ISSN 2959-409X

Applications of Lipid Nanoparticles in CRISPR Technology

Tianhao Zhang[1, *]

[1]School of Life Sciences, Jilin University, Jilin, China
*Corresponding author: zhangth1322@jlu.edu.cn

Abstract:

Clustered, regularly interspaced short palindromic repeat (CRISPR) genome editing is one of the most popular gene editing techniques with its simpleness, convenience, and efficiency. Nowadays, CRISPR-Cas9 technology has been used in agriculture, medicine, biology, and many other fields for screening target genes, creating modal animals, and gene therapy. However, there are still obstacles before clinical applications of CRISPR-Cas9, and a safe and effective delivery system is required for transportation. Studies have shown that lipid-nanoparticle-based delivery, using lipid nanoparticle (LNP) as the carrier, is a good method of transportation. LNP is a vesica-like globule composed of a lipid shell adorned with signal proteins and goods inside it. LNP improved the stability and immunogenicity of the CRISPR-Cas9 system and has the merits of easy production and high modifiability, making it an ideal carrier with high potential in the future. This review introduces four basic components of LNP: localizable cationic lipids, polyethylene glycol (PEG) lipids, zwitterionic phospholipids, and cholesterol. This review focuses on the applications of LNP, including lipid-encapsulated gold nanoparticles, biocompatible monosized lipid-coated stellate mesoporous silica nanoparticles (LC-MSNs), biodegradable lipid and messenger RNA Nanoparticles, Mulberry leaf lipid nanoparticles, phenylboronic acid-derived lipid nanoparticles, lipid-polymer hybrid nanoparticles combined with ultrasound-mediated microbubble destruction, cationic lipid-assisted PEG-b-PLGA nanoparticles, multi-valent N-Acetylgalactosamine-Lipid nanoparticles, etc. Further research in LNP and CRISPR systems is required to optimize the delivery properties for clinical applications.

Keywords: CRISPR, lipid nanoparticles, carrier, gene editing.

1. Introduction

CRISPR genome editing is an important technology for modern research and application, and it has a profound effect on medicine, biology, agriculture, etc. As a genome editing technology, it can be used to delete specific segments of DNA or RNA. Scientists have used this function to establish animal models and genes, gene editing, and gene knockout [1]. What's more, by combining some editing enzymes with Cas9 protein in the gene editing system, this technology can be used in technologies of changing or fixing a specific nucleobase effectively in the genome without cutting off the DNA chain. Moreover, this nucleobase editing technology can be applied to fixing and researching gene mutations, gene therapy, and decoding gene functions. It has been regarded as an efficient and accurate gene editing tool [2].

However, safe and effective delivery systems are required for future clinical applications. A traditional way to transport the drug into the body is using some kind of virus carrier, but it may cause a lethal immune response. In addition, the durability of the transporter building by virus is also problematic. Viruses may recover their ability to copy by genetic recombination after slathering for a long period. Thus, it can also cause immune response and mutation. In order to achieve therapies based on CRISPR in the human body, a safe and effective way is required to transport the drug carrier into vivo. The research found that lipid-nanoparticle-based delivery is a very good delivery vector. A lipid nanoparticle (LNP) is a nano lipid globule that carries therapeutic protein, nucleic acid, and other therapeutic drugs [3]. The property is like a vesica and can be modified by some signal molecule so that it can be transported to a specific area *in vivo* and assimilated by target cells by endocytosis [4]. It has the advantages of being small in size, easy to produce, stable in a blood environment, highly biocompatibility, modifiable, controllable, and strong function [4]. Nowadays, since the development of the LNP technology, its immunogenicity and toxicity have been reduced distinctly, and its stability as well as sustainability have been enhanced [3]. All these advantages make LNP one of the most successful and reliable transport platforms at present and can be used in cancers and other scenarios [5]. Currently, these methods

include solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLCs), liposomes, and niosomes [4]. SLN is a type of spherical particle which packs drugs inside.

The lipids that constitute SLN are positively charged so that the nucleic acid inside them can be electrostatically attracted, and the endocytosis between the particle and cells can be promoted [5]. NLCs are second-generation lipid carriers that are structured by both solid and liquid lipids in order to overcome the problem of burst release in SLN [6]. What's more, the crystalline structure of NLCs is not as perfect as SLN. Thus, it has a larger drug-loading capacity while avoiding lipid crystallization [6]. Liposomes are structured by phospholipid bilayer [3]. The drugs are put inside the lipid bilayer. Niosomes are structured by cholesterol bilayer and nonionized surface activators.

This review summarizes the characters and functions of four primary lipid components and eight types of LNP.

2. LNP Composition

LNP composition typically includes four basic lipid components: localizable cationic lipids, polyethylene glycol (PEG) lipids, zwitterionic phospholipids, and cholesterol (Figure 1).

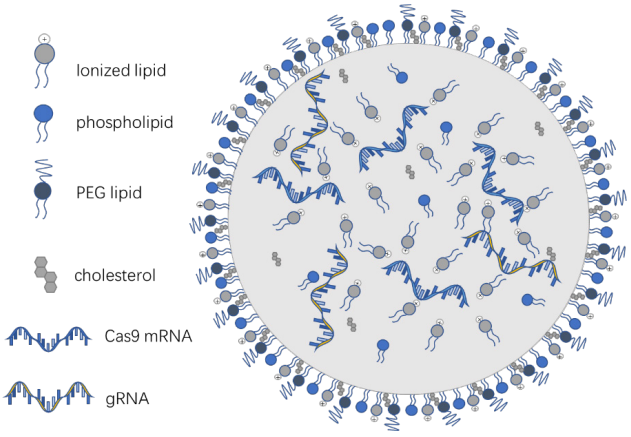

Fig. 1 Structure of lipid nanoparticle (LNP). Figure credit: original.

2.1 Localizable Cationic Lipids

Localizable cationic lipids, compared to permanently charged lipids, have different charges in different environmental pH and specific lipid equilibrium constants. Nucleic acid inside LNP can combine with the hydrophilic part of localizable cationic lipids, and the hydrophobic part is exposed to the outside so that it can form the glycol shape [7]. What's more, the ability to change the charge according to the environment of localizable cationic lipids facilitates protecting the drug inside against immune cells and lysosomal enzymes *in vivo*. It assists more drugs to arrive at the target area. By modifying the structure of the lipid, the effect and safety of LNP can be enhanced (Table 1) [7].

2.2 PEG Lipids

PEG lipids refer to lipids that combine PEG in their cephalic group. PEG molecule has functions of keeping LNP size and forming a water molecular barrier for avoiding clusters of particles and reducing immunogenicity (Figure 1). Therefore, PEG lipids can lengthen the retention time of LNP and keep the concentration *in vivo* so that it can promote the effectiveness of LNP (Table 1) [8].

2.3 Zwitterionic Phospholipids

Zwitterionic phospholipids are also called helper lipids. They exist in the strata extremum of the lipid, and they are used to promote the stability and efficiency of LNP by changing its size and charge during production (Table 1) [9].

2.4 Cholesterol

Cholesterol is another substance used to promote the stability of LNP by filling the gap between the phospholipids (Figure 1). Moreover, cholesterol can also promote the endocytosis by strengthen the activity of localizable cationic lipids (Table 1) [7].

Table 1. Four LNP compositions comparing

Composition	Feature of structure	Function	References
Localizable Cationic lipids	Charged in different environments	Protect the drug to the target area	[7]
PEG lipids	Lipids combined with PEG	Keeping LNP size protect the efficient	[8]
Zwitterionic phospholipids	Has a variable amount of charge	To adjust LNP size for its stability	[9]
Cholesterol	Filling the void between phospholipids	Promoting the stability and endocytosis	[7]

3. Applications

3.1 Lipid-Encapsulated Gold Nanoparticles

Encapsulating gold nanoparticles with lipids is a strategy for promoting the transport effectiveness of the CRISPR-Cas9 system. In this type of LNP, the Cas9-sgPlk-1 plasmid (CP) is combined with gold nanoparticles modified by TAT peptide and form AuNPs/CP(ACP), and coats lipids forming the lipid-encapsulated AuNPs-condensed CP(LACP). While LACP is irradiated by laser light, AuNPs are released into the environment and enter into tumor cells. The TAT peptide can lead CP to enter the cell nucleus and play a role. AuNPs are designed as a heat source so that during laser lighting, they would have a photothermal effect and release the goods [10]. LACP can be used to cure melanoma by knocking down the Plk-1 gene and restraining tumor cells‘ growth and proliferation.

3.2 Biocompatible Monosized Lipid-Coated Stellate Mesoporous Silica Nanoparticles (LC-MASNs)

LC-MSNs is a kind of MSN that loads Cas9-gRNA compound and expression carrier. It has a stable stellate shape and a bigger superficial area so that it can be degraded rapidly into silicic acid. This function makes LC-MSNs have low immunogenicity and are biocompatible [11]. Silica inside of the LNP can transport the drug into tumor cells more effectively. However, there are some drawbacks, such as low express efficiency and limited by specific issue areas [11].

3.3 Biodegradable Lipids and Messenger RNA Nanoparticles

Biodegradable lipid and messenger RNA nanoparticles are a new kind of LNP using BAMEA-O16B as the encapsulated lipid, which encapsulates both mRNA and sgRNA inside the LNP with Cas9 protein [12]. mRNA in the LNP can avoid off-target effects and complement DNA plasmids to reduce mutagenesis risk, transient effects, and complexity. However, the stability of the mRNA plasmid is lacking compared to the DNA plasmid. Thus, BAMEA-O16B is used to solve this problem. BAMEA-O16B is a kind of lipid particle that is produced by a synthesis of amines and acrylates or acrylamide with disulfide bonds [13]. It can encapsulate mRNA by electrostatic interaction and release goods *in vivo* by disulfide bond exchange mechanisms responding to reducing chemical signals [12]. All these new technologies in this LNP give it an effective and rapid editing process both inside and outside the body and widen the therapeutic field of CRISPR-Cas9 technology.

3.4 Mulberry Leaf Lipid Nanoparticles

Mulberry leaf LNP is a kind of LNP using pluronic copolymer as the cover lipid. Pluronic can promote the stability of LNP in the digestive system environment and help LNP go through the intestinal wall so that taking CRISPR-Cas9 system drugs orally comes to realize [14]. In addition, the galactose terminal group on it can promote the endocytosis of LNP. Taking LNP orally can release immunoreaction caused by LNP and is beneficial for treating intestinal diseases and cancer [14].

3.5 Lipid Nanoparticles Derived from Phenylboric Acid

Cellular surface sialic acid (SA) is a molecule that exists on glycoprotein on the cell surface and has a higher content on the tumor cell surface [15]. PBA is a kind of molecule that can combine with SA so that it can be a recognition molecule to SA [15]. Thus, phenylboronic acid-derived lipid nanoparticles are designed for better recognition of the tumor cells and for promoting the transportation of mRNA into the cells. This type of LNP can be used to target tumor cells and contribute to future cancer gene treatment strategies.

3.6 Lipid-Polymer Hybrid Nanoparticles Technology Bound to Ultrasound-Mediated Microbubble Destruction

CRISPR interference (CRISPRi) is a technology of silencing gene expression using nuclease deficiency Cas9 (dCas9) to target the specific gene in the genome and shut it down instead of normal Cas9 used in CRISPR-Cas9 technology. Compared to the normal CRISPR-Cas9 technology, CRISPRi would not cause DNA damage, which showed a higher safety and specificity. However, the volume dCas9 with the addition of sgRNA in the same LNP will increase the LNP size. Thus, it hampers the transport process [16]. PH-responsive lipid-polymer hybrid nanoparticles (PLPNs) are a kind of LNP that has the structure of a nuclear membrane. In the core area of PLPNs is the pDNA combined with PBA-functionalized low molecular weight polyethyleneimine (PEI-PBA), which can target tumor cells accurately and release the goods into tumor cytoplasm [16]. The shell area has pH-responsive PEOz lipids, which protect PLPNs against immune cells. When PLPNs come into the tumor issue, their shell structure will be decomposed in an acid environment around the tumor cell. What’s more, ultrasound-mediated microbubble destruction can be used to enhance the efficiency of gene expression and transportation by enhancing DNA osmosis into tumor cells [17]. This LNP system has a lot of potential in cancer therapy.

3.7 Cationic lipid-assisted PEG-b-PLGA nanoparticles (CLAN)

CLAN are a type of LNPs that uses PEG-b-1-polylactide (mPEG-PLA) as the matrix, loading siRNA as the gene silence drug assisted by cationic lipid [18]. SiRNA is an interfering RNA that has excellent expression of the silent target gene. However, siRNA usually has a big size with anionic properties, making it hard to transport into cells. This structure of LNP can promote the efficiency of loading siRNA and make it stable *in vivo*. The cationic lipid used in the LNP is N, N-bis (2-hydroxyethyl)-N-methyl-N-(2-cholesteryloxycarbonyl aminoethyl) ammonium bromide, which can promote the packing efficiency of siRNA and avoid leakage during LNP transporting *in vivo* [18].

3.8 Multi-Valent N-Acetylgalactosamine (GalNAc)-Lipid Nanoparticles

Multi-valent GalNAc-lipid nanoparticles are a type of LNPs that adds GalNAc targeting ligand on its surface [19]. GalNAc targeting ligand can combine with the asialoglycoprotein receptor (ASGPR) and then rapidly trigger endocytosis of liver cells [20]. What's more, because of the independence of lipoprotein receptors, this kind of LNP can be used for patients that have less density of lipoprotein receptors on liver cells and Homozygous familial hypercholesterolemia [19].

4. Conclusion

There are four primary lipid compositions of LNP, including localizable cationic lipids, PEG lipids, zwitterionic phospholipids, and cholesterol. Currently, there are multiple applications of the LNP delivery system, including lipid-encapsulated gold nanoparticles, biocompatible monosized LC-MSNs, biodegradable lipid and messenger RNA Nanoparticles, Mulberry leaf lipid nanoparticles, lipid nanoparticles derived from phenylboronic acid, lipid-polymer hybrid nanoparticles technology bound to ultrasound-mediated microbubble destruction, CLAN, multi-valent GalNAc-lipid nanoparticles, etc. LNP application varies based on their structures and characteristics. These applications focused on the decoration of LNP by new types of lipid molecules, proteins, or metallics in order to promote its efficiency and reliability during transportation and the duration of therapy. Thus, LNP is a powerful and potential carrier of the CRISPR system and has bright prospects for development. However, the cost and method of mass production of LNP have not yet been mentioned. Future research may focus on combining the innovations and benefits of each application to produce more clinically appropriate LNP, as well as improving the production system for mass production and industrialization for large-scale clinical use. Moreover, since CRISPR systems have a highly off-targeted effect, further research is required to explore how to accurately target cells and genes in the delivery system and carry out effective therapy.

References

[1]Wang JY, Doudna JA. CRISPR technology: A decade of genome editing is only the beginning. Science, 2023, 379(6629): eadd8643.

[2]Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature, 2016, 533(7603): 420-424.

[3]Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nature Reviews Drug Discovery, 2021, 20(2): 101-124.

[4]Aziz A, Rehman U, Sheikh A, Abourehab MAS, Kesharwani P. Lipid-based nanocarrier mediated CRISPR/Cas9 delivery for cancer therapy. Journal of Biomaterials Science, Polymer Edition, 2023, 34(3): 398-418.

[5]Yonezawa S, Koide H, Asai T. Recent advances in siRNA delivery mediated by lipid-based nanoparticles. Advanced Drug Delivery Reviews, 2020, 154-155: 64-78.

[6]Ghasemiyeh P, Mohammadi-Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages, and disadvantages. Research in Pharmaceutical Sciences, 2018, 13(4): 288-303.

[7]Kazemian P, Yu SY, Thomson SB, Birkenshaw A, Leavitt BR, Ross CJD. Lipid-Nanoparticle-Based Delivery of CRISPR/Cas9 Genome-Editing Components. Molecular Pharmacology, 2022, 19(6): 1669-1686.

[8]Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Advanced Drug Delivery Reviews, 2016, 99(Pt A): 28-51.

[9]Cheng X, Lee RJ. The role of helper lipids in lipid nanoparticles (LNPs) designed for oligonucleotide delivery. Advanced Drug Delivery Reviews, 2016, 99(Pt A): 129-137.

[10]Wang P, Zhang L, Zheng W, Cong L, Guo Z, Xie Y, Wang L, Tang R, Feng Q, Hamada Y, Gonda K, Hu Z, Wu X, Jiang X. Thermo-triggered Release of CRISPR-Cas9 System by Lipid-Encapsulated Gold Nanoparticles for Tumor Therapy. Angewandte Chemie-international Edition, 2018, 57(6): 1491-1496.

[11]Noureddine A, Maestas-Olguin A, Saada EA, LaBauve AE, Agola JO, Baty KE, Howard T, Sabo JK, Espinoza CRS, Doudna JA, Schoeniger JS, Butler KS, Negrete OA, Brinker CJ, Serda RE. Engineering of monosized lipid-coated mesoporous silica

nanoparticles for CRISPR delivery. Acta biomaterialia, 2020, 114: 358-368.

[12]Liu J, Chang J, Jiang Y, Meng X, Sun T, Mao L, Xu Q, Wang M. Fast and Efficient CRISPR/Cas9 Genome Editing In Vivo Enabled by Bioreducible Lipid and Messenger RNA Nanoparticles. Advanced Materials, 2019, 31(33): e1902575.

[13]Wang M, Zuris JA, Meng F, Rees H, Sun S, Deng P, Han Y, Gao X, Pouli D, Wu Q, Georgakoudi I, Liu DR, Xu Q. Efficient delivery of genome-editing proteins using bioreducible lipid nanoparticles. Proceedings of the National Academy of Sciences of the United States of America, 2016, 113(11): 2868-2873.

[14]Ma L, Ma Y, Gao Q, Liu S, Zhu Z, Shi X, Dai F, Reis RL, Kundu SC, Cai K, Xiao B. Mulberry Leaf Lipid Nanoparticles: A Naturally Targeted CRISPR/Cas9 Oral Delivery Platform for Alleviation of Colon Diseases. Small, 2024: e2307247.

[15]Matsumoto A, Stephenson-Brown AJ, Khan T, Miyazawa T, Cabral H, Kataoka K, Miyahara Y. Heterocyclic boronic acids display sialic acid selective binding in a hypoxic tumor relevant acidic environment. Chemical Science, 2017, 8(9): 6165-6170.

[16]Li Y, Wu P, Zhu M, Liang M, Zhang L, Zong Y, Wan M. High-Performance Delivery of a CRISPR Interference System via Lipid-Polymer Hybrid Nanoparticles Combined with Ultrasound-Mediated Microbubble Destruction for Tumor-Specific Gene Repression. Advanced Healthcare Materials, 2023, 12(10): e2203082.

[17]Sirsi SR, Borden MA. Advances in ultrasound mediated gene therapy using microbubble contrast agents. Theranostics, 2012, 2(12): 1208-1222.

[18]Yang XZ, Dou S, Sun TM, Mao CQ, Wang HX, Wang J. Systemic delivery of siRNA with cationic lipid assisted PEG-PLA nanoparticles for cancer therapy. Journal of Controlled Release, 2011, 156(2): 203-211.

[19]Kasiewicz LN, Biswas S, Beach A, Ren H, Dutta C, Mazzola AM, Rohde E, Chadwick A, Cheng C, Garcia SP, Iyer S, Matsumoto Y, Khera AV, Musunuru K, Kathiresan S, Malyala P, Rajeev KG, Bellinger AM. GalNAc-Lipid nanoparticles enable non-LDLR dependent hepatic delivery of a CRISPR base editing therapy. Nature Communications, 2023, 14(1): 2776.

[20]Nair JK, Willoughby JL, Chan A, Charisse K, Alam MR, Wang Q, Hoekstra M, Kandasamy P, Kel'in AV, Milstein S, Taneja N, O'Shea J, Shaikh S, Zhang L, van der Sluis RJ, Jung ME, Akinc A, Hutabarat R, Kuchimanchi S, Fitzgerald K, Zimmermann T, van Berkel TJ, Maier MA, Rajeev KG, Manoharan M. Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. Journal of the American Chemical Society, 2014, 136(49): 16958-16961.