Development Of Lipid Nanoparticles and Application in Drug Delivery Systems

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Abstract. The most clinically developed non-viral gene delivery method available today is lipid nanoparticles (LNPs), which can efficiently encapsulate a variety of medications, including proteins, nucleic acids (like mRNA and siRNA), and small molecules. LNPs greatly enhance drug stability, targeting and bioavailability, and through modification of the specific ligands on the surface, enable precise delivery to specific cells or tissues with reduced uptake by off-target cells and reduced side effects. By modifying specific ligands on their surfaces, they can achieve precise delivery to specific cells or tissues, reduce uptake in non-target cells and minimize side effects. The composition of LNPs is described in this paper along with their usage in cancer treatment, where gene editing is made possible through the CRISPR/Cas9 technology and LNPs can be combined with immunotherapeutic and chemotherapeutic medicines for multimodal therapy. Applications of LNPs in the treatment of neurological illnesses and in nucleic acid drug delivery systems are also covered. LNPs can be used to deliver siRNAs and mRNAs and protect them from degradation for targeted delivery. LNPs can also be employed for brain-targeted medication delivery by altering ligands such as lactoferrin, transferrin and others.

Keywords: Lipid nanoparticles; Cancer therapy; Drug delivery system; Mrna; Neurological diseases.

1. Introduction

The research and application of LNPs has experienced a rapid development process from basic exploration to clinical application (See Figure 1). Bangham et al.'s 1965 creation of liposomes marked the beginning of major developments in LNP technology for drug delivery. The use of liposomes as encapsulated enzymes in 1971 paved the way for their application in drug delivery. Subsequently, the discovery of immunoliposomes and the development of liposome-targeted studies further expanded their application potential [1]. In 1990, a breakthrough in extending the circulatory period of LNPs in vivo was proposed with the proposal of stealth liposomes. When the U.S. FDA authorized Doxil, the first liposomal drug, in 1995 to treat severe fungal infections, it set a precedent for the use of liposomal medications in clinical therapy. After years of deep cultivation in academia and industry, Onpattro® was approved by the US FDA in 2018, officially marking a milestone breakthrough in LNP technology. In 2020, two well-known COVID-19 mRNA vaccines were authorized by regulatory authorities in many countries. This marks the mature application of LNP technology in nucleic acid drug delivery and promotes its wide application in the field of life sciences.

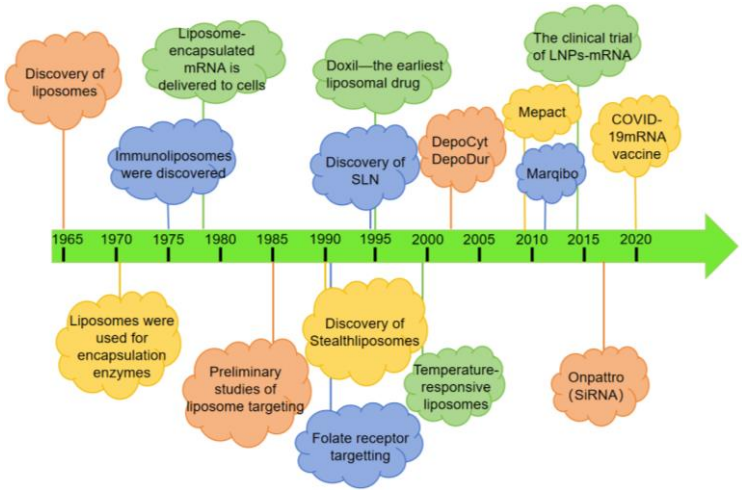

Figure 1. The development and history of LNPs [1]

Compared to alternative drug delivery methods, LNPs have a number of advantages. First off, LNPs work well for encapsulating a variety of medications, like as proteins, nucleic acids (such mRNA and siRNA), and tiny compounds. This significantly improves the stability and bioavailability of the drug. Second, the surface modification capability of LNPs allows them to achieve precise targeting. By conjugating specific ligands (e.g., antibodies, peptides, or aptamers) to the surface, LNPs can specifically bind to cancer cells or specific tissues, reducing uptake by non-target cells and reducing side effects. In addition, the controlled-release function and endosomal escape mechanism of LNP further boost the accumulation and efficacy of drugs at the lesion site. LNPs can be protonated in acidic endosomal surroundings, which promotes the diffusion of drugs from endosomes into the cytoplasm and increases the intracellular concentration of drugs. This mechanism is particularly important for nucleic acid drugs, which need to enter the cytoplasm to work. In summary, LNPs have the advantages of versatility, precise targeting, endosomal escape mechanism, low immunogenicity, and high biocompatibility in drug delivery systems, making them occupy an important position in modern drug delivery technologies [1]. This paper highlights the composition of LNPs and their applications in cancer therapy, nucleic acid drug delivery and vaccine development and the difficulties and challenges faced by LNPs in drug delivery system applications.

2. The Composition of LNPs

Ionized lipids, phospholipids, cholesterol, and pegylated lipids are the four main constituents of LNPs (See Figure 2).

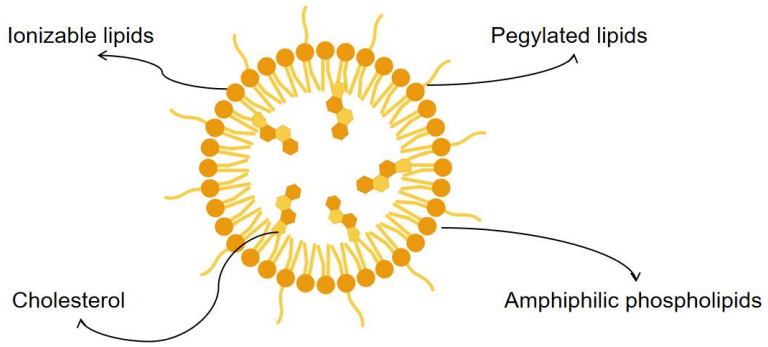

Figure 2. The composition of LNPs [2]

Ionized lipids are key components of LNPs, and they offer unique advantages in drug delivery systems. Traditionally, permanently charged cationic lipids may interact with negative charges on cell membranes due to their persistent positive charge. This causes cell membranes to be destroyed, causing cytotoxicity. However, ionizable lipids are not charged at physiological pH. This enhances biocompatibility, lowers the chance of aggregation and harmful side effects, and lessens interactions

with blood's negatively charged serum proteins [2]. During endocytosis, LNPs enter endosomes. Due to the low local pH of endosomes, the amine groups in the LNPs undergo protonation [3]. LNPs become positively charged, which not only helps LNPs escape from endosomes but also facilitates drug release.

Amphiphilic phospholipids are a crucial part of LNPs and have hydrophilic head groups and hydrophobic tails. In addition to affecting how LNPs work, the makeup and characteristics of phospholipids also have an impact on how long a medicine works and how quickly the body eliminates LNPs. This significantly affects LNPs and how they are used in drug delivery systems.

Cholesterol, which accounts for 20%-50% of total lipids, is the main component of the shell of LNP preparations [2]. By changing the fluidity of the bilayer, cholesterol helps regulate the stiffness and integrity of the LNP membrane and enhance stability. It can also prevent aggregation due to electrostatic effects and spatial repulsion, maintaining the dispersion of LNPs. In addition, cholesterol prevents permeability from decreasing with increasing temperature [2]. Studies have found that cholesterol can also alter the order of phospholipids within the bilayer, which affects the fluidity of the bilayer membrane, which in turn regulates membrane-protein interactions. Cholesterol not only improves the stability and membrane fusion ability of LNPs but also enhances the delivery efficacy of LNPs by optimizing their structure or incorporating cholesterol derivatives, giving them special functions.

Another significant component of LNPs is polyethylene glycol. PEG reduces the metabolic inactivation and degradation of the kidney and MPS by forming an external polymer layer on the surface of LNPs, thereby hindering the adsorption of serum proteins and mononuclear phagocytic cell systems [4]. PEGylation also allows LNP surfaces to be conjugated to ligands or biomacromolecules for precise drug delivery.

3. The Application of LNPs in Drug Delivery

3.1. Cancer Treatment

3.1.1 CRISPR/Cas9 system

The potential of CRISPR/Cas9 technology is to eradicate cancer genes entirely, get beyond the need for repeated dosages in traditional cancer treatments, and enhance therapeutic effectiveness. LNPs have been used as a delivery system to deliver CRISPR/Cas9 systems to treat tumors in preclinical studies.

The system can alter the genetic makeup of creatures by specifically altering genes in cells and organisms through the use of gene editing technologies. The Cas9 protein is responsible for DNA double-strand cleavage. The Cas9 protein is guided to target particular DNA sequences by the guiding function of guide RNA (gRNA). When the Cas9 protein and sgRNA are produced intracellularly, the Cas9 protein attaches itself to the sgRNA to target and disrupt the desired DNA sequence. The intracellular DNA damage repair pathway is triggered following DNA cleavage, predominantly by the use of non-homologous end joining (NHEJ) to fix the damaged DNA strand [5]. During the repair process, several bases may be inserted or deleted from the end of the DNA, resulting in a mutation of the gene and loss of function, resulting in gene editing.

LNPs are prepared by using novel ionizable cationic lipids to encapsulate Cas9 mRNA and sgRNA targeting PLK1[6]. It has been found that in experiments with a single intracerebral injection of CRISPR-LNP (0.05 mg/kg) into aggressive glioblastoma in situ, CRISPR-LNP is distributed throughout tumor tissue and shows efficacy and potent anti-tumor effects in PLK1 gene editing [5].

Then, LNPs form siFAK + CRISPR PD-L1-LNPs by encapsulating siRNAs targeting focal adhesion kinases (FAKs) and Cas9 mRNAs and sgRNAs targeting PD-L1 which is used for tumor delivery and to enhance gene editing [6]. Inhibition of FAK activity has been found to reduce the contractility and membrane tension of tumor cells while reducing the stiffness of the extracellular matrix (ECM). By significantly increasing CRISPR gene editing in tumor cells in both in vitro and

in vivo experiments, this LNP significantly reduced the levels of PD-L1 and enhanced immune cell penetration. Furthermore, in MYC-driven liver cancer models, ovarian cancer xenograft models, and ovarian cancer mice metastatic models, the LNP markedly suppressed tumor development and metastasis [5].

However, the development of CRISPR/Cas9-LNPs also currently faces some problems. The first is the encapsulation of ribonucleoproteins (RNPs). Cas9 RNPs with anionic properties are composed of Cas9 protein and sgRNA and can be encapsulated into LNPs. However, LNPs prepared using a buffer at pH 5.2 have a larger particle size (approximately 300 nm) and only demonstrate the editing effect of fluorescent protein genes in vitro [5]. Then the most important issue is stability. RNP has a large molecular weight (about 200 kDa) and is susceptible to denaturation due to pH changes, so the ratio of LNP components needs to be modified during development.

3.1.2 Targeted delivery

The creation of excess vascular permeability factors and the development of aberrant blood arteries within tumor tissue cause the enhanced permeability and retention (EPR) effect. The ability of the liposomal drug delivery system (DDS) to selectively target cancer cells and enrich tumor regions while reducing toxicity to normal tissues and side effects is mostly due to the absence of lymphatic drainage [7]. However, there are limitations to the EPR effect, which exhibits significant heterogeneity among different tumor types and individual patients [8]. This has led to the poor targeting effect of many nanomedicines based on EPR effects in practical applications. As a nanomaterial, liposomal DDS has basic properties such as a high surface-to-volume ratio, enhanced biocompatibility, and better bio-barrier penetration. However, liposomes themselves lack advantages such as electrical conductivity, superparamagnetism, or optical properties.

To overcome the limitations of the EPR effect, actively targeting liposomal DDS has been extensively studied. Conjugating certain ligands to the liposome surface that can bind to cancer cells or receptors in the tumor microenvironment (TME) is how active targeting is accomplished. Fibroblasts, immunological cells, cancer-associated endothelial cells, and extracellular matrix make up the TME. These elements communicate with cancer cells, promote tumor invasion and metastasis, and suppress immune responses that fight cancer. Surface modifications of liposomal DDS include molecules like proteins, aptamers, peptides, and antibodies that selectively bind to enzymes that promote tumor growth [7]. It is notable that although the EPR effect is the foundation of the active targeting technique, through these modified molecules, the internalization process of liposomes within tumor cells can be further enhanced, thereby improving the targeting and therapeutic effect of the drug.

3.1.3 Combination therapy

LNP can also be used in combination with chemotherapy drugs, photothermal therapy drugs or immunotherapy drugs to achieve multimodal therapy.

(1) The use of LNPs in the field of chemotherapy

LNPs can keep medications from breaking down and increase how long they stay in the body. LNP reduces side effects by accumulating at the tumor site through regulated release. For example, chitosan-coated nano lipid carriers (NLCs) significantly enhance skin permeability, cellular uptake, and cytotoxicity of breast cancer cells when tetrahydrocurcumin (THC) is delivered [9]. In addition, NLCs co-loaded with doxorubicin (DOX), docosahexaenoic acid (DHA), and α-tocopheryl succinate (TS) demonstrated the effect of inhibiting tumor growth and preventing lung metastasis in the therapy of carcinoma of the breast, while attenuating the cardiotoxicity and hepatotoxicity of DOX. As the first liposomal platinum preparation to enter clinical trials, aroplatin (L-NDDP) exerts cytotoxic effects by cross-linking with DNA, inhibiting the synthesis of DNA from tumor cells, exhibiting lower toxicity and higher bioavailability. In contrast, doxorubicin hydrochloride liposomal injection (Doxil) encapsulates doxorubicin through liposomal technology, mitigating toxic effects on healthy tissues such as the heart [9].

(2) Application of LNPs in gene therapy

As an efficient delivery vehicle, LNPs can protect siRNA and mRNA from enzymatic degradation and achieve selective targeting through surface modification. For example, amino acid-modified LNPs have excelled in protecting siRNAs from serum degradation and significantly inhibiting the expression of the IKBKE gene in prostate and pancreatic cancers [9]. In addition, iLP181 LNPs have made progress in delivering the CRISPR-Cas9 system, enabling long-term and efficient vivo gene editing [10]. LNP-based gene therapies have entered clinical trials, such as Patisiran (Onpattro®), the world's first siRNA-LNP drug.

(3) Application of LNP in photothermal therapy and photodynamic therapy

As a carrier of photosensitizers, LNPs enhance cell permeability and targeted delivery of photosensitizing molecules, improving the therapeutic effect. For example, gold-BODIPY coordination liposome nanocomposites (LABs) effectively solve the challenges faced by traditional photothermal agents. LAB significantly increased the level of intracellular reactive oxygen species and induced apoptosis under laser irradiation. In addition, cetuximab-conjugated chitosan-based iron oxide nanoparticles (Cet-CINPs) combine the advantages of chemotherapy and photothermal therapy [9]. Targeted killing of colorectal cancer cells was achieved through surface modification of anti-epidermal growth factor receptor (EGFR) antibodies.

(4) Application of LNPs in Immunotherapy

LNPs enable precise targeted delivery to tumor cells by encapsulating tumor antigens or immunostimulants, improving the bioavailability of drugs and improving treatment outcomes. For example, heterocyclic LNPs are capable of efficient delivery of oncology vaccines and STAT3 siRNAs. By activating the STING pathway, it induces the production of type I interferons, which in turn promotes dendritic cell maturation and antigen presentation and attenuates the immunosuppressive tumor microenvironment [11]. In addition, the U-101-LNP/IL-2F mRNA formulation releases IL-2F at the tumor site via MMP-14-mediated cleavage, significantly enhancing antitumor activity while reducing systemic toxicity [12]. Together, these findings highlight the enormous potential of LNPs in cancer treatment, especially in terms of improving drug delivery efficiency, reducing side effects, and enhancing treatment outcomes.

3.2. Nucleic Acid Drug Delivery

3.2.1 LNPs for siRNA delivery

siRNA is a 21-nucleotide, a double-stranded RNA with one of the guide strands capable of binding to the RISC. This induces RISC degradation or inhibits translation, resulting in gene silencing. siRNA therapeutics have great potential in cancer treatment. However, exposed siRNAs are easily degraded by nucleases in vivo, and there are problems such as renal clearance and reticuloendothelial system clearance, resulting in poor siRNA stability and low delivery efficiency [5].

Through tissue-targeted design, LNPs provide the effective distribution of siRNAs to target cells while shielding them from destruction. As a promising siRNA delivery vector, LNPs can enhance the stability and targeted delivery ability of siRNAs.

In tumor therapy, siRNA-LNPs play a role primarily by specifically inhibiting oncogene expression. Preclinical research focuses on areas such as liver cancer, pancreatic cancer, and prostate cancer. For example, siRNA-LNPs targeting centromere-associated protein 2 (KNTC2) can significantly inhibit hepatocellular carcinoma cell growth [5]. siRNA-LNPs targeting the KRAS gene delay pancreatic cancer progression. siARfl-LNPs targeting androgen receptor splice variants significantly inhibit prostate cancer growth. In addition, siRNA-LNPs can also promote anti-tumor immune responses by regulating the polarization of tumor-associated macrophages.

At present, siRNA-LNP-based clinical trials are mainly focused on solid tumors. For instance, LNP-encapsulated siRNAs that target vascular endothelial growth factor (VEGF) and KIF11 were used to treat solid liver tumors in the Phase I clinical trial. In phase I/II clinical studies for liver cancer,

TKM-080301 has demonstrated effective tumor suppression; nevertheless, overall survival has not improved [13]. By encapsulating siRNAs that target the MYC gene with LNPs, DCR-MYC was used to treat a number of tumors. However, because of disappointing trial results, the product was abandoned. Nevertheless, more research is needed to determine how siRNA-LNP can be used to treat hematological malignancies.

3.2.2 LNPs for mRNA delivery

The effective and safe distribution of mRNA is one of the primary challenges facing the development of mRNA treatment. Although naked mRNA can be spontaneously taken up by many cell types, most of the mRNA accumulates in lysosomes, and only a few are able to enter the cytoplasm. In addition, naked mRNA is susceptible to degradation by extracellular ribonucleases (RNases), so a suitable delivery strategy is needed to help mRNA enter cells.

Traditional cationic lipids, such as DOTMA or DOTAP, bind to mRNA through electrostatic action to form a delivery system that is widely used in early clinical research. However, the cytotoxicity and shorter blood circulation time of these cationic lipids limit their clinical application. To address these issues, researchers have developed various novel ionizable lipids that help mRNA escape from acidic endosomes, improving delivery efficiency. For example, the development of the lipids SM-102 and ALC-0315 has contributed to the success of COVID-19 mRNA vaccines. LNP is currently the most deeply researched and clinically advanced mRNA delivery vector. In the 90s of the 20th centuries, it was found that LNPs could effectively transport mRNA to the liver and produce proteins. In 2015, LNP was first used as an mRNA vector method. LNPs elicit an immune response by transfecting antigen-presenting cells, and when mRNA is translated into proteins in the cytoplasm, it can activate cytotoxic T cells and helper T cells, thereby stimulating the immune system.

The target organ or cell and the injection route have a significant impact on mRNA's effectiveness. Targeted mRNA distribution to non-hepatic tissues is essential to expanding the use of mRNA therapeutics because the majority of LNPs preferentially gather in the liver following intravenous administration. A platform for selective organ-targeting (SORT) nanoparticles has been created by researchers. By introducing particular SORT molecules, it permits the targeted distribution of mRNA in the liver, spleen, or lungs of mice [14]. Additionally, the creation of innovative LNP formulations or the application of cell-specific ligands can be used to distribute mRNA in a cell type-specific manner. For use in cancer immunotherapy, imidazole-based LNPs, for instance, can target the delivery of mRNA to T cells. Notably, the study found that therapeutic mRNA can be targeted and delivered to specific inflammatory leukocytes or tumor cells based on the ASSET platform. For example, therapeutic mRNA can be targeted and delivered to Ly6c+ inflammatory leukocytes in mice for the treatment of inflammatory bowel disease by conjugating an anti-Ly6c-targeting ligand to LNPs [15]. In order to deliver Cas9 mRNA and sgRNA to EGFR-expressing ovarian tumors for targeted gene editing, tumor growth inhibition, and improved survival, the ASSET platform can conjugate ligands targeting EGFR to LNPs.

3.2.3 LNPs for the development of mRNA vaccines

LNPs are mainly used in vaccine development to encapsulate mRNA or protein antigens to improve their stability and immunogenicity. LNPs form stable nanocomplexes by encapsulating mRNA, protecting them from nuclease degradation and prolonging their cycle time in vivo. LNPs are also able to facilitate the entry of mRNA into the cell and release the mRNA into the cytoplasm through endosomal escape mechanisms. This allows the mRNA to be translated into proteins by ribosomes, activating the immune response. The translated protein acts as an antigen that activates the immune system through multiple pathways.

LNPs can encapsulate not only mRNA but also protein antigens. This makes the protein antigen more stable in the body and prevents degradation by proteases. The encapsulated protein antigen can be more effectively taken up and processed by immune cells, enhancing its immunogenicity, thereby activating a stronger immune response and improving the protective effect of vaccines.

Modification of specific ligands (e.g., antibodies, peptides, or aptamers) on the surface of LNPs enables targeted delivery of specific immune cells. This targeting not only improves the immune efficacy of the vaccine but also reduces the uptake of non-target cells, thereby reducing side effects. Targeted delivery of mRNA or protein antigens to dendritic cells can more effectively activate the immune system, which in turn enhances the protective effect of vaccines.

Additionally, by encapsulating the mRNA encoding the influenza viral antigen, researchers are creating LNP-based influenza mRNA vaccines to increase the vaccine's durability and immunogenicity. In the field of cancer vaccines, LNP-encapsulated mRNAs can encode tumor-specific antigens for use in cancer immunotherapy [5]. When these antigens are produced in cells, they can trigger T cells, which are part of the immune system, to identify and combat cancer cells. In addition, researchers are developing LNP-based influenza mRNA vaccines to improve vaccine stability and immunogenicity by encapsulating the mRNA of influenza virus antigens. In the field of cancer vaccines, LNP-encapsulated mRNAs can encode tumor-specific antigens for use in cancer immunotherapy. These antigens, when expressed intracellularly, are able to activate the immune system, specifically T cells, to recognize and attack cancer cells.

3.3. Drug Delivery for Neurological Disorders

3.3.1 The introduction of Blood-brain barrier (BBB)

The extremely selective BBB separates the brain's blood arteries from its tissue. The BBB shields the brain's nerve tissue from dangerous toxins. BBB is mainly composed of five structures: cerebral vascular endothelial cells, pericytes, astrocytes, basement membrane and tight junctions. Cerebral vascular endothelial cells form the inner layer of cerebral capillaries and are the main components of BBB. Cerebrovascular endothelial cells are tightly connected to form a continuous endothelial layer, which restricts the passage of macromolecules and water-soluble substances [16]. Pericytes are encased around endothelial astrocytes and interact with endothelial cells to regulate vascular stability and permeability. The terminal foot of astrocytes wraps around the capillaries and regulates the barrier function of endothelial cells by secreting signaling molecules and intercellular connections. Endothelial cells and pericytes are structurally supported by the basement membrane, an extracellular matrix made of proteins and polysaccharides. Tight junctions exist between endothelial cells and prevent most substances from entering the brain through the intercellular space.

3.3.2 Targeted ligand modifications

Via receptor-mediated intracellular transport, transferrin (Tf) binds to the Tf receptor on the BBB and crosses it, increasing the drug's concentration in the brain. It was discovered that Tf-modified liposomes greatly enhanced the drug's brain targeting, raising its level in the brain while also enhancing its stability and bioavailability [17]. For instance, Tf-modified liposomes are utilized to deliver the neuroprotective short peptide Pep63, which can alleviate cognitive deficits in AD and lessen the Aβ burden in the hippocampus. In addition, Tf-modified liposomes are also used to deliver ostholetin, which enhances the intracerebral delivery of drugs and improves drug stability and bioavailability.

A positively charged glycoprotein, lactoferrin (Lf) has a strong affinity for brain cells, particularly BBB endothelial cells. Lf-modified liposomes enhance drug delivery in the BBB by binding to the lactoferrin receptor (LfR). Lf-modified liposomes have been found to exhibit higher targeting under pathological conditions such as AD and Parkinson's disease [17]. This is because these diseases lead to increased LfR expression. For instance, nerve growth factor (NGF) is delivered via Lf-modified exosomes to enhance neuronal survival in rats and prevent cholinergic neurons from degenerating. Researchers have shown that cyclic D, L-α peptide (CP-2)-modified liposomes can specifically target Aβ oligomers with high BBB permeability, which can prevent Aβ aggregation and lessen Aβ-mediated toxic effects, in addition to the previously described targeted changes [18].

4. Conclusion

This article details the composition, characterization and wide application of lipid nanoparticles (LNPs) in drug delivery systems. LNP has shown outstanding performance in cancer treatment, nucleic acid drug delivery, and neurological disease treatment. In cancer treatment, LNP is used to deliver CRISPR/Cas9 systems for gene editing to improve drug targeting and therapeutic efficacy. It is also used in combination with chemotherapy and immunotherapy drugs to achieve multimodal therapy. In nucleic acid drug delivery, LNPs are used to deliver siRNA and mRNA for targeted delivery. In the treatment of neurological disorders, LNPs achieve brain targeted drug delivery by modifying Tf, Lf, and other ligands. LNPs increase the concentration and bioavailability of drugs in the brain, providing a new strategy for treating diseases such as Alzheimer's disease. However, LNPs also face some challenges in drug delivery systems. More clinical data are needed to support immune response and long-term safety. The preparation process of LNP is complex, and the quality control is difficult, which increases the production cost. LNPs are less efficient for delivery to non-liver tissues and need to be further optimized for efficient delivery to other tissues. The drug release mechanism and endosomal escape efficiency of LNP encapsulation are insufficient, which affects the therapeutic effect. The storage and biostability of LNPs need to be addressed to ensure the performance of LNPs in long-term storage. In terms of clinical translation, LNPs face dual pressures of trial design and regulatory requirements, increasing the difficulty of moving from laboratory to clinic. However, with the further optimization of LNP composition and structure, as well as the development of new targeted ligands, it is anticipated that LNPs will become more important in precision and personalized medicine. To increase the targeting and delivery effectiveness of LNPs, researchers are investigating novel lipid composition and surface modification techniques. In addition, the development of combination therapy strategies will further expand the application scope of LNPs and provide efficient and safe drug delivery solutions for more disease areas. As technology continues to advance, LNPs are expected to overcome current challenges and bring better treatment outcomes and quality of life to patients.

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