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Application of Lipid Nanoparticles (LNPs) in Nucleic Acid Delivery and Precision Medicine

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Abstract. Lipid nanoparticles (LNPs), as a benchmark platform for non-viral vectors, have become a core technology for the delivery of nucleic acid drugs (mRNA, siRNA) and gene editing tools, owing to their excellent biocompatibility, structural designability, and broad payload compatibility. Their development spans over six decades, progressing from early liposomes to innovations in ionizable lipids and further to multifunctional targeted LNPs, gradually establishing a comprehensive technological system encompassing structural design, preparation techniques, functional regulation, and clinical translation. This article systematically reviews the component design, structural engineering, preparation technologies, clinical applications, and future development directions of LNPs, aiming to provide theoretical support and practical guidance for vector development in the era of precision medicine.

Keywords: Lipid nanoparticles, Nucleic acid delivery, Precision medicine, Clinical application

1. Introduction

Nucleic acid drugs (e.g., mRNA, siRNA, gene editing components) have demonstrated enormous therapeutic potential in fields including tumor immunotherapy, prevention and control of infectious diseases, and treatment of rare diseases. Their clinical use, however, has been hindered by the efficiency and safety of delivery systems for a long time [1]. Lipid nanoparticles (LNPs), owing to their high nucleic acid encapsulation capacity, favorable biocompatibility, and tunable in vivo fate, have become the "gold standard" for nucleic acid delivery [2]. Specifically, the commercialization of COVID-19 mRNA vaccines (e.g., Comirnaty and Spikevax) worldwide, through emergency use authorization (EUA) [3, 4], represented the leap of LNP technology from the lab to large-scale industrial manufacturing and population-wide use. This not only validated their safety and efficacy but also greatly accelerated the iteration and innovation of lipid nanocarrier technology. However, LNPs still encounter many challenges: low efficiency of endosomal escape (<3%) [5], immunogenicity issues (especially the PEG-related accelerated blood clearance phenomenon [6]), targeting mostly limited to the liver [7, 8], and issues of quality control in large-scale production of LNPs [9]. LNPs are currently undergoing an unprecedented transformation from empirical to rational formulation, from single function to multifunctional synergy, in recent years, thanks to the cross-integration of lipid chemistry [10] with materials science, microfluidics, and artificial

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intelligence [11, 12]. This article summarizes the latest advances in this field from four aspects: LNP component design and structural basis, new progress in preparation technology, clinical applications, as well as challenges and opportunities in the future, and analyzes its prospects for transforming precision medicine.

2. Design principles and structural optimization of LNPs

Lipid nanoparticles (LNPs) are made up of four categories of lipids that often act in synergy: ionizable lipids, helper phospholipids, cholesterol, and PEGylated lipids. These four components can self-assemble into nanoparticles with a core-shell structure in which the nucleic acid molecules are entrapped inside, protected from nuclease degradation, and then delivered with the help of a precise ratio targeted to the cells and released inside the cells [13].

The most critical functional constituent of LNPs is the ionizable lipids, which influence the ability to efficiently encapsulate the nucleic acid, the ability to escape the endosomes, and the tissue targeting [14, 15]. These lipids are usually made up of one amine-containing hydrophilic head and multiple hydrophobic tails. One of the important properties is the acid dissociation constant of the amine group (pKa). The pKa can be controlled in the range of 6.0 to 6.5, which makes the LNP surface neutral or weakly positive at physiological pH (7.4), and consequently decreases non-specific interactions with serum proteins and increases circulation time. Once endosomes/lysosomes (pH 5.0-6.5), the pH triggers the protonation of the ionizable lipids, and this causes them to have a positive charge. This results in electrostatic interactions with anionic lipids in the endosomal membrane, facilitating membrane fusion and disruption, thus releasing nucleic acids into the cytosol by the "proton sponge effect" or membrane pore formation mechanisms. It is believed that the classical proton sponge effect involves buffering of protons in the endosome by the presence of ionizable lipids to create a massive influx of water and chloride ions into the endosome, causing the endosome to swell and rupture and releasing its contents. More recently, however, a series of single-molecule studies indicate that the lipid-induced fusion of membranes in the endosomal pathway might be more efficient, with LNPs fusing directly with the endosomal membrane and nucleic acids injected into the cytosol. But at the heart of it, it's structural optimization of ionizable lipids that is key to improving delivery efficiency, regardless of the mechanism. In the case of clinically approved products, examples include the clinically approved product MC3 lipid in Patisiran, ALC-0315 in Comirnaty, and SM-102 in Spikevax, which are all different generations of ionizable lipid design strategies [16]. MC3 contains biodegradable linoleic acid chains, while in ALC-0315 and SM-102, the ester bond is hydrolyzable to promote faster clearance in vivo, improve the tail length and unsaturation of the membrane fluidity to optimize the transfection activity.

Helper phospholipids are typically saturated or monounsaturated phospholipids, such as distearoylphosphatidylcholine (DSPC) and dioleoylphosphatidylethanolamine (DOPE). DSPC's high phase transition temperature ensures that LNP membranes are rigid and cargo does not leak and that particles remain intact during storage and circulation. On the other hand, DOPE, as a result of its conical geometry, has a tendency to form hexagonal phases, which favor membrane fusion and therefore endosomal escape. In practice, for siRNA delivery where long-term stability is required, DSPC is more commonly used, while for mRNA delivery, where efficient release is desired, DOPE may have advantages. Recently, responsive helper phospholipids have been synthesized which can be degraded by reactive oxygen species or enzymes to allow controlled intracellular release.

Cholesterol is an essential part of LNPs. It is not only used to fill the gaps in the lipid membrane and to influence the fluidity and rigidity of the membrane but also plays a role in LNP interactions with the cell membrane [17]. The cholesterol molecules in the lipid bilayer make the membrane

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more compact, inhibit the adsorption of serum proteins, and inhibit the separation of lipid phases. Furthermore, cholesterol contains a hydroxyl group that can hydrogen-bond with nucleic acids and enhances the encapsulation efficiency. In vivo performance of LNPs has been demonstrated to be highly sensitive to minor changes in the structures of cholesterol derivatives (e.g., double bond position, side chain modifications). For instance, using β-sitosterol instead of cholesterol in mRNA vaccines can increase their immunogenicity, which is thought to be due to its role in modulating complement activation.

PEGylated lipids (PEG-lipids) are lipids with a hydrophilic PEG chain attached to a hydrophobic lipid by a linker. They are used primarily to provide steric hindrance on the surface of the LNP, to prevent opsonization by serum proteins and quick clearance by the reticuloendothelial system (RES), and thus prolong the half-life in circulation. The commonly used PEG-lipids are DMG-mPEG$_{2000}$, DSG-mPEG$_{2000}$, and DPG-mPEG$_{2000}$. This dissociation rate of PEG-lipids from the particle depends on the length of the lipid anchors: shorter anchors dissociate faster. Obviously, this is an important feature since PEG-lipids may give a stealth effect at first, but if the PEG dissociates too quickly, particles will not be protected before reaching the target; if PEG-lipids do not dissociate fast enough, particles will not be taken up by cells or escape from endosomes. Therefore, it is important to consider keeping the PEG-lipid stable in circulation while at the same time providing intracellular release. Moreover, PEG immunogenicity (the accelerated blood clearance of PEG, the ABC phenomenon) is a potential problem for clinical translation [18, 19] and the development of degradable PEG-lipids or non-PEG alternatives (e.g., polyglycerol, polyamino acids, polysaccharides) to avoid immune responses while maintaining delivery efficiency.

Apart from the four-component formulation, fine regulation of the internal structure of LNP represents a new challenge to be addressed to overcome the endosomal escape obstacle. According to the traditional model, LNPs are in a simple core-shell structure. Recent synchrotron small-angle X-ray scattering (SAXS) studies, however, have shown that in LNPs, several liquid crystalline phases can be present, such as lamellar, hexagonal, and bicontinuous cubic phases. The geometry and ratios of the different lipids in the formulation are important, as ionizable lipids (with CPP > 1) are more likely to form non-lamellar structures, while helper phospholipids and cholesterol are more likely to form lamellar structures. The protonation of ionizable lipids, upon entering acidic endosomes, causes a change in lipid molecular geometry and a lamellar to hexagonal phase transition. The topological defects and mismatch created during this phase transition strongly lower the energy barrier to LMP fusion with the endosomal membrane, allowing efficient cytosolic release. Other investigations have revealed an exponential increase in transfection efficiency with increasing percentage of hexagonal phase in LNPs, indicating that tuning the phase behaviour can yield optimised delivery performance. Based on these findings, lipid structures have been designed that form non-lamellar phases, or small-molecule adjuvants (e.g., vitamin E, glyceryl monooleate) have been added to induce the desired phase state. Furthermore, the new concept of acid-sensitive reversible crosslinking technology provides an alternative strategy to improve LNP stability: the use of pH-sensitive chemical bonds (e.g., hydrazone, acetal bonds) between the lipid molecules can keep LNPs crosslinked during storage or circulation, thus preventing leakage of cargo, and crosslinks can be broken upon arrival at acidic endosomes, rapidly dissociating to release nucleic acids [20]. This approach will substantially enhance freeze-drying tolerance and long-term storage stability, while ensuring efficient delivery.

The development of new lipids further stretches the limits of LNPs. Ionizable lipids with cyclic head groups, like AMG1541, help to increase the precision of pKa by providing a rigid cyclic structure and increasing the affinity to nucleic acids, thereby improving protein expression levels

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compared to SM-102 following intramuscular injection. In addition to promoting mRNA delivery, Vitamin E-derived lipid VN_2C_1 can also improve vaccine efficacy by tuning local inflammatory responses due to its antioxidant and immunomodulatory properties, which are conferred by the Vitamin E component. Another interesting development is the push towards minimalist formulations: a group at the Chinese Academy of Sciences and Peking University reported a two-part formulation that contained only peptide-based ionizable lipids and cholesterol. Spleen-specific delivery was obtained by varying the hydrophobic tail length and the headgroup charge density of the peptide lipids, which did not accumulate in the liver and offered a new tool for spleen-specific immunotherapy. These formulations are minimalist in nature, which makes manufacturing processes easier, and leaves behind relatively few potential toxicity concerns while offering promising applications.

3. Preparation technologies and process innovations

The preparation method of LNPs directly impacts the particle size, polydispersity, encapsulation efficiency, and batch-to-batch consistency, which is the basis for in vitro and in vivo performance. The traditional liposome preparation methods used for laboratory-scale screening, including thin-film hydration and sonication/extrusion, are not effective for precise control of the size distribution and the encapsulation efficiency and are difficult to scale up for production. Microfluidics is the current mainstream platform as LNPs head towards clinical and commercial applications [21, 22]. The lipids in an ethanol phase and the nucleic acids in an aqueous phase are mixed at controlled flow rates on the microfluidic chips using precisely designed microchannels. Lipid molecules self-assemble into nanoparticles, encapsulating nucleic acids, by instantaneous dilution of ethanol, through the use of hydrodynamic focusing or staggered herringbone structures to promote rapid mixing. The benefits of the microfluidic technology are: (1) mixing times in the millisecond range, resulting in homogeneous particle formation; (2) adjustable flow rate ratio and overall flow rate, leading to precise adjustment of particle size (20-200nm) and polydispersity (PDI<0.1); (3) simple continuous production and scale-up, conforming to GMP requirements. At present, the microfluidic-based LNP production platforms are widely used in the industrial production of mRNA vaccines.

Other preparation methods for the lipid-polymer hybrid nanoparticles (LPHNPs) are nanoprecipitation and emulsion-solvent evaporation. LPHNPs are composed of a polymer core (such as PLGA, PCL) and a lipid shell. The polymer core allows for sustained or responsive release, and the lipid shell enhances biocompatibility and cellular uptake. Usually, the polymer and lipids are dissolved in an organic solvent, and an anti-solvent is added to precipitate the polymer, or the solution is emulsified into an oil-in-water emulsion and the solvent is removed in preparation [23]. LPHNPs have recently been demonstrated to possess some unique advantages about multi-drug synergistic delivery and gene-drug combination therapy, including co-encapsulation of chemotherapeutic drugs and siRNA to combat tumor multidrug resistance.

To achieve efficient delivery, preparation parameters need to be optimized. The molar ratio of the components of the lipids used to make LNPs is important for the LNP structure and performance, with typically 30–50% ionizable lipids, 20–40% cholesterol, 10–20% helper phospholipids, and 1–5% PEG-lipids. The ratio of nucleic acid to lipid needs to be optimized, as either a low N/P ratio (molar ratio of protonatable nitrogen in ionizable lipids to phosphate groups in nucleic acids) can result in poor encapsulation, or a high N/P ratio can be toxic to cells, with the recommended range for mRNA and siRNA generally being 5–10 [24]. The size of the particles should be adjusted according to their intended application: liver targeting is generally done using particle sizes of 100–150nm, exploiting the fenestrations in the liver sinusoids, while sizes of 50–100nm may be useful

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for targeting tumors, where the EPR effect is present, and even smaller (20–50nm) sizes may facilitate targeting for pulmonary delivery, where the alveolar mucus barrier is encountered. In addition, the buffer pH, ionic strength, and temperature are also parameters that influence the formation of particles and their stability and must be optimized for the particular formulation.

Since the advent of high-throughput screening technologies has significantly sped up the formulation development of LNPs. The traditional approach that uses sequential synthesis and testing is inefficient. Using DNA/RNA barcoding technologies (e.g., SORT, FIND), hundreds of LNP formulations can be evaluated in a single experiment. Organ distribution and delivery efficiency of each LNP formulation can be quantitatively resolved by encapsulating different sequences of oligonucleotides as barcodes and recovering and sequencing after injection from different tissues [25]. This technology promises to be a great opportunity for the discovery of novel organ-targeting LNPs. At the same time, artificial intelligence (AI) and machine learning are making more and more inroads into many areas of LNP research and development. AI can also enhance the R&D cycle by over 50% by training models to learn the relationship between known lipid structures and their corresponding properties, and then recommend optimal combinations for formulation, which can predict important parameters of new lipids including pKa, toxicity, and transfection efficiency. For instance, graph neural networks can analyse chemical graphs of lipids and accurately predict their delivery efficiency in certain cell lines for experimental validation [10, 26]. AI can even be used in combination with automated synthesis platforms to create a closed-loop optimization of "design-synthesis-test-feedback", which will advance LNP research and development to a new paradigm of data-driven.

4. Progress in clinical applications

LNPs have come a long way in the clinical translation from local to systemic delivery, from rare disease to common disease, and from single-gene to multigene delivery. The first siRNA-LNP drug, Patisiran (Onpattro), was approved in 2018 to treat hereditary transthyretin-mediated amyloidosis and was the first RNAi therapeutic drug into clinical trials in 2009, representing a major step forward in RNAi therapeutics and LNP technology [27]. Patisiran's success proved the feasibility of systemically administered LNPs for delivering siRNA to the liver and silencing target genes. More recently, Inclisiran (Leqvio), with the same backbone, was also approved in 2021 for the reduction of low-density lipoprotein cholesterol, which can be administered twice a year, and illustrates the long-acting effects that can be achieved with LNPs in chronic disease treatment [28].

The COVID-19 emergency use authorization of vaccines based on mRNA-LNPs has been a major factor driving the explosive growth of mRNA-LNPs. In both Comirnaty (BioNTech/Pfizer) and Spikevax (Moderna), the LNPs are designed to deliver mRNA that codes for the entire Spike protein and show efficacy >90% in clinical trials, with both having been administered billions of doses globally, fully proving the safety and scalability of LNP technology. Use of mRNA-LNPs is fast growing in other infectious diseases (such as influenza, Zika, HIV), cancer immunology, and protein replacement therapy. For instance, personalized cancer vaccines are based on the delivery of mRNA that codes for neoantigens that are specific to an individual patient using LNPs, to induce adaptive immune responses, and have proceeded to Phase II clinical trials in combination with immune checkpoint inhibitors. LNPs have also been applied to co-deliver Cas9 mRNA and single-guide RNA (sgRNA) to successfully correct genetic mutations in animal models in the field of gene editing. The 2024 launch of a lung-targeted CRISPR-mRNA-LNP clinical trial to treat CF marks a pivotal moment in advancing the use of LNPs beyond the liver.

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Apart from being the vehicles to deliver nucleic acids, LNPs are also used in cancer therapy to co-deliver chemotherapeutic drugs, immunomodulators, and photothermal agents. LNPs can be passively or actively targeted to achieve concentration of drugs in tumour tissues and simultaneous minimisation of systemic toxicity. For instance, paclitaxel or doxorubicin as a cargo of folate receptor-targeted LNPs has demonstrated improved antitumor efficacy in ovarian cancer models. Lipid-polymer hybrid nanoparticles (LPHNPs) are next-generation nanoparticles that can incorporate both the advantages of sustained release of polymers and the high uptake efficiency of lipids; for example, doxorubicin and BCL-2 siRNA were co-delivered to overcome apoptosis resistance [29].

Breakthroughs in organ-specific delivery have broadened the therapeutic scenarios for LNPs [30]. Researchers have been able to modify lipid structures or surface modifications to target organs like the liver, spleen, lung, and placenta with LNPs. The organ-targeting properties of LNPs can be modified, for example, by including positively charged molecules (permanent cationic lipids); e.g., addition of cationic lipids like DOTAP will switch organ-targeting of LNPs from liver to lung; addition of anionic lipids will switch organ-targeting to spleen. Lung-targeting LNPs have been used to deliver the mRNA encoding the components of CRISPR to the lung epithelial cells, and were able to repair CFTR mutations by using this strategy. Placenta-targeting LNPs may provide opportunities to develop therapies for gestational diseases, e.g., delivering mRNA encoding growth factors to treat growth restriction in fetuses.

5. Challenges and future perspectives

Despite LNPs' success in many therapeutic areas, there remain systemic problems with the development of LPNs. The first problem is the efficiency of endosomal escape: it is estimated that only about 2% of internalized LNPs manage to get their nucleic acids into the cytosol where they can exert a therapeutic effect, with the majority of LNPs being degraded by lysosomes, and this severely limits therapeutic efficacy and increases dose-related toxicity [31]. Future studies should involve a deeper understanding of the molecular mechanisms of endosomal escape, the design of novel lipids able to induce the endosomal membrane to fuse or to disrupt it, or the incorporation of photosensitive or sonosensitive components within LNPs for endosomal escape to be physically-assisted. The second is that of immunogenicity: PEG-lipids are immunogenic, and anti-PEG antibodies produced in response can result in more rapid clearance of LNP, diminished efficacy, and even hypersensitivity reactions. To solve this, one can try to develop degradable PEG-lipids, to replace PEG with low-immunogenicity hydrophilic polymers, such as polysarcosine or polyzwitterions [32], or to use immune tolerance mechanisms to induce tolerance to PEG. Third is the delivery efficiency to other organs (e.g., brain, deep tumor tissues), which is still difficult to achieve with most LNPs. The blood-brain barrier (BBB) is the biggest challenge in the treatment of central nervous system (CNS) diseases. Future studies could involve LNPs towards transferrin or insulin receptors, or the use of ultrasound to transiently open the BBB to aid delivery. Fourth, large-scale manufacturing and storage stability: the cold chain for LNPs adds to global distribution expenses. While lyophilized formulations have potential to solve this problem, lyophilization may affect the structure and activity of LNP; thus, lyoprotectants and mild lyophilization processes need to be developed. Fifth is the extensive study of clinical translation: long-term safety of LNPs in gene therapy, immune memory formation following repeated administrations, and possible reproductive toxicity need to be studied in long-term follow-up and multicenter studies.

Future directions of LNP development will involve (1) Smart responsive LNPs: Incorporating chemical bonds or domains sensitive to the microenvironment conditions, such as pH, enzyme,

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redox potential, or light, can make LNPs sense the microenvironment change and trigger the release or activation for on-demand delivery and spatiotemporally controlled therapy. (2) Multifunctional integrated lipid design: Lipid molecules can be designed with therapeutic functions such as immunomodulation and photothermal conversion, which allows LNPs to be multifunctional, simplifies formulations, and enhances synergistic effects. (3) AI in end-to-end R&D: From designing lipids to screening formulations for optimal performance, to predicting in vivo behavior and optimizing drug doses, AI will be present throughout the entire R&D process, significantly improving success rates and reducing the costs of trial and error. (4) Search for new administration methods: Apart from classic intravenous and intramuscular injections, new methods are being actively sought, such as administration through inhalation, via the oral route, or through transdermal application. Inhaled LNPs can be used to treat pulmonary diseases (e.g., asthma, cystic fibrosis), and oral LNPs must withstand the harsh environment of the gastrointestinal system. (5) Cell-subtype specific targeting: Screening of novel targeting ligands such as nanobodies, aptamers, and carbohydrates can allow for the targeting of specific subpopulations of immune cells (e.g., dendritic cells, regulatory T cells) and stem cells for cell immunotherapy and regenerative medicine purposes [33].

6. Conclusion

Lipid nanoparticles have emerged as a fundamental technology for the delivery of nucleic acids, with tremendous impact and potential in basic research, technological translation, and clinical applications. LNP technology is rapidly progressing and developing, from the precise tuning of classical four-component formulations, to internal phase engineering and smart responsive design, from microfluidic scalable manufacturing to AI-driven high-throughput screening. While there are still obstacles like endosomal escape, immunogenicity, and targeting, with the deep involvement of fundamental sciences, materials engineering, and clinical medicine, these obstacles are likely to be overcome in the near future. LNPs have the potential to enable more breakthrough therapies for human health, and it is foreseeable that they will have an even more transformative impact in the future on gene therapy, precision immunomodulation, personalized medicine, and more.

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