

LIPID-BASED NANOPARTICLE FORMULATION STRATEGIES FOR TARGETED DRUG, MRNA, AND GENE DELIVERY; A REVIEW

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Abstract

Lipid-based nanoparticles have emerged as a transformative platform for precision medicine, enabling the targeted delivery of diverse therapeutic payloads including small molecule drugs, mRNA, siRNA, and gene-editing components. This review systematically examines the formulation strategies, biological barriers, and therapeutic applications of lipid nanoparticles (LNPs) and cell-derived nanovesicles for gene delivery. LNPs are typically composed of four essential constituents—ionizable or cationic lipids, helper phospholipids, cholesterol, and PEGylated lipids—each playing a critical role in encapsulation efficiency, cellular uptake, endosomal escape, and in vivo stability. The clinical success of FDA-approved products such as Onpattro (patisiran) and the COVID-19 mRNA vaccines (BNT162b2 and mRNA-1273) has unequivocally validated LNP technology as a safe and effective nucleic acid delivery platform. We have discussed the key biological barriers that LNPs must overcome, including nuclease degradation, opsonization and clearance by the mononuclear phagocyte system, cellular internalization, and the rate-limiting step of endosomal escape. Targeting strategies, both passive (enhanced permeability and retention effect) and active (ligand-mediated), are explored for their roles in enhancing therapeutic precision. The review also provides a comparative analysis of synthetic LNPs versus cell-derived nanovesicles (exosomes), highlighting differences in targeting efficiency, biocompatibility, immunogenicity, and stability. Emerging hybrid and bio-inspired systems, including cell membrane-coated nanoparticles and exosome-mimetic vesicles, are discussed as next-generation platforms that synergistically combine the advantages of synthetic and biological carriers. Finally, we have examined the preclinical and clinical progress of LNP-based therapeutics, ongoing translational challenges related to storage stability, immunogenicity, and scalable manufacturing, and future directions incorporating artificial intelligence and selective organ targeting. By integrating fundamental principles of lipid chemistry with advances in nanotechnology and molecular medicine, this review provides a comprehensive framework for the rational design of lipid-based nanoparticles for next-generation gene therapies.

1. INTRODUCTION

Lipid-based nanoparticles represent a revolutionary platform in precision medicine, enabling the targeted delivery of diverse therapeutic payloads. From traditional chemotherapeutic drugs to modern nucleic acid medicines like mRNA and gene therapies, these nanocarriers overcome fundamental biological barriers that limit

treatment efficacy. By encapsulating fragile cargo within protective lipid bilayers and incorporating smart targeting ligands, these systems can direct therapeutics to specific cells and tissues while minimizing off-target effects. This review systematically examines the formulation strategies underpinning lipid nanoparticles, analyzing how

their design dictates success in delivering small molecule drugs, mRNA, and genetic material to intended biological targets. We evaluate both established approaches and emerging innovations, highlighting how rational nanoparticle engineering translates to improved therapeutic outcomes across disease applications.

The ability to precisely manipulate and edit the sequence of DNA in human cells could enable powerful new classes of genomic medicines. Millions of people worldwide suffer from heritable genetic disorders (Korf et al., 2019). The promise of therapeutic gene editing has motivated intense efforts to bring gene editing therapies to the clinic. Recent advances include the development of robust tools for gene editing in mammalian cells, including programmable nucleases, base editors, and prime editors (Anzalone et al., 2020; Doudna, 2020; Newby and Liu, 2021). These gene editing agents have been widely applied to treat numerous disorders with a genetic component across a variety of animal models (Newby and Liu, 2021; Rees et al., 2021). Some of these therapeutic gene editing strategies have already entered clinical trials, with promising early results (Gillmore et al., 2021), and many additional clinical and pre-clinical gene editing programs are underway.

1.1 Rationale for Targeted Gene Delivery

The rationale for targeted gene delivery is rooted in the need to safely and efficiently deliver therapeutic nucleic acids to specific cells where they can exert their intended effect. Considering the possibility of silencing the expression of almost any gene in a convenient, potent, and moderately specific manner, it is not surprising that researchers have been actively tapping into the application potential of RNA interference (RNAi) (Xue et al., 2015). Small interfering RNAs (siRNAs) and microRNAs (miRNAs) have become standard experimental tools and hold immense therapeutic potential for diverse gene-related medical conditions including cancer, autoimmune diseases, neuropathological conditions, and viral infections (Xue et al., 2015; Khan et al., 2023). Messenger RNA (mRNA)-based therapeutics have also emerged as a highly appealing new class of drugs, revolutionizing cancer immunotherapy by finding application in therapeutic vaccines, monoclonal antibodies, immunomodulatory drugs, and chimeric antigen receptor (CAR) cell therapies (Guevara et al., 2020;

Van Lint et al., 2012). In comparison to other functional biomolecules such as plasmid DNA (pDNA) and recombinant proteins, mRNA exhibits several therapeutic benefits, thereby rendering it highly desirable for the development of a new generation of drugs (Pastor et al., 2018). To achieve therapeutic effects, these nucleic acid molecules have to reach specific target cells and produce sufficient proteins of interest or silence target genes, highlighting the critical need for safe and effective delivery materials (Hou et al., 2021).

1.2 Limitations of Viral vs Non-Viral Vectors

The delivery of genetic material can be achieved through viral and non-viral vectors, each with distinct advantages and limitations. Viral vectors, such as adeno-associated virus (AAV) and lentivirus, are effective for gene transfer but exhibit underlying potential carcinogenicity, high self-immunogenicity, and high production costs (Khan et al., 2023). Because viral carriers are associated with a higher risk of triggering lethal immunogenic responses, non-viral nanocarriers have been the current choice for RNA delivery (Xue et al., 2015). Encouragingly, lipid-based nanoparticles stand out, possessing advantages of definite structure, ease of scale-up, targeting flexibility, minimal toxicity, and high in vivo delivery efficacy (Hou et al., 2021).

1.3 Emergence of Lipid Nanoparticles and Cell-Derived Nanovesicles

Lipid-based nanocarriers made of lipids and/or phospholipids are often used for drug and gene delivery because of various favorable properties. To begin with, the cell membrane mainly consists of lipids and phospholipids; lipid-based nanocarriers therefore have a natural tendency to interact well with the cell membrane to facilitate cellular uptake of therapeutic agents (Xue et al., 2015). There are many choices of highly biocompatible and biodegradable lipids and phospholipids that are commercially available without the need for chemical synthesis (Xue et al., 2015). Several classes of lipid-based nanocarriers have been studied for RNA delivery, the most notable ones being liposomes, lipid-based nanoparticles (such as solid lipid nanoparticles and nanostructured lipid carriers), and lipid nanoemulsions (Xue et al., 2015). Liposomes, first discovered in 1965 by Bangham and colleagues, are self-assembling nanosized lipid vesicles comprising one or more

concentric phospholipid bilayers that enclose discrete aqueous spaces (Mehta et al., 2023). Solid lipid nanoparticles (SLNs) were initially formulated as small spherical particles with a solid lipid core at room temperature, offering advantages such as enhanced physical stability and controlled drug release (Mehta et al., 2023). Nanostructured lipid carriers (NLCs) were developed as a second generation of lipid nanoparticles, composed of a mixture of solid and liquid lipids to address the limitations of SLNs by creating an imperfect matrix with enhanced drug loading capacity (Mehta et al., 2023).

2. Biological Barriers to Targeted Gene Delivery

For lipid-based nanoparticles (LNPs) to successfully fulfill their therapeutic promise, they must navigate a complex and hostile series of obstacles between the site of administration and the intracellular location where their payload—be it a drug, mRNA, or gene-editing component—must act (Hou et al., 2021). These barriers can be broadly categorized as extracellular, occurring before the nanoparticle reaches its target cell, and intracellular, which must be overcome after cellular uptake. A deep understanding of these physiological hurdles is fundamental to the rational design of effective LNP formulations. To function *in vivo*, lipid nanoparticle-mRNA formulations need to overcome multiple extracellular and intracellular barriers (Hajj and Whitehead, 2017; Kowalski et al., 2019).

2.1 Extracellular Barriers (Circulation, Opsonization, Clearance)

Upon administration, especially via the intravenous route, LNPs immediately encounter the bloodstream, a hostile environment rich in nucleases and proteases. A primary function of the LNP is to protect its fragile nucleic acid cargo from enzymatic degradation, a task achieved through encapsulation within the particle's interior core (Hajj and Whitehead, 2017; Kowalski et al., 2019). First, mRNA needs to be protected from nuclease degradation in physiological fluids (Hajj and Whitehead, 2017; Kowalski et al., 2019). Lipid nanoparticle-mRNA formulations manufactured by rapid mixing exhibit a stable nanostructure, in which mRNA molecules can be encapsulated in the interior core through electrostatic interactions with the lipids, a structural feature that protects mRNA

molecules from nuclease degradation and increases nanoparticle stability in physiological fluids (Hou et al., 2021; Hajj and Whitehead, 2017).

Second, the formulation should evade interception by the mononuclear phagocyte system (MPS) and clearance by renal glomerular filtration post-systemic administration (Hajj and Whitehead, 2017; Kowalski et al., 2019). The body's immune system recognizes and clears foreign particles through a process called opsonization, where serum proteins bind to the nanoparticle surface, triggering recognition and subsequent phagocytosis by macrophages (Blanco et al., 2015). Incorporating PEGylated lipids (PEG-lipids) into the LNP formulation is a critical strategy to address this challenge. PEGylation enhances the biocompatibility of LNPs, reduces cytotoxicity, and decreases the interaction of LNPs with blood plasma (Khan et al., 2023). The PEG coating on the outer layer of nanoparticles prevents the adsorption and recognition of serum proteins and impedes recognition by the immune system, thereby reducing clearance by the MPS and renal filtration (Hou et al., 2021). This steric stabilization is crucial for prolonging the circulation half-life of LNPs, allowing them more time to reach their intended target tissues.

2.2 Cellular Uptake and Endosomal Escape

Once LNPs have successfully circulated and reached the vicinity of their target cells, they must be internalized. LNPs undergo cellular uptake via the endocytic pathway (Khan et al., 2023). Endocytosis pathways can be broadly divided into phagocytosis and pinocytosis, with pinocytosis further divided into clathrin-mediated, caveolin-mediated, clathrin/caveolin-independent endocytosis, and micropinocytosis (Khan et al., 2023). The particular pathway that particles enter depends on several characteristics such as size, shape, particle surface, and rigidity of the nanoparticles (Mao et al., 2013). Clathrin-mediated endocytosis relies on receptor-mediated, hydrophobic, or electrostatic interactions between ligands and extracellular receptors and is a common pathway for many lipid-based NPs (Khan et al., 2023). The initial interaction of nanoparticles with the cell plasma membrane can be promoted or accelerated by the presence of positive charges or active targeting ligands on the outer surface, which can interact with the negatively charged cell

membrane components or with specific proteins exposed on the target cell (Hajj and Whitehead, 2017).

Following cellular internalization, lipid nanoparticles are usually trapped in endosomal compartments (Sahay et al., 2013; Gilleron et al., 2013). After entering cells, internalized nanoparticles are typically trafficked first into early endosomes, then into late endosomes, and finally into lysosomes where the cargo is enzymatically degraded (Khan et al., 2023). Therefore, endosomal escape is a crucial and often rate-limiting step for effective mRNA delivery, as the payload must be released into the cytoplasm to be functional (Hou et al., 2021). It has been estimated that only a small fraction (1-2%) of lipid nanoparticles can evade the endosomal pathway before they reach the lysosomes, and this tends to vary between cell types (Gilleron et al., 2013). The mechanism of endosomal escape is complex and not fully understood. The proton sponge effect was initially considered the dominant mechanism, but increasing evidence indicates that it involves the docking of lipid nanoparticles at the endosomal membrane, triggering membrane fusion and destabilization of the endosomal lipid bilayer (Zelphati and Szoka, 1996; Gilleron et al., 2013). Positively charged lipids, such as ionizable lipids that become protonated in the acidic endosomal environment, may facilitate electrostatic interaction and fusion with negatively charged endosomal membranes, resulting in the leak of mRNA molecules into the cytoplasm (Hou et al., 2021; Kowalski et al., 2019). Endosomal escape can be increased by optimizing the pKa values of ionizable lipids and by modulating the type and ratio of helper lipids like DOPE, which tends to adopt an inverted hexagonal phase that destabilizes endosomal membranes (Hou et al., 2021; Jayaraman et al., 2012).

2.3 Intracellular Trafficking and Nuclear Entry

For mRNA therapeutics, the final destination is the cytoplasm, where the cellular machinery translates the mRNA into the therapeutic protein (Guevara et al., 2020; Hou et al., 2021). Once endosomal escape is achieved, the mRNA must be released from the nanoparticle complex in its intact, functional form to be accessible to ribosomes for translation. For other nucleic acid therapies, such as DNA-based gene therapies or CRISPR-Cas9 components for gene editing, an additional major hurdle exists: trafficking to and entry into the nucleus. Unlike RNA, DNA must reach the inside of the nucleus to be transcribed, which presents a significant advantage for mRNA-based approaches, as they do not require nuclear entry (Khan et al., 2023). For gene editing, components like plasmid DNA encoding a nuclease must cross the nuclear membrane, a process that is inefficient in non-dividing cells where the nuclear envelope remains intact. This challenge underscores the rationale for using mRNA to encode gene-editing tools, as it is active in the cytoplasm and bypasses the need for nuclear delivery (Khan et al., 2023). The complex interplay of uptake mechanism, endosomal maturation, and intracellular trafficking means that mRNA transfection mediated by lipid nanoparticles is an intricate process, strongly influenced by several factors and varying widely between different cell types (Guevara et al., 2020).

3. Lipid Nanoparticles for Gene Delivery

Lipid nanoparticles have been actively studied for decades as an effective carrier capable of delivering various therapeutic agents, including nucleic acids like DNA, mRNA, and siRNA (Khan et al., 2023). Chemical structures of various components used in LNP formulations are shown in Figure 1.

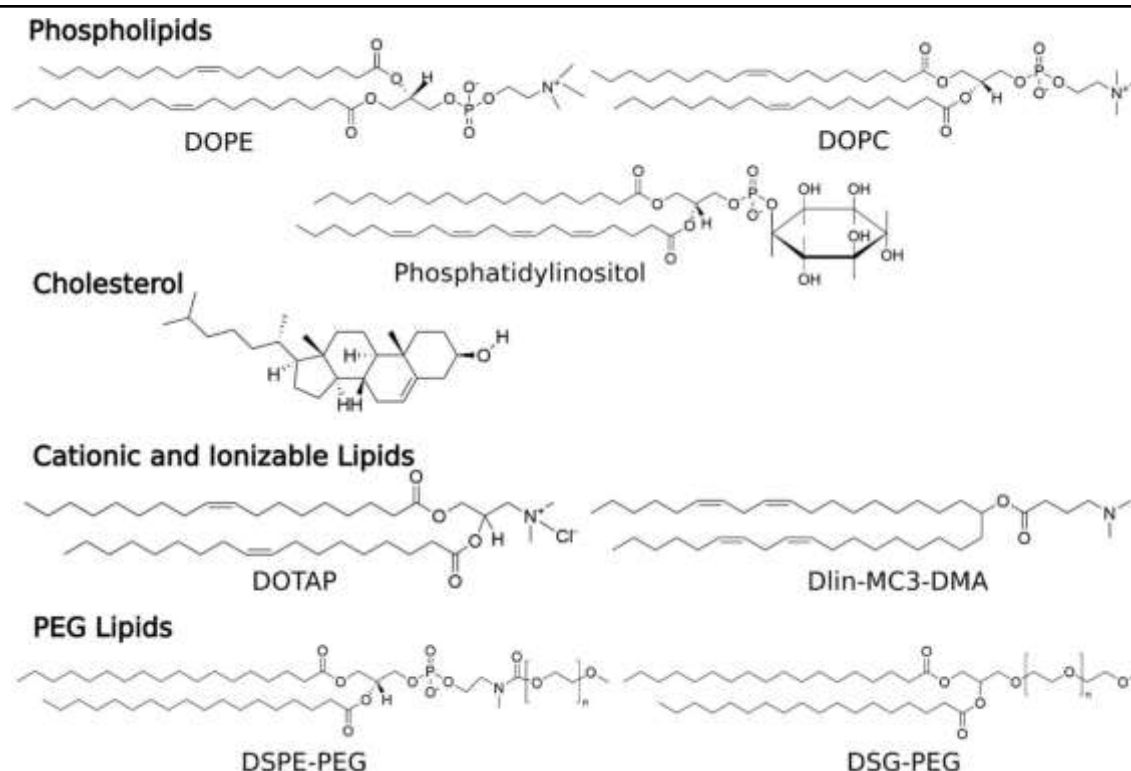


Figure 1. Chemical structures of various components used in LNP formulations. These include phospholipids (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), and phosphatidylinositol), cholesterol, cationic and ionizable lipids (1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), (1,2-dilinoleyloxy-3-dimethylaminopropane) (Dlin-MC3-DMA)), and PEG lipids (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)](DSPE-PEG), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[succinyl(polyethylene glycol)] (DSG-PEG)) (Mehta et al., 2023)

3.1 Composition and Structural Design of LNPs

Typical LNPs comprise four essential constituents: ionizable or cationic lipids, phospholipids (helper lipids), cholesterol, and polyethylene glycol-derived lipids (PEG-lipids) (Hou et al., 2021; Mehta et al., 2023). Each constituent plays a significant role in determining the effectiveness of the LNP systems. The chemical structures of various components used in LNP formulations include phospholipids (such as DOPE, DOPC, and phosphatidylinositol), cholesterol, cationic and ionizable lipids (such as DOTAP and Dlin-MC3-DMA), and PEG lipids (such as DSPE-PEG and DSG-PEG) (Mehta et al., 2023).

3.1.1 Ionizable Lipids, Helper Lipids, Cholesterol, PEGylation

Ionizable and Cationic Lipids: Cationic lipids have a head group with permanent positive charges and were first used for nucleic acid delivery (Guevara et al., 2020; Hou et al., 2021). For example, 1,2-di-Octadecenyl-3-trimethylammonium-propane

(DOTMA) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) have been applied for mRNA delivery in multiple cell types (Hou et al., 2021).

Ionizable lipids are protonated at low pH, which makes them positively charged, but they remain neutral at physiological pH (Hou et al., 2021). The pH-sensitivity of ionizable lipids is beneficial for mRNA delivery in vivo, because neutral lipids have less interaction with the anionic membranes of blood cells and, thus, improve the biocompatibility of lipid nanoparticles (Hou et al., 2021; Kowalski et al., 2019). Trapped in endosomes, in which the pH is lower than in the extracellular environment, ionizable lipids are protonated and, therefore, become positively charged, which may promote membrane destabilization and facilitate endosomal escape of the nanoparticles (Hou et al., 2021). The ionizable lipid 1,2-dilinoleyloxy-*N,N*-dimethyl-3-aminopropane (Dlin-DMA) was initially synthesized for siRNA delivery, and delivery efficacy was improved by modification of the linker and hydrophobic regions, resulting in Dlin-KC2-DMA

(Hou et al., 2021). Further optimization of the amine head group led to (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino) butanoate (DLin-MC3-DMA; MC3), which is a key delivery component of Onpattro, the first FDA-approved siRNA drug (Hou et al., 2021; Akinc et al., 2019). Biodegradable lipids heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino) octanoate (SM-102) and ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate) (ALC-0315) have better in vivo delivery efficacy and pharmacokinetics than MC3 and are the ionizable delivery components in the mRNA-1273 and BNT162b2 COVID-19 vaccines, respectively (Hou et al., 2021; Sabnis et al., 2018; Hassett et al., 2019).

Helper Lipids (Phospholipids): The phospholipids commonly used in LNPs, such as 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), change from a stable layered phase to a non-stable hexagonal phase after the phase transition temperature is reached in vivo, aiding in fusion with the endo/lysosomal membrane and enabling effective endosomal escape (Koltover et al., 1998). DSPC, an approved pharmaceutical excipient, is used in marketed mRNA vaccines (mRNA-1273 and BNT162b2), while DOPE shows higher delivery efficiency for mRNA (Kauffman et al., 2015). DOPE is a phosphoethanolamine with two unsaturated tails, which has a melting temperature of $\sim 30^{\circ}\text{C}$ and a conical shape, tending to adopt an inverted hexagonal H(II) phase, which destabilizes endosomal membranes and facilitates endosomal escape of lipid nanoparticles (Hou et al., 2021).

Cholesterol: Cholesterol is an important component of the cell membrane and is responsible for membrane fluidity (Khan et al., 2023). Adding cholesterol impacts the stability, longevity, and structural integrity of LNPs, and it also helps in cellular uptake, as shown in some studies, because it aids in higher membrane fusion (Khan et al., 2023; Farzaneh et al., 2018). Recent findings have unveiled cholesterol's involvement in endosomal escape through the promotion of membrane fusion (Patel et al., 2020). The COVID-19 LNP mRNA vaccines developed by Moderna, Inc. and Pfizer-BioNTech contain cholesterol as their ingredients (Khan et al., 2023).

PEGylated Lipids: A challenge often linked with the use of liposomes is their short circulation time when administered intravenously (Chatzikleanthous et al., 2021). In order to overcome this issue, liposomes are often modified with PEG hydrophilic polymer moieties (Chatzikleanthous et al., 2021). PEG present on the surface of LNPs is associated with increased hydrophilicity, reduced electrostatic interaction, and prolonged LNP circulation (Khan et al., 2023). PEG enhances the biocompatibility of LNPs, reduces cytotoxicity, and decreases the interaction of LNPs with blood plasma (Khan et al., 2023). PEG incorporation also prevents the aggregation and coalescence of nanoparticles (Khan et al., 2023). The amount of PEG-lipids can affect particle size and zeta potential, and they contribute to particle stability by decreasing particle aggregation (Hou et al., 2021). Certain PEG modifications prolong the blood circulation time of nanoparticles by reducing clearance mediated by the kidneys and the mononuclear phagocyte system (MPS) (Hou et al., 2021; Knop et al., 2010). Finally, PEG-lipids can be used to conjugate specific ligands to the particle for targeted delivery (Hou et al., 2021). For the case of cationic liposomes where the depot effect is involved in their immunostimulatory mechanism, pegylation may not be beneficial, depending on the PEG density and length used, as higher PEG concentrations can lead to adverse effects (Chatzikleanthous et al., 2021; Kaur et al., 2012).

3.2 Mechanisms of Nucleic Acid Encapsulation and Release

3.2.1 Electrostatic Interactions

The encapsulation of nucleic acids into LNPs is primarily driven by electrostatic interactions. Thanks to their cationic amino groups, ionizable or cationic lipids can electrostatically interact with the negatively charged phosphate groups of mRNA molecules and allow their entrapment in a lipid-based nanoparticle (Guevara et al., 2020). The use of delivery systems based on permanently cationic lipids has proven to be effective for mRNA in vitro transfection and was described for the first time more than 30 years ago (Guevara et al., 2020; Malone et al., 1989). Ionizable cationic lipids, which are positively charged at acidic pH and neutral at physiological pH, are particularly effective. During the manufacturing process, LNPs are formed by ethanol injection nanoprecipitation,

where lipids dissolved in ethanol at an appropriate ratio and mRNA dissolved in an acidic aqueous buffer are mixed together (Guevara et al., 2020; Cullis and Hope, 2017). The acidic pH is necessary to ensure the protonation of ionizable lipids so that, after mixing, electrostatic interactions drive the formation of inverted micelles containing the mRNA surrounded by cationic lipids (Guevara et al., 2020).

3.2.2 Endosomal Escape Mechanisms

Following cellular internalization, lipid nanoparticles are usually trapped in endosomal compartments, and only a small amount may be able to escape from the endosome (Hou et al., 2021; Sahay et al., 2013). Thus, endosomal escape is crucial for effective mRNA delivery. Although the mechanism has not yet been fully understood, positively charged lipids may facilitate electrostatic interaction and fusion with negatively charged endosomal membranes, resulting in the leak of mRNA molecules into the cytoplasm (Hou et al., 2021; Kowalski et al., 2019). The proton sponge effect was initially considered the dominant mechanism leading to the endosomal escape of RNA-loaded lipid nanoparticles (Guevara et al., 2020). However, increasing evidence indicates that the endosomal escape mechanism of lipid nanoparticles is much more complex and involves the docking of the lipid nanoparticles at the endosomal membrane, triggering membrane fusion and destabilization of the endosomal lipid bilayer, with consequent release of the genetic cargo into the cytosol (Guevara et al., 2020; Zelphati and Szoka, 1996). Ionizable lipids play a key role here; they are protonated in the low pH endo/lysosomes and interact with negatively charged endo/lysosomal membranes, resulting in membrane instability and endosomal escape (Sahay et al., 2010).

3.3 Targeting Strategies for LNPs

3.3.1 Passive Targeting

Passive targeting leverages the unique properties of cancer cells to deliver therapeutic agents by nanocarriers (Bazak et al., 2014). For example, doxorubicin-loaded LNPs can accumulate in renal cancer in situ through an EPR effect, with concentrations 4 to 6 times higher than in normal kidney tissue (Sakurai, 2019).

3.3.2 Ligand-Mediated Active Targeting

Folate receptors exhibit significant overexpression in the proximal tubules of renal tumors, and consequently, folate-modified nanoparticles demonstrate preferential accumulation in damaged kidney cells (Huang et al., 2019). By improving the target specificity, it is possible to lower the dose of an RNA nanoformulation so the toxicity issue will be addressed at the same time (Xue et al., 2015). The more recently developed RNA nanocarriers therefore are typically surface-modified with active-targeting moieties, including receptor substrates, monoclonal antibodies, and cell-penetrating peptides (Xue et al., 2015). The surface modifications are technically not difficult for lipid-based systems as the phospholipid molecules, especially those PEGylated ones, can be easily functionalized to provide useful sites for conjugation to the targeting moieties (Xue et al., 2015). For example, mannose, which binds with high affinity to the Lectin Receptor DC-SIGN exposed on the surface of dendritic cells, can be introduced in the formulation to target these cells in vivo (Guevara et al., 2020; Wang et al., 2018).

3.4 Therapeutic Applications of LNP-Based Gene Delivery

3.4.1 mRNA Therapeutics

mRNA-based gene therapy holds the promise to revolutionize the field of cancer immunotherapy by addressing current manufacturing limitations and offering novel therapeutic solutions (Guevara et al., 2020). The relative rapidity of its production is considered one of the central advantages of mRNA compared with other immunotherapeutic strategies; indeed, the synthesis of mRNA-based treatments can be achieved within weeks using a cell-free and scalable process, once the sequence encoding the immunogene is available (Guevara et al., 2020; Wadhwa et al., 2020). Currently, mRNA constructs have been employed to express tumor-associated antigens (TAA) and neoantigens for the development of therapeutic and prophylactic vaccines, for the in-situ production of potent monoclonal antibodies, and for the engineering of CAR T cells (Guevara et al., 2020; Kranz et al., 2016). Two types of mRNA platforms have been proposed for cancer immunotherapy: non-amplifying mRNA (conventional mRNA) with an open reading frame (ORF) flanked by 5' and 3' untranslated regions (UTRs), and self-amplifying

mRNA (saRNA) derived from the positive-stranded alphavirus RNA genome (Guevara et al., 2020; Kowalski et al., 2019). The application of lipid-based nanovectors has enabled the integration of mRNA-based technology in many pre-existing anti-cancer immunotherapeutic approaches (Guevara et al., 2020). A proof-of-concept study demonstrated that dendritic cells can be passively targeted in vivo using intravenously injected mRNA-lipoplexes based on DOTMA/DOPE or DOTAP/DOPE formulations, and the loading of TAA-mRNA onto the lipoplex nanostructure efficiently protected the mRNA from extracellular ribonucleases and enhanced its uptake and expression by different dendritic cell subsets (Guevara et al., 2020; Kranz et al., 2016).

3.4.2 siRNA and Antisense Oligonucleotides

RNA-interference (RNAi) agents such as small-interfering RNA (siRNA) and micro-RNA (miRNA) have strong potential as therapeutic agents for the treatment of a broad range of diseases such as malignancies, infections, autoimmune diseases, and neurological diseases that are associated with undesirable gene expression (Xue et al., 2015). For systemic administration of these poorly permeable and easily degradable macromolecules, a safe and efficient delivery platform is highly desirable (Xue et al., 2015). Single-stranded antisense oligonucleotides (ASOs) are examples of therapies that aim to target nucleic acids that are short stretches of modified DNA

made of about 13-25 building blocks or nucleotides, which block protein synthesis by preventing mRNA translation (Khan et al., 2023). The first RNA-based therapeutic tool is Patisiran, ALN-TTR02, which was approved to be used in adults in 2018 for the treatment of hereditary amyloidogenic transthyretin amyloidosis with polyneuropathy, delivering siRNA using a liposome formulation (Khan et al., 2023; Akinc et al., 2019). In terms of clinical development, LNP (lipid nanoparticle, in earlier reports also known as SNALP, stabilized nucleic acid lipid particle) is probably the most successful RNA nanocarrier studied up to date, with many clinical trials of LNP for a range of diseases conducted (Xue et al., 2015).

4. Comparative Analysis of Lipid Nanoparticles and Cell-Derived Nanovesicles

Both synthetic lipid nanoparticles (LNPs) and naturally occurring cell-derived nanovesicles (such as exosomes) have emerged as powerful platforms for gene delivery, each offering distinct advantages and facing unique challenges. A comparative analysis of these two classes of nanocarriers is essential for guiding the selection of the most appropriate delivery system for specific therapeutic applications. While LNPs offer the advantages of well-defined composition, scalability, and tunability, cell-derived nanovesicles provide inherent biocompatibility and complex biological functionality (Khan et al., 2023).

Table1: Comparison between Lipid-Based Systems (Chatzikleanthous et al., 2021)

Feature/Type of Nanoparticle	Liposome	Lipid Nanoparticle
Easy surface modification	+++	+++
Loading of hydrophilic and hydrophobic molecules	+++	+++
Biocompatible and biodegradable	+++	+++
Improve stability of therapeutic agents	+++	+++
Controlled drug release	+	+
Large scale production and sterilization	+	++
Manufacturing cost	+	++
Physical and biological stability	++	+++
Avoidance of organic solvents during manufacturing when desired	+	+++
Drug leakage	+	+
Toxicity	+	++

Key:

- +++ = Excellent / High
- ++ = Good / Moderate
- + = Fair / Low

4.1 Biocompatibility and Immunogenicity

Biocompatibility and immunogenicity are paramount considerations for any therapeutic intended for in vivo use. LNPs are formulated from biocompatible and biodegradable lipids, such as phospholipids and cholesterol, which are naturally occurring components of cell membranes (Xue et al., 2015; Mehta et al., 2023). This biomimetic architecture gives LNPs a natural tendency to interact well with cell membranes and reduces the risk of undesirable immunogenic reactions compared to most polymeric materials (Xue et al., 2015). However, some LNP components, particularly cationic lipids and PEGylated lipids, can still trigger immune responses. Cationic lipids have been associated with cytotoxicity and the activation of pro-inflammatory pathways (Xue et al., 2015; Lv et al., 2006). PEGylation, while essential

for prolonging circulation time, can lead to the production of anti-PEG antibodies and the phenomenon of accelerated blood clearance (ABC) upon repeated administration, which may compromise therapeutic efficacy and cause hypersensitivity reactions (Ishida et al., 2006). Their surface composition, which includes self-markers such as CD47, can help them evade immune recognition and phagocytosis, potentially resulting in longer circulation times and reduced immunogenicity compared to some synthetic nanoparticles. This natural "stealth" property is a significant advantage for therapeutic applications. However, the immunogenicity of exosomes can vary depending on their cellular origin. For example, tumor-derived exosomes may carry immunosuppressive molecules that could potentially interfere with cancer immunotherapy,

while exosomes from other sources might be well-tolerated.

4.2 Stability and Storage

The stability and storage requirements of a nanomedicine formulation are critical for its practical application, distribution, and shelf-life. LNPs, particularly those formulated for mRNA delivery, have well-characterized stability profiles. However, they often require stringent storage conditions. For instance, the Pfizer-BioNTech COVID-19 vaccine (BNT162b2) initially required storage at ultra-low temperatures (-80°C to -60°C), while the Moderna vaccine (mRNA-1273) required storage at -20°C (Schoenmaker et al., 2021). This need for cold chain distribution poses significant logistical challenges. The instability can arise from lipid oxidation, hydrolysis, and the inherent fragility of the mRNA payload (Mehta et al., 2023; Packer et al., 2021). Strategies to improve LNP stability include lyophilization (freeze-drying) with cryoprotectants like sucrose or trehalose, which can enable storage at higher temperatures, though this process must be carefully optimized to maintain particle integrity and delivery efficacy (Zhao et al., 2020).

Cell-derived nanovesicles face even more pronounced stability challenges. Their complex biological composition, including proteins, lipids, and nucleic acids, makes them susceptible to degradation, aggregation, and loss of function during storage and handling. Maintaining the integrity and bioactivity of exosomes over time requires carefully controlled conditions, and there is currently no consensus on optimal storage buffers, temperatures, or protocols. While some studies have shown that exosomes can be stored at -80°C for extended periods, freeze-thaw cycles can be detrimental.

5. Hybrid and Bio-Inspired Nanovesicle Systems

Recognizing the distinct advantages and limitations of both synthetic lipid nanoparticles and naturally derived cell vesicles, researchers have increasingly turned to hybrid systems that combine the strengths of both platforms. These bio-inspired nanovesicles represent an advanced generation of drug delivery systems that synergistically integrate the stability, scalability, and tunability of synthetic nanomaterials with the biocompatibility, targeting capabilities, and complex biological functionality of

natural membranes (Mehta et al., 2023).

5.1 Cell Membrane-Coated Lipid Nanoparticles

Cell membrane-coated nanoparticles represent an innovative biomimetic strategy where synthetic nanoparticle cores are enveloped in naturally derived cell membranes.

This coating confers several key advantages. The cell membrane coating effectively camouflages the synthetic nanoparticle, allowing it to inherit the surface properties and functionalities of the source cell (Fang et al., 2018). For example, coating with red blood cell membranes provides immune evasion capabilities due to the presence of "self-markers" like CD47, which inhibit macrophage-mediated phagocytosis and prolong circulation time (Hu et al., 2011). Coating with platelet membranes can enable targeting to vascular injury sites or circulating tumor cells (Hu et al., 2015). Importantly, the membrane coating can be derived from cancer cells themselves, imparting the nanoparticle with homotypic targeting capabilities—the ability to specifically target and bind to the same type of cancer cell due to the presence of surface adhesion molecules (Fang et al., 2014).

5.2 Exosome-Mimetic Nanoparticles

Exosome-mimetic nanoparticles are another class of bio-inspired nanovesicles designed to emulate the structural and functional properties of natural exosomes while enabling more controlled and scalable manufacturing (Garcia-Manrique et al., 2018). Natural exosomes possess inherent advantages for drug delivery, including efficient cellular uptake, low immunogenicity, and the ability to cross biological barriers. However, their clinical translation is hampered by low production yields, complex isolation procedures, and batch-to-batch variability (Luan et al., 2017). Exosome-mimetics aim to capture the key beneficial features of exosomes while circumventing these production challenges.

These biomimetic systems are typically fabricated using top-down or bottom-up approaches. Top-down methods involve the physical extrusion of cells through porous membranes to shear off cell membranes and generate large quantities of nanovesicles that closely resemble exosomes in size and surface composition (Jo et al., 2014). These cell-derived nanovesicles often contain the same

membrane proteins as natural exosomes and can be loaded with therapeutic cargo. Bottom-up approaches, in contrast, involve the self-assembly of lipids and membrane proteins to form synthetic vesicles that mimic exosome structure (van der Meel et al., 2014). A key aspect of this strategy is the incorporation of specific targeting ligands or adhesion molecules onto the vesicle surface to replicate the homing capabilities of natural exosomes.

6. Preclinical and Clinical Progress

The features of lipid nanoparticle-mRNA formulations have been thoroughly preclinically and clinically investigated, which has allowed the rapid development and clinical use of the COVID-19 LNP-mRNA vaccines (Hou et al., 2021). A variety of lipid nanoparticles have been explored and optimized for mRNA delivery, providing valuable information for the future design of mRNA therapeutics. Based on the lessons and experiences from clinical studies, lipid nanoparticle-mRNA formulations can be further improved (Hou et al., 2021). The clinical-grade COVID-19 vaccine, mRNA-1273, was produced within a month after the SARS-CoV-2 genome sequence was available, and about 2, 5, and 6 months from sequence availability, clinical trial phases I, II, and III were initiated, respectively (Hou et al., 2021; Gebre et al., 2021). Many other LNP-mRNA formulations are in clinical trials for the treatment of infectious diseases, cancer, and genetic disorders (Hou et al., 2021; Pardi et al., 2018; Sahin et al., 2014).

6.1 In Vitro and In Vivo Evaluation Models

The preclinical development of LNP-based therapeutics relies on a combination of in vitro and in vivo models to assess efficacy, safety, and mechanism of action. In vitro studies are essential for initial screening and optimization of LNP formulations. Cell-based assays allow researchers to evaluate critical parameters such as cellular uptake, transfection efficiency, cytotoxicity, and intracellular trafficking (Guevara et al., 2020). For example, the delivery efficiency of TT3 lipid-like nanoparticles (LLNs) was evaluated by in vitro screenings in Hep3B cells, where TT3 LLNs induced the highest luminescence intensity compared to other formulations (Wang et al., 2021; Li et al., 2015). Similarly, vitamin-derived lipid

nanoparticles (VLNPs) were screened and optimized in RAW264.7 macrophages for mRNA delivery, demonstrating over 70- and 300-fold higher efficiency than Lipofectamine 3000 and electroporation, respectively (Wang et al., 2021; Hou et al., 2020). These in vitro models provide rapid, cost-effective platforms for down-selecting lead candidates and understanding structure-activity relationships before progressing to more complex animal studies.

In vivo evaluation in animal models is a critical step in the preclinical development pipeline, providing essential data on biodistribution, pharmacokinetics, efficacy, and toxicity. Rodent models, particularly mice and rats, are the most commonly used species for initial in vivo studies due to their availability, cost-effectiveness, and well-characterized genetics. For example, the biodistribution of TT3 LLNs was evaluated in mice, showing notably higher signals in spleen and liver compared to control groups (Wang et al., 2021). In a hemophilia B mouse model, TT3-hFIX mRNA LLNs produced 608 and 1740 ng/mL hFIX at mRNA doses of 0.55 and 1.1 mg/kg, respectively, successfully recovering hFIX levels to the normal range (Wang et al., 2021; Li et al., 2015). For infectious disease applications, mouse models are used to assess vaccine immunogenicity and protective efficacy. Studies with TT3-NASAR LLNs encoding SARS-CoV-2 antigens demonstrated over 300-fold higher anti-S1 antibodies compared to DLin-MC3-DMA LNP vaccines, with dose-dependent effects observed from 0.012 to 12 µg (Wang et al., 2021; Zeng et al., 2020). Larger animal models, such as non-human primates, are often employed for confirmatory studies before advancing to clinical trials, providing more predictive data on human responses (Hou et al., 2021; Sabnis et al., 2018).

6.2 Approved and Ongoing Clinical Trials

The clinical translation of LNP-based therapeutics has accelerated dramatically in recent years, with several products now approved and numerous others in active clinical development. Currently, five LNP-based medicinal products have received FDA and/or EMA approval, including three mRNA vaccines for COVID-19, an RNA interference (RNAi) therapy for hereditary transthyretin amyloidosis, and a vaccine for

Respiratory Syncytial Virus (RSV) (Mehta et al., 2023; Jung et al., 2022).

The first FDA-approved LNP-based therapy was Onpattro (patisiran), developed by Alnylam Pharmaceuticals, which received approval in 2018 for the treatment of hereditary transthyretin-mediated amyloidosis (Hou et al., 2021; Akinc et al., 2019). Onpattro uses LNP technology to deliver siRNA to hepatocytes, silencing the production of transthyretin protein. This landmark approval marked the first systemic delivery of an RNAi therapeutic and validated LNP technology for nucleic acid delivery (Xue et al., 2015; Akinc et al., 2019). The success of Onpattro paved the way for subsequent LNP-based products.

The most prominent clinical success of LNP technology came with the rapid development and authorization of mRNA vaccines for COVID-19. The Pfizer/BioNTech vaccine (BNT162b2) and the Moderna vaccine (mRNA-1273) both received Emergency Use Authorization in late 2020 and have since been administered to billions of people worldwide (Hou et al., 2021; Khan et al., 2023; Polack et al., 2020; Baden et al., 2021). These vaccines use ionizable lipid nanoparticles to deliver nucleoside-modified mRNA encoding the full-length spike protein of SARS-CoV-2, demonstrating approximately 95% efficacy in phase III clinical trials (Hou et al., 2021). Other COVID-19 vaccines based on LNP-mRNA formulations are also under evaluation in different clinical phases, including CVnCoV from CureVac and ARCT-021 from Arcturus Therapeutics (Chatzikleanthous et al., 2021).

Beyond infectious diseases, LNP-mRNA formulations are being actively investigated for cancer immunotherapy and genetic disorders. For cancer, numerous clinical trials are evaluating LNP-based vaccines and immunomodulators. FixVac, a systemic mRNA vaccine encoding four non-mutated melanoma antigens, is in clinical trials (NCT02410733) and has shown that more than 75% of patients generated immune responses against at least one tumor-associated antigen (Hou et al., 2021; Sahin et al., 2020). Personalized cancer vaccines, such as mRNA-4157, which encodes up to 34 neoantigens, are being evaluated in phase I and II studies (NCT03897881) (Hou et al., 2021). For genetic disorders, clinical trials of protein replacement therapies using LNP-mRNA formulations have focused on inherited metabolic

disorders, including ornithine transcarbamylase deficiency (NCT03767270), methylmalonic acidemia (NCT03810690), and propionic acidemia (NCT04159103) (Hou et al., 2021). Translate Bio has initiated a phase I/II trial (NCT03375047) to evaluate nebulized LNP-mRNA formulations (MRT5005) in patients with cystic fibrosis, with interim reports showing improvements in lung function (Hou et al., 2021). Most recently, Intella Therapeutics initiated a phase I clinical trial (NCT04601051) to study NTLA2001, LNP encapsulating CRISPR-Cas9 gene-editing components, in patients with hereditary transthyretin amyloidosis, marking the first systemic delivery of CRISPR-Cas9 in humans (Hou et al., 2021).

6.3 Translational Challenges

Despite the remarkable clinical successes, the translation of LNP-based therapeutics from bench to bedside faces several significant challenges that must be addressed to expand their clinical utility (Hua et al., 2018). Lipid crystallization and polymorphic transitions can lead to structural changes, resulting in drug leakage and reduced encapsulation efficiency (Mishra et al., 2018). Currently, commercially available mRNA vaccines generally necessitate ultra-low temperatures for storage (Pfizer/BioNTech's BNT162b2 at -80°C to -60°C; Moderna's mRNA-1273 at -20°C), posing significant logistical challenges for global distribution, particularly in low-resource settings (Schoenmaker et al., 2021).

Overcoming biological barriers remains a major hurdle. For systemic administration, LNPs must evade immune clearance, extravasate from the bloodstream, penetrate target tissues, and achieve efficient cellular uptake and endosomal escape (Hou et al., 2021; Hajj and Whitehead, 2017). Targeting extrahepatic tissues is particularly challenging, as most systemically administered LNPs naturally accumulate in the liver (Cheng et al., 2020). While strategies such as selective organ targeting (SORT) and ligand-mediated targeting are being developed to redirect LNPs to other tissues like the lungs, spleen, or tumors, these approaches require further optimization to achieve clinically relevant efficiency and specificity (Cheng et al., 2020).

Toxicity and immunogenicity of LNP components also pose safety concerns (Wang et al., 2024). While

ionizable lipids reduce toxicity compared to permanently charged cationic lipids, immune activation and complement system engagement must be carefully managed to avoid adverse reactions (Kedmi et al., 2010). Cationic and ionizable lipids have been reported to stimulate the secretion of pro-inflammatory cytokines and reactive oxygen species, and in vivo application of lipid nanoparticles has been reported to induce liver and lung injuries in rodents (Hou et al., 2021; Dokka et al., 2000; Abrams et al., 2010). PEG-lipids, while essential for prolonging circulation, could induce hypersensitivity reactions by stimulating the complement system and may lead to the production of anti-PEG antibodies, resulting in accelerated blood clearance upon repeated administration (Hou et al., 2021; Ishida et al., 2006).

Manufacturing and reproducibility issues further complicate clinical applications. Large-scale production requires stringent control of particle size, composition, and batch consistency, as minor variations can significantly affect pharmacokinetics and bioactivity (Khairnar et al., 2022). The development of robust quality control systems and the implementation of Quality by Design (QbD)

and Design of Experiments (DoE) approaches are essential for ensuring reproducible manufacturing and regulatory compliance (Gurba-Bryskiewicz et al., 2023). The regulatory landscape for nanomedicines also requires refinement to accelerate approval pathways while ensuring patient safety, and harmonizing regulatory policies for LNP use in clinical settings at a global level is essential (Mehta et al., 2023; Foulkes et al., 2020).

7. Safety, Toxicity, and Regulatory Considerations

As lipid nanoparticles advance through preclinical development and into clinical practice, a comprehensive understanding of their safety profile, potential toxicity, and the regulatory landscape governing their use is paramount. The safety profile of LNP-mRNA formulations correlates with the lipid components and mRNA molecules themselves (Hou et al., 2021). While LNPs offer significant advantages as drug delivery systems, their components can sometimes trigger adverse biological responses that must be carefully managed to ensure patient safety and therapeutic efficacy (Xue et al., 2015).

Table 2: Toxicity of LNP-Based Marketed Drugs (Mehta et al, 2023)

Marketed Formulation	Active Drug	Toxicity Symptoms
Doxil	doxorubicin	hand-foot syndrome, stomatitis, skin toxicity (facial swelling, itching), hypersensitivity reactions, mild myelosuppression and alopecia, dyspnea
Myocet	doxorubicin	neutropenia, mild cardiotoxic
Abelcet, Amphotec, AmBisome	amphotericin B	nephrotoxicity, hypersensitivity reactions such as rash, flushing, facial edema, bronchospasm, persistent fever and rigors, hypokalemia
Linhaliq/Pulmaquin	ciprofloxacin	dyspnea, bronchospasm, hemoptysis, cough, taste disorders
DaunoXome	daunorubicin	hematological toxicity such as neutropenia, back pain
Visudyne	verteporfin	back pain, chest pain, dyspnea, dizziness, rash
Arikace	amikacin	ototoxicity including deafness, dizziness, vertigo, dysphonia, pneumonitis, laryngitis

Marketed Formulation	Active Drug	Toxicity Symptoms
Abraxane	paclitaxel	neutropenia, hypersensitivity reactions, neuropathy, severe myelosuppression

7.1 Immunogenicity and Off-Target Effects

Immunogenicity is a primary safety consideration for LNP-based therapeutics. Both the lipid components and the nucleic acid cargo can potentially activate the immune system, leading to unwanted inflammatory responses or reduced therapeutic efficacy (Hou et al., 2021). Lipid components may activate host immune responses following systemic or local administration (Hou et al., 2021). Cationic and ionizable lipids have been reported to stimulate the secretion of pro-inflammatory cytokines and reactive oxygen species (Hou et al., 2021; Dokka et al., 2000). For example,

first-generation monoalkyl cationic lipid, stearyl amine, has been shown to cause hemolysis and hemagglutination of human erythrocytes (Mehta et al., 2023). Another commonly used cationic lipid, DOTAP, has the propensity to bind to serum proteins, lipoproteins, and the extracellular matrix, leading to aggregation or premature drug release (Mehta et al., 2023). Although the immunogenicity of these lipids has not yet been fully understood, complement system and Toll-like receptors may participate in innate immune activation (Hou et al., 2021; Kedmi et al., 2010; Loney et al., 2014).

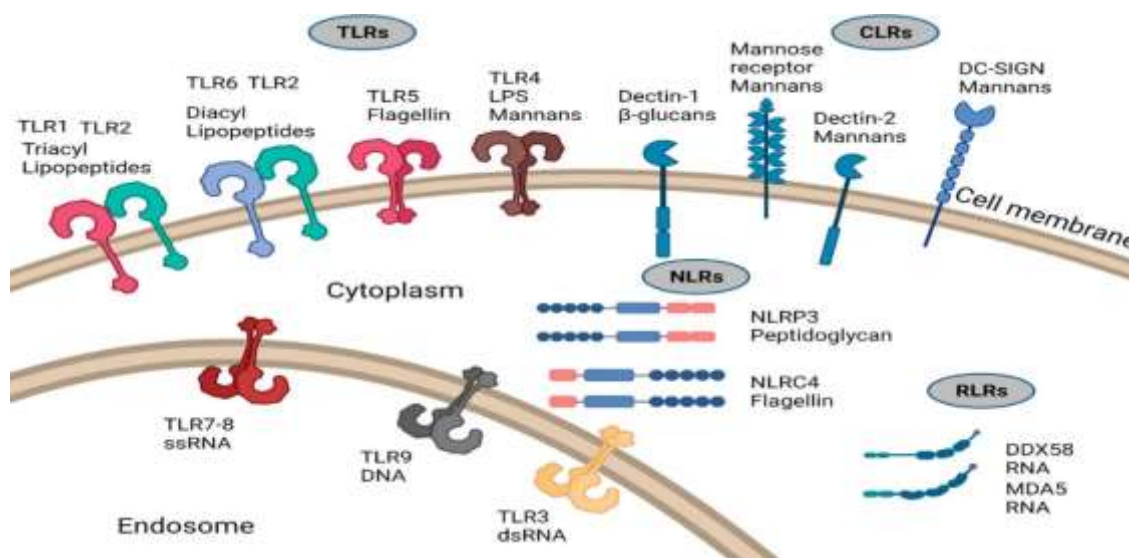


Figure 2. Schematic representation of the major Pattern recognition receptors and their corresponding ligands. (Chatzikleanthous et al., 2021)

PEGylated lipids, while crucial for prolonging circulation time, present their own immunogenicity concerns. PEG-lipids could induce hypersensitivity reactions by stimulating the complement system, a phenomenon known as complement activation-related pseudoallergy (CARPA) (Hou et al., 2021; Szebeni, 2014). Moreover, anti-PEG antibodies could result in fast systemic clearance of subsequently administered PEGylated nanoparticles by accelerated blood clearance (ABC), which may change the

bioavailability and biodistribution of the drug and cause side effects (Hou et al., 2021; Ishida et al., 2006). The ABC phenomenon has been documented with empty PEGylated liposomes administered systemically, accelerating the clearance of subsequently injected liposomes (Xue et al., 2015; Dams et al., 2000). A study on multi-dosing of stabilized plasmid lipid particles also revealed loss of expression in the targeted tumor and increased expression in the liver in later doses as a result of production of IgM and IgG against

PEG (Xue et al., 2015; Judge et al., 2006). To ameliorate safety concerns, numerous natural and synthetic polymers are being investigated as alternatives to PEG, with several under evaluation in clinical trials (Hou et al., 2021; Knop et al., 2010).

The immunogenicity of in vitro transcribed (IVT) mRNA itself is another safety consideration. While eliciting cellular and humoral immunity may be advantageous for vaccination, immune responses to IVT mRNA may also suppress antigen expression and negatively affect vaccine efficacy (Hou et al., 2021; Pollard et al., 2013; Lokugamage et al., 2020). Moreover, immune activation is undesirable for some mRNA applications, such as protein replacement therapies and genome editing (Hou et al., 2021). To minimize the immunogenicity of mRNA, two approaches are commonly used. Chemical modifications of specific IVT mRNA nucleotides, such as pseudouridine (ψ) and N1-methylpseudouridine (m1 ψ), can reduce innate immune sensing of exogenous mRNA translation (Hou et al., 2021; Kariko et al., 2005). Chromatographic purification can remove double-stranded RNA, an analogue of viral genome, in IVT mRNA preparations, diminishing immune activation and increasing translational efficiency (Hou et al., 2021; Kariko et al., 2011). The IVT mRNA molecules used in the mRNA-1273 and BNT162b2 COVID-19 vaccines were prepared by replacing uridine with m1 ψ , and their sequences were optimized to encode a stabilized pre-fusion spike protein (Hou et al., 2021; Baden et al., 2021; Polack et al., 2020).

7.2 Long-Term Safety Concerns

Beyond acute immunogenicity, the long-term safety of LNP-based therapeutics requires careful evaluation, particularly for applications requiring repeated administration or for chronic diseases. Cytotoxicity of lipid materials is a significant safety concern, depending on the dose, lipid properties, and cell types (Hou et al., 2021; Lv et al., 2006). In vivo application of lipid nanoparticles has been reported to induce liver and lung injuries in rodents, which may be attributed to the cytotoxicity of the materials and the induction of pro-inflammatory factors (Hou et al., 2021; Kedmi et al., 2010; Li et al., 1999). Accumulation of LNPs in healthy tissues can lead to cytotoxicity and genotoxicity, potentially due to the cationic lipid

components utilized in LNP formulations (Mehta et al., 2023).

The biodegradability of lipid components is critical for minimizing long-term toxicity. To improve the biocompatibility of lipid nanoparticles, biodegradable lipids can be applied (Hou et al., 2021; Maier et al., 2013; Zhang et al., 2020). Incorporating biodegradable lipids improves the tolerability of lipid nanoparticles by allowing fast metabolism while retaining mRNA delivery efficacy (Hou et al., 2021). The biodegradability of lipids can be increased by introducing ester motifs; for example, introducing ester bonds in the linker and lipidic tails of MC3 results in the lipid L319, which shows better delivery efficacy and faster elimination from the liver and plasma in vivo in comparison with MC3 (Hou et al., 2021; Maier et al., 2013). SM-102 and ALC-0315, the ionizable delivery components in the mRNA-1273 and BNT162b2 COVID-19 vaccines, incorporate biodegradable ester bonds to enhance safety profiles (Hou et al., 2021; Sabnis et al., 2018; Hassett et al., 2019). Biodegradable lipids can also be made of both ester and disulfide motifs, where cleavage of the disulfide bonds drives intraparticle nucleophilic attack on the ester linker, accelerating degradation (Hou et al., 2021; Tanaka et al., 2020; Liu et al., 2019).

Long-term storage stability is another factor impacting safety, as degradation products from unstable formulations could contribute to toxicity. Most LNP formulations have a relatively short shelf life of less than one year, and existing LNP-formulated drugs often necessitate ultracold storage temperatures (Mehta et al., 2023; Schoenmaker et al., 2021). During the COVID-19 pandemic, mRNA-LNP vaccines like Comirnaty (BNT162b2) and Spikevax (mRNA-1273) required storage at ultracold temperatures (-80°C and -20°C , respectively) to ensure their safety and efficacy as per regulatory guidelines (Mehta et al., 2023). The distribution and storage of these vaccines require advanced packaging technologies and specialized containers capable of withstanding ultralow temperatures and minimizing breakage during transportation (Mehta et al., 2023; Ramakanth et al., 2021). Lyophilization with cryoprotectants such as sucrose or trehalose offers a potential solution for improving long-term stability, but this process must be carefully optimized to maintain particle integrity and delivery efficacy (Zhao et al., 2020).

8. Conclusion

Lipid-based nanoparticles have emerged as a transformative and clinically validated platform for drug and gene delivery, fundamentally altering the therapeutic landscape for a wide range of diseases. From the first description of liposomes in 1965 to the recent global deployment of LNP-mRNA vaccines against COVID-19, the field has witnessed remarkable progress driven by decades of interdisciplinary research in lipid chemistry, materials science, nanotechnology, and molecular biology. The clinical success of products like Onpattro, the first FDA-approved siRNA drug, and the mRNA vaccines from Pfizer/BioNTech and Moderna has unequivocally validated LNP technology as a safe and effective platform for nucleic acid delivery, paving the way for a new class of genetic medicines.

The remarkable versatility of LNPs stems from their rational design and composition. By carefully selecting and optimizing ionizable lipids, helper lipids, cholesterol, and PEGylated lipids, researchers can engineer nanoparticles with tailored properties for specific therapeutic applications. Ionizable lipids have revolutionized nucleic acid delivery by enabling efficient encapsulation, protecting cargo from degradation, facilitating endosomal escape, and minimizing toxicity through pH-dependent charge conversion. Helper lipids like DOPE and DSPC play critical roles in stabilizing particle structure and promoting membrane fusion, while cholesterol modulates membrane fluidity and enhances particle stability. PEGylated lipids provide steric stabilization, prolonging circulation time and reducing immune recognition, though their immunogenicity remains an important consideration for repeated dosing.

The ability of LNPs to overcome biological barriers is central to their therapeutic efficacy. These nanoparticles protect labile nucleic acid cargo from nuclease degradation in physiological fluids, evade clearance by the mononuclear phagocyte system, and facilitate cellular uptake through endocytic pathways. The critical step of endosomal escape, enabled by the protonation of ionizable lipids in the acidic endosomal environment, allows mRNA and other nucleic acids to reach the cytoplasm where they can exert their therapeutic function. Targeting strategies, both passive and active, further enhance the precision of LNP-based therapies, enabling selective accumulation in tumors via the

EPR effect or specific binding to overexpressed receptors through ligand-mediated targeting.

The therapeutic applications of LNPs have expanded far beyond initial expectations. mRNA-based therapeutics are now being investigated for protein replacement therapy, cancer immunotherapy, and gene editing, in addition to their well-established role in vaccination. siRNA and antisense oligonucleotide delivery via LNPs offers a powerful means to silence disease-causing genes, with multiple clinical programs underway. The ability to deliver CRISPR-Cas components for in vivo genome editing represents one of the most exciting frontiers, with early clinical trials already demonstrating the potential for durable correction of genetic disorders.

Despite these remarkable advances, significant challenges remain. The long-term stability of LNP formulations, particularly the need for ultra-cold storage of mRNA vaccines, poses logistical and economic hurdles for global distribution. Immunogenicity and toxicity of LNP components, including PEG and cationic lipids, require ongoing monitoring and mitigation strategies, especially for chronic dosing regimens. Scalable manufacturing with consistent quality attributes remains a critical requirement for widespread clinical translation, necessitating robust process control and Quality by Design approaches. The regulatory landscape for nanomedicines continues to evolve, requiring harmonized guidelines and clear pathways for approval.

Looking forward, the future of LNP technology is exceptionally bright. Emerging trends in precision targeting, including selective organ targeting and ligand-mediated delivery, promise to expand the range of tissues accessible to LNP-based therapies. The integration of artificial intelligence and machine learning into nanoparticle design will accelerate the discovery of novel lipids and optimize formulations with unprecedented efficiency. Next-generation gene editing delivery systems, enabled by LNPs, hold the potential to cure genetic diseases at their source. Hybrid and bio-inspired systems, combining synthetic LNPs with cell-derived membranes or exosome-mimetic designs, offer enhanced biocompatibility and targeting capabilities.

In conclusion, lipid-based nanoparticles stand as a cornerstone of modern nanomedicine, offering a powerful and adaptable platform for the delivery of

diverse therapeutic payloads. The convergence of advances in lipid chemistry, formulation science, and biotechnology is driving the development of increasingly sophisticated LNP systems with enhanced efficacy, safety, and targeting precision. As these technologies continue to mature and new applications emerge, LNPs are poised to revolutionize the treatment of cancer, infectious diseases, genetic disorders, and beyond, ushering in a new era of precision medicine that will benefit patients worldwide. The journey from the first liposomes to the global deployment of mRNA vaccines is a testament to the power of interdisciplinary collaboration and rational design, and the future holds even greater promise for this remarkable class of nanomedicines.

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