096)	https://clinicaltrials.gov/ct2/show/NCT01669096
mRNA-1010	Seasonal influenza	im	LLPs	I/II (NCT04956575)	https://clinicaltrials.gov/ct2/show/NCT04956575
mRNA-1325	Zika virus	–	LLPs	I (NCT03014089)	https://clinicaltrials.gov/ct2/show/NCT03014089
mRNA-1345	Respiratory syncytial virus	im	LLPs	I (NCT04528719)	https://clinicaltrials.gov/ct2/show/NCT04528719
mRNA-1440	Influenza H10N8	im	LLPs	I (NCT03076385)	https://clinicaltrials.gov/ct2/show/NCT03076385
mRNA-1653	Human metapneumovirus and human parainfluenza infection	im	LLPs	I (NCT03392389)	https://clinicaltrials.gov/ct2/show/NCT03392389
mRNA-1647	Cytomegalovirus infection	im	LLPs	II (NCT04232280)	https://clinicaltrials.gov/ct2/show/NCT04232280
mRNA-1851	Influenza H9N9	im	LLPs	I (NCT03345043)	https://clinicaltrials.gov/ct2/show/NCT03345043
mRNA-1893	Zika virus	im	LLPs	I (NCT04064905)	https://clinicaltrials.gov/ct2/show/NCT04064905
VAL-181388	Chikungunya virus	im	LLPs	I (NCT03325075)	https://clinicaltrials.gov/ct2/show/NCT03325075
RG-SAM	Rabies	im	LLPs	I (NCT04062669)	https://clinicaltrials.gov/ct2/show/NCT04062669
BI 1361849	Metastatic non-small cell lung cancer	intratumoral injection	LLPs	I/II (NCT03164772)	https://clinicaltrials.gov/ct2/show/NCT03164772
BNT113	Unresectable head and neck squamous cell carcinoma, metastatic head and neck cancer, recurrent head and neck cancer	iv	LPs	II (NCT04534205)	https://clinicaltrials.gov/ct2/show/NCT04534205
HARE-40	Squamous cell carcinoma, head and neck neoplasm, cervical neoplasm, penile neoplasms malignant	id	LLPs	I/II (NCT03418480)	https://clinicaltrials.gov/ct2/show/NCT03418480
Lipo-MERIT	Melanoma	iv	LLPs	I (NCT02410733)	https://clinicaltrials.gov/ct2/show/NCT02410733
mRNA-2416	Relapsed/refractory solid tumor malignancies or lymphoma, ovarian cancer	intratumoral injection	LLPs	I/II (NCT03323398)	https://clinicaltrials.gov/ct2/show/NCT03323398
mRNA-2752	Relapsed/refractory solid tumor malignancies or lymphoma	intratumoral injection	LLPs	I (NCT03739931)	https://clinicaltrials.gov/ct2/show/NCT03739931
mRNA-4157	Melanoma	im	LLPs	II (NCT03897881)	https://clinicaltrials.gov/ct2/show/NCT03897881
mRNA-4650	Melanoma, colon cancer, gastrointestinal cancer, genitourinary cancer, hepatocellular cancer	im	LLPs	I/II (NCT03480152)	https://clinicaltrials.gov/ct2/show/NCT03480152
mRNA-5671/V941	Non-small-cell lung carcinoma, pancreatic	im	LLPs	I (NCT03948763)	https://clinicaltrials.gov/ct2/show/NCT03948763

Table 4 (*continued*)

Drug	Disease	Administration route	Lipid-based carriers	Trial phase	Ref.
	neoplasms, colorectal neoplasms				
RNA-LPs	Adult glioblastoma	iv	LPs	I (NCT04573140)	https://clinicaltrials.gov/ct2/show/NCT04573140
RO7198457	Advanced melanoma	iv	LLPs	II (NCT03815058)	https://clinicaltrials.gov/ct2/show/NCT03815058
RO7198457	Melanoma, non-small-cell lung cancer, bladder Cancer, colorectal cancer, triple negative breast cancer, renal cancer, head and neck cancer and other solid Cancers	iv	LLPs	I (NCT03289962)	https://clinicaltrials.gov/ct2/show/NCT03289962
SAR441000	Metastatic neoplasm	intratumoral injection	LLPs	I (NCT03871348)	https://clinicaltrials.gov/ct2/show/NCT03871348
TNBC-MERIT	Triple negative breast cancer	iv	LPs	I (NCT02316457)	https://clinicaltrials.gov/ct2/show/NCT02316457
W_ova1	Ovarian cancer	iv	LPs	I (NCT04163094)	https://clinicaltrials.gov/ct2/show/NCT04163094
ARCT-810	Ornithine transcarbamylase deficiency	iv	LLPs	I (NCT04442347)	https://clinicaltrials.gov/ct2/show/NCT04442347
Low Density Lipoprotein Receptor mRNA Exosomes	Familial hypercholesterolemia	iv	Exosomes	I (NCT05043181)	https://clinicaltrials.gov/ct2/show/NCT05043181
mRNA-3927	Propionic acidemia	iv	LLPs	I/II (NCT05130437)	https://clinicaltrials.gov/ct2/show/NCT05130437
UX053	Glycogen storage disease type III	iv	LLPs	I/II (NCT04990388)	https://clinicaltrials.gov/ct2/show/NCT04990388
mRNA-6231	Various autoimmune disorders	sc	LLPs	I (NCT04916431)	https://clinicaltrials.gov/ct2/show/NCT04916431

−, not applicable.
Id, intradermal injection; im, intramuscular injection; iv, intravenous injection; LPs, liposomes; LLPs, liposome-like nanoparticles; sc, subcutaneous injection.

this cryopreservation at low or even ultra-low temperatures impedes vaccine transport, storage and distribution[233]. For the improvement of mRNA-LLPs' stability, the mRNA nucleotide composition should be optimized primarily. A report indicated that the introduction of modified uridines inside the mRNA could minimize the recognition of mRNA by cells and thus reduce the natural immune response induced by foreign mRNA[193]. In contrast, the mRNA biostability was elevated. Secondly, a better understanding of the environment of mRNA in the core of LLPs helps adjust the structure of LLPs reasonably to maintain the integrity of mRNA. In addition, more mature and accurate preparation techniques, such as lyophilization, remain to be explored.

Globally, various kinds of COVID-19 vaccines have been on the market, including mRNA vaccines, recombinant protein

Table 5 Lipid composition of the LLP carriers of the COVID-19 mRNA vaccines.

Vaccine name (company)	Lipids	Abbreviation or lab code	Role
mRNA-1273 (Moderna)	Heptadecan-9-yl 8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy)hexyl)amino) octanoate	SM-102	Ionizable lipid
	1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000	PEG2000-DMG	PEG-lipid
	1,2-Distearoyl-*sn*-glycero-3-phosphocholine	DSPC	Helper lipid
	Cholesterol	Chol	Helper lipid
BNT162b2 (Pfizer/BioNTech)	(4-Hydroxybutyl)azanediyl bis(hexane-6,1-diyl)bis(2-hexyldecanoate)	ALC-0315	Ionizable lipid
	(2-Hexyldecanoate),2-[(polyethylene glycol)-2000]-*N*,*N*-ditetradecylacetamide	ALC-0159	PEG-lipid
	1,2-Distearoyl-*sn*-glycero-3-phosphocholine	DSPC	Helper lipid
	Cholesterol	Chol	Helper lipid

Table 6 Clinical trials of the lipid-based mRNA vaccines for COVID-19.

Vaccine name (company)	Lipid-based carrier	Registration number/phase
ChulaCov19 (Chulalongkon University)	LLPs	NCT04566276/phase I/II
CoV2 SAM (LNP) (GlaxoSmithKline)	LLPs	NCT04758962/phase I
SAM-LNP-S (Gritstone)	LLPs	NCT04776317/phase I
ARCT-021 (Arcturus)	LLPs	NCT04480957/phase I/II
LNP-nCoVsaRNA (Imperial College London)	LLPs	ISRCTN17072692/phase I
LNP-nCOV saRNA-02 (MRC/UVRI and LSHTM Uganda Research)	LLPs	NCT04934111/phase I
ARCoVax (Abogen/Walvax)	LLPs	ChiCTR2000039212/phase I/II/III
LVRNA009 (Aimbio)	LLPs	ChiCTR2200057782/phase I/II
SW0123 (Stemirna)	LPR	ChiCTR2100047917/phase I
COVID-19 mRNA vaccine (RiboBio/AgnaBio)	LLPs	Phase I
CvnCoV (CureVac AG)	LLPs	NCT04674189/phaseIII
SYS6006 (CSPC)	LLPs	Preclinical
COVID-19 mRNA vaccine (CanSino Biologics)	LLPs	Preclinical
RQ3013 (RNAcure)	LLPs	Preclinical
R520A (Recbio)	LLPs	Preclinical

LLPs, liposome-like nanoparticles; LPR, lippolyplex.

vaccines, recombinant adenovirus vector vaccines, and inactivated vaccines[234]. Among them, the clinical data on the protective efficacy of mRNA vaccines has excellent performance. The protection against mutant strains was also significantly improved after the acupuncture was strengthened. mRNA-1273 and BNT162b1 vaccines have been approved in the United States and the European Union. So far, none of the mRNA-based vaccines have been approved for marketing in China, but the development of mRNA vaccines has achieved remarkable progress recently. Several mRNA vaccines have entered the clinical trial (Table 6).

4. Summary and prospect

The most extensively considered RNA drugs are divided into two categories according to their molecular weight, siRNA and mRNA. siRNA has short sequences and a molecular weight ranging from thousands to tens of thousands of daltons, which can easily be synthesized by chemical preparation *in vitro* and then act using enzymes in the cell[235]. siRNA has been approved for disease therapy *via* gene silencing. These double-stranded RNAs with a molecular weight of approximately 13 kDa can repress protein translation by recruiting the RNA-induced silencing complex (RISC) to the mRNA through base pairing[235]. siRNA-lipid NPs have been approved by the FDA or the European Medicines Agency (EMA), including patisiran for treating hereditary transthyretin-mediated amyloidosis (hATTR)[236], givosiran for treating acute hepatic porphyria[237], lumasiran for treating primary hyperoxaluria type 1[238], and inclisiran for treating hypercholesterolemia[239]. As a therapeutic drug, siRNA works by suppressing the translation of target genes or degrading mRNA to reduce abnormal-protein production, mainly at the post-transcriptional and translational levels[240]. MicroRNAs (miRNAs) act similarly. However, miRNA is an inherent component of intracellular RNA with a single-stranded RNA, whereas siRNA is double-stranded RNA. Due to the incomplete complementarity of miRNA, miRNA mainly acts on the 3'UTR of mRNA to regulate mRNA, while siRNA can work on any position of mRNA. Regarding the action mode, miRNA works by inhibiting the translation of the target gene or degrading the target gene, performing at the post-transcriptional and translational levels; in contrast, siRNA modulates the post-transcription by discomposing the target gene[241].

Unlike siRNA or miRNA, mRNAs can be directly translated into target proteins in cells, such as gene editing-related CRISPR proteins, SARS-CoV-2, etc. mRNA has shown therapeutic potential in various applications, including viral vaccines, protein replacement therapies, and cancer immunotherapies[54]. For therapeutic-effect achievement, mRNA molecules must reach specific target cells and produce sufficient proteins of interest. mRNA can be used for precise and personalized therapy, enabling patients to produce therapeutic proteins in their bodies without struggling with the associated manufacturing problems of recombinant proteins. mRNA production is more cost-effective, faster, and more flexible than current treatments because it can be quickly produced by *in vitro* transcription. Whereas mRNA has a longer sequence and higher molecular weight changing from the hundreds of thousands to millions of daltons compared with siRNA or miRNA[235]. Therefore, mRNA does not possess the advantages of a shorter chemical synthesis cycle, flexible modification sites, and high yields like siRNA and miRNA. While the toxicity and activity of RNAi depend significantly on the present or absent sequences in the transcriptome of animal models. siRNA that works well at silencing mouse genes may have little activity against human genes[242]. Meanwhile, after reaching the cytoplasm, siRNA must be integrated into RISC to trigger RNAi. This process demands that the most unstable siRNA strand of 5′end hybridization is specially loaded into RISC while the other strand is degraded. Therefore, attaching the delivery material to the 5′ end of the antisense strand in siRNA delivery should be prevented. siRNA-backbone modifications and siRNA sequences must be selected carefully to guarantee that RISC accurately targets the strands and avoid hybridization to non-target mRNA moieties, which can lead to off-target gene silencing[243]. In contrast, mRNA therapy just needs a translation to produce a specific target protein, without considering the off-target effects. In addition, mRNA vaccines allow the rapid development of personalized medicines based on sequencing results and/or personalized conditions[244]. However, key issues must be addressed, including stability, immunogenicity, translation efficiency, and intracellular delivery, to elevate mRNA effectiveness[244]. Besides, the drawback is that mRNA only offers a short duration of protein expression and short-term transient gene expression, limiting its therapeutic use. Co-delivery of mRNA and other nucleic acid has the potential to address this issue. For instance, the combination of mRNA and

DNA can integrate their properties into one system. mRNA-mediated protein expression starts quickly but lasts for a limited time, while DNA transfection has higher and sustained protein expression but starts later[245]. In addition, the emergence of a new generation of mRNA vaccines, saRNA, which can generate consistent and robust immune responses at low doses, marks mRNA development[246]. Nevertheless, improving the efficacy of the endosomal escape and intracellular delivery is still the main interest of carrier-mediated mRNA delivery.

Lipid carriers play an essential role in mRNA delivery, along with a few lipid carriers approved for clinical use[247]. However, the *in vivo* fate of lipid carrier is poorly demonstrated, compromising the formulation design efficacy[248]. When entering the body, mRNA lipid NPs undergo biological processes, including absorption, distribution, metabolism, and excretion. The size and surface charge significantly impact the absorption of mRNA lipid NPs. Particles smaller than 10 nm may escape from the loose lymphatic vessels and accumulate in the spleen[249], while particles larger than 100 nm are difficult to diffuse into lymphatic capillaries[250]. So, particles having a size of 10−100 nm are more efficient in targeting lymph nodes[251,252]. In addition, the components, such as collagen fibers and glycosaminoglycan, in the lymph node are negatively charged; thus, neutrally or negatively charged mRNA NPs are always utilized for lymphatic delivery[251]. mRNA lipid-NPs have limited treatment potential due to liver accumulation. Recent findings displayed that mRNA lipid-NPs decorated with organ-selective protein could deliver drugs beyond the liver, predominantly depending on the protein properties[253]. mRNA lipid-NPs adsorb serum proteins in the blood and are recognized by receptors on targeting organs[253]. Hence, mRNA lipid-NPs could be designed to target specific organs such as the lung and spleen. Metabolism and elimination are also critical steps in the fate of mRNA lipid-NPs *in vivo*. High biodegradability is critical and required to ensure their safety while using. Maier et al.[254] reported a novel ionizable lipid, DLin-MC3-DMA5, by introducing ester bonds into lipids. *In vivo* experiments showed that DLin-MC3-DMA5 was rapidly metabolized and eliminated by urine and feces in 72 h after being intravenously administered to mice.

Furthermore, the administration route significantly impacts their *in vivo* fate. Im and iv are the most widely used administration routes for mRNA lipid NPs delivery (Table 4). For the im administration, the lipid NPs go through the epidermis and subcutaneous tissues and reach the deep muscle tissues[54]. Muscle tissues have rich lymph vessels with high permeability. Therefore, mRNA lipid-NPs primarily diffuse into lymphatic circulation and then distribute to lymph nodes[255]. Besides the size and charge mentioned above, the ligand modification affects the targeting ability of mRNA lipid-NPs and distribution efficiency. Zhu et al.[256] developed a lipid-based nanovaccine modified with mannose to target mannose receptors on APCs. Mannose-decorated NPs demonstrated prolonged retention time at the injection site, which could be detected at 96 h after im injection to mice, and increased distribution to inguinal lymph nodes compared to the non-modification nanovaccine. The results indicated that mannose modification considerably enhanced the transport of lipid NPs in lymph nodes after im dosing[256]. Recent studies discovered that the deformability of lipid NPs may affect the antigen transport after im injection. Song et al.[257] reported a 330-nm nanoemulsion composed of albumin, squalene, and lipopeptide ovalbumin. Due to the highly flexible deformability, the NPs could adjust the size to cross the endothelial gaps (20−100 nm), leading to higher lymph-node transfer compared to solid albumin particles[257]. Additionally, tracing lipid components indicated that NPs were metabolized in the liver and spleen after im administration[76]. A further parallel study of mRNA distribution should be conducted to confirm the finding[40]. For iv injection, the lipid carriers enter the blood and are quickly taken up by the reticuloendothelial system (RES)[210]. The size and charge are the most important factors affecting the *in vivo* fate of lipid NPs. NPs with a size over 100 nm are primarily retained in the liver Kupffer cells or trapped in the lung[1]. Cationic lipid NPs are easily captured by serum protein. PEG modification is promising to compromise the interaction between lipid NPs and serum protein and extend the circulation time[1]. Also, the modification of lipid NPs could alter the biodistribution. Perche et al.[258] decorated lipid-based formulation with glycomimetics, named Man_{11}-LPR100, to improve the transfer efficiency of spleen DCs. The results revealed that mice injected with a green fluorescent protein (EGFP) mRNA-loaded Man_{11}-LPR100 possessed four times DC-expressing EGFP than those injected with sugar-free LPR100[258]. However, liver accumulation remains the major challenge for disease therapy. The liver biodistribution of LPR100 or Man_{11}-LPR100 is over 80%, while merely 8% NPs accumulated in the spleen, owing to the interactions between positive charge in LPR and serum proteins and erythrocytes in the liver. Strategies such as using a glycolipid containing a PEG spacer between the mannose residue and the lipid moiety could decrease the LPR's positive charge and reduce the liver's capture[258]. Regardless of the im or iv administration, the development of lipid NPs for mRNA delivery remains challenging, including liver accumulation, mRNA internalization and intracellular trafficking[1], etc. Undoubtedly, a deeper understanding of the fate of mRNA lipid-NPs *in vivo* benefits lipid carriers' contribution to mRNA delivery.

Despite the approval of ionizable lipids, several challenges still need to be addressed. First, ionizable lipids in LLPs often cause acute immune responses, such as complement activation-related pseudoallergy (CARPA), an acute immunological response that can lead to anaphylactic-like shock[259] and long-term toxicity in other mRNA therapy applications[260]. Although degradable ionizable lipids can alleviate the unwanted effects, long-term treatment still requires premedication with glucocorticoids and antihistamines[261]. Consequently, amino lipid optimization is required[260]. By optimizing the lipid tail and introducing a primary lipid (similar to that observed with an MC3-based series), Sabnis et al.[90] present amino lipids with good pharmacokinetics. The lipids demonstrated little adverse effect in a one-month toxicological evaluation, providing the potential for long-term administration without activating the immune system[90]. In addition, Chen et al.[262] formulated dexamethasone-lipid prodrug into LLPs, suppressing the production of pro-inflammatory cytokines such as TNFα, IL-1β, and IL-6. This study offers an appropriate means to prevent immune stimulation initiated by dosing mRNA formulations. Second, ionizable lipid-mediated extra hepatic delivery is another challenge for mRNA application. Many ester-based ionizable lipids could transfect the liver; therefore, the proper design of ionizable lipids with targeted ability is expected to improve non-liver drug delivery. For instance, a neurotransmitter-derived ionizable lipid exhibits a transporting ability to cross the blood−brain barrier (BBB). This brain delivery platform allowed mice brain delivery of amphotericin B (AmB), antisense oligonucleotides (ASOs) against tau and genome-editing fusion protein (−27) GFP-Cre recombinase *via* iv injection[263]. The results demonstrated that the vectors based on these derived ionizable lipids have great potential for brain delivery and treating central nervous system diseases. Furthermore, protein libraries such as

phage display and bar-coding assays have been instrumental in finding proteins with affinity to other organs (tissues). These efforts are expected to reveal effective targeting ligands and thereby improve the targeting capability of LLPs beyond the liver. Third, the synthesis of ionizable lipids is cumbersome, causing scalable manufacturing challenges. *E.g.*, synthesizing MC3 requires four steps and one week of intensive labor[77]. This problem might be addressed using combinatorial chemistry for simplified synthesis. As a consequence, the optimization and innovation of ionizable lipids are still highly required for mRNA delivery.

To sum up, additional efforts should be involved to get efficient vectors for mRNA delivery. As our best knowledge goes, the effectiveness of mRNA delivery lies in the following aspects: 1) mRNA/carriers complexation efficacy determines the degradation of mRNA; 2) the moderate binding strength enables the delivery system to release mRNA at the target destination; 3) efficient cellular uptake and endosomal escape[18]. Therefore, lipid carriers possessing these criteria could effectively deliver mRNA.

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Author contributions

Wanting Zhang, Yuxin Jiang and Yonglong He were responsible for data collection and writing the manuscript. Hamza Boucetta, Jun Wu and Zhongjian Chen participated in correction. Wei He supervised and revised the manuscript. All of the authors have read and approved the final manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2022.11.026.

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