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mRNA Lipid Nanoparticle Delivery Systems: Composition, Optimization and Advances in Clinical Translation

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Abstract. Lipid nanoparticles (LNP) represent critical non-viral carriers for mRNA delivery, capable of encapsulating, protecting mRNA, facilitating cellular uptake and enabling cytosolic release. This review summarizes the composition and delivery mechanisms of mRNA-LNP. Optimization strategies including ionizable lipid structure, component ratio tuning, endosomal escape improvement and tissue-targeted modification are analysed in depth. Its applications in vaccines, tumour therapy, protein replacement and gene-editing are further outlined. Current bottlenecks contain limited endosomal-escape efficiency, insufficient extra-hepatic delivery, safety concerns for repeated administration, and consistency challenges in large-scale manufacturing. Future efforts should focus on lipid-structure and formulation optimization, alongside improved safety evaluation and quality-control frameworks to accelerate clinical translation.

Keywords: mRNA, lipid nanoparticles, delivery system

1. Introduction

Messenger RNA (mRNA) acts as a template for protein translation within the cytoplasm and exerts preventive or therapeutic functions by encoding specific antigens or functional proteins. It features flexible design, relatively short production cycles, and independence from nuclear entry. In the 1990s, Wolff et al. directly injected in-vitro transcribed mRNA into mouse skeletal muscle and detected exogenous protein expression, which firstly proved that exogenous mRNA could be efficiently translated in mammalian organisms and laid the foundation for mRNA-drug research [1]. Since then, advances in nucleoside modification, sequence optimization and delivery technologies have promoted mRNA research from fundamental studies to infectious-disease vaccines, tumour immunotherapy, protein-replacement therapy and gene-editing applications.

Nevertheless, bare mRNA suffers from poor stability, large molecular weight and negative charge, making direct transmembrane penetration difficult. Once administered in vivo, unformulated mRNA is readily degraded by ribonucleases and may activate innate immune responses via pattern-recognition receptors, compromising its stability, expression efficiency and biosafety. Even after cellular internalization, mRNA is frequently trapped within the endosome-lysosome pathway, and only a minor fraction escapes into the cytosol. Previous investigations have demonstrated that cytosolic release efficiency is substantially lower than cellular-uptake efficiency, indicating endosomal escape as a major rate-limiting step for nucleic-acid delivery [2]. Accordingly, delivery

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platforms that protect mRNA, promote cellular uptake and enhance cytosolic release are indispensable for in-vivo mRNA applications.

Lipid nanoparticles (LNP) are representative non-viral carriers for mRNA delivery. A typical LNP formulation consists of ionizable lipids, helper phospholipids, cholesterol and PEG-lipids. Synergistic effects among these components enable mRNA encapsulation, structural stabilization and in-vivo delivery. Ionizable lipids become protonated under acidic conditions to facilitate mRNA complexation and participate in endosomal-membrane interactions; helper phospholipids and cholesterol sustain particle architecture and membrane properties; PEG-lipids modulate colloidal stability and surface characteristics. LNP technology greatly improves mRNA stability and delivery performance and accelerates clinical translation. mRNA-LNP vaccines such as BNT162b2 can elicit neutralizing-antibody and antigen-specific T-cell immune responses, validating the clinical feasibility of this delivery platform [3].

Despite remarkable progress, the delivery performance and clinical utility of mRNA-LNP are constrained by multiple factors. The structure of ionizable lipids and component ratios govern particle size, encapsulation efficiency, colloidal stability and tissue distribution. Insufficient endosomal escape still restricts cytosolic mRNA release and subsequent protein production. Intravenous LNP administration tends to accumulate in the liver, which creates obstacles for precise delivery towards extra-hepatic organs. Moreover, long-term safety upon repeated dosing, inter-individual variability, batch consistency and quality control during large-scale production remain critical barriers hindering further clinical adoption of mRNA-LNP.

This review describes the composition and intracellular delivery mechanisms of mRNA-LNP, highlights optimization approaches covering ionizable-lipid engineering, formulation adjustment, endosomal-escape enhancement and tissue-targeting modification. Its applications in vaccines, tumour therapy, protein-replacement therapy and gene-editing are summarized. Challenges including long-term safety and scalable manufacturing are also discussed, aiming to provide references for rational design and further optimization of mRNA-LNP delivery platforms.

2. Composition and delivery mechanism of mRNA-lipid nanoparticles

mRNA serves as an intermediate carrier for genetic-information transfer and functions as cytoplasmic templates for protein synthesis. Naked mRNA is vulnerable to ribonuclease degradation, and its large size and negative charge impede membrane penetration. Therefore, efficient delivery is essential for in-vivo mRNA utilization. Lipid nanoparticles (LNP) can encapsulate and shield mRNA, enhance cellular uptake and mediate cytosolic release, and have become leading non-viral delivery vehicles for mRNA.

Conventional mRNA-LNP comprises four categories of lipids: ionizable lipids, helper phospholipids, cholesterol and PEG-conjugated lipids. Ionizable lipids are core components determining mRNA encapsulation and intracellular delivery performance. They are protonated under acidic environments and electrostatically interact with negatively-charged mRNA, while maintaining near-neutral charge under physiological pH to mitigate non-specific biological interactions. After entering acidified endosomes, re-protonation of ionizable lipids triggers interactions with endosomal-membrane anionic lipids. Helper phospholipids support particle assembly and membrane stability; cholesterol modulates lipid packing and membrane fluidity to strengthen nanoparticle integrity; surface-localized PEG-lipids suppress particle aggregation and tune particle size and in-vivo behaviours. The combined effects and relative proportions of these four lipid classes jointly determine physicochemical traits and delivery potency of LNP.

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mRNA-LNP is assembled via self-assembly driven by electrostatic attraction between ionizable lipids and mRNA under rapid-mixing conditions. In typical preparation workflows, ionizable lipids, helper phospholipids, cholesterol and PEG-lipids are dissolved in ethanol (organic phase), whereas mRNA is dissolved in weakly acidic aqueous solution. Rapid mixing of two phases protonates ionizable lipids that bind to phosphate backbones of mRNA, and lipids co-assemble into nanoparticles for mRNA encapsulation. Buffer exchange is subsequently performed to adjust pH toward physiological values, and ethanol and unincorporated materials are removed by dialysis or ultrafiltration to yield mature mRNA-LNP formulations [4].

After cellular entry, the delivery cascade of mRNA-LNP includes cellular uptake, endosomal trafficking, endosomal escape and cytosolic translation. LNPs are internalized via endocytosis and enclosed within endosomes. As endosomes acidify, ionizable lipids undergo protonation and interact with anionic lipids residing on endosomal membranes, perturbing local membrane structures and promoting mRNA liberation into cytosol. Successfully escaped mRNA binds to ribosomes to initiate target-protein translation, and lipid components gradually dissociate and get metabolized. Since only a portion of internalized mRNA achieves effective cytosolic release, endosomal-escape efficiency strongly governs overall delivery efficacy and protein expression for mRNA-LNP.

3. Progressive optimization logic for mRNA-LNP delivery systems

Optimization of mRNA-LNP performance primarily originates from structural engineering of ionizable lipids. Ionizable lipids exert profound influences on mRNA encapsulation, nanoparticle formation, endosomal escape and in-vivo safety. Modifications on head groups, linkers and hydrophobic tails alter biological behaviours of resultant LNPs. Maier et al. introduced degradable ester bonds into lipid hydrophobic tails and constructed a panel of biodegradable lipids. L319-derived LNPs maintained high nucleic-acid encapsulation capacity and exhibited potent gene-silencing activity in animal studies. This work demonstrated that lipid structural modification can improve metabolic profiles without sacrificing delivery performance [5].

Beyond lipid-structure engineering, component ratios represent another pivotal determinant for LNP characteristics. Weight or molar ratios among ionizable lipids, helper phospholipids, cholesterol and PEG-lipids influence particle dimension, polydispersity index, surface potential, encapsulation efficiency and colloidal stability, further shaping in-vivo biodistribution and cellular uptake. Improper formulation ratios may induce particle aggregation, mRNA leakage or compromised delivery potency. Hence formulation parameters should be tuned according to targeted quality attributes to generate stable, high-performance LNPs [6].

Intracellular delivery is further limited by inadequate endosomal escape. After endocytic uptake, most LNP-loaded mRNA remains trapped inside endosome-lysosome compartments; merely increasing cellular uptake cannot guarantee proportional elevation of protein expression. Upon acidification within endosomes, protonated ionizable lipids interact with anionic endosomal lipids and trigger membrane perturbation for cytosolic mRNA liberation. The pKa value and molecular architecture of lipids dominate this process. Boosting endosomal-escape efficiency is consequently a key target for improving mRNA-LNP functionality [7-9].

On the basis of enhanced intracellular delivery, tissue-targeting capability becomes the next optimization priority. Following intravenous infusion, many LNPs preferentially accumulate in the liver, restricting their applicability towards extra-hepatic sites such as lung and tumour tissue. Adjusting lipid compositions or decorating LNPs with targeting moieties including peptides and saccharides can reshape interactions between LNPs with cell-surface receptors and circulating biomolecules, improving biodistribution and augmenting mRNA expression at desired sites [10].

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Nevertheless, ligand-mediated targeting faces obstacles such as low conjugation efficiency, protein-corona formation and poor in-vivo stability. Efficient and specific delivery to extra-hepatic organs remains a major research challenge for mRNA-LNP development.

4. Application evaluation and clinical-translation challenges of mRNA-LNP

mRNA-LNP delivery platforms have achieved concrete clinical success in infectious-disease vaccine development. Clinical deployment of BNT162b2 and mRNA-1273 COVID-19 vaccines proves that LNPs can efficiently encapsulate and deliver mRNA to express target antigens and evoke protective immune responses, establishing clinical proof-of-concept for LNP-based mRNA technologies [11]. Building on these achievements, mRNA-LNP platforms are expanding to other infectious-disease vaccines, tumour therapy, protein-replacement therapy and gene-editing interventions. Investigations reveal that LNP-delivered tumour-antigen mRNA can provoke specific cellular immune responses. For gene-editing applications, LNPs are exploited for delivering Cas9 mRNA and other nucleic-acid components [12, 13]. Compared with vaccine scenarios, these therapeutic applications impose stricter requirements for tissue targeting, cytosolic-delivery efficiency and safety upon repeated dosing. Effective delivery to extra-hepatic tissues still constitutes a prominent bottleneck.

As mRNA-LNP applications shift from prophylactic vaccines toward long-term therapeutic interventions, safety risks and inter-subject variation attract growing attention. Current clinical experience is largely derived from vaccine use cases; therapies for chronic disorders often demand repeated administration, which raises concerns over lipid accumulation, persistent inflammation and anti-carrier immune reactions that require thorough risk assessment [14]. Furthermore, pre-existing anti-PEG antibodies and heterogeneous immune backgrounds among human populations may alter LNP clearance and drug exposure, leading to inter-individual divergence in therapeutic outcomes and adverse events [15]. Robust long-term safety-evaluation systems suitable for repeated dosing are prerequisites for expanding therapeutic indications of mRNA-LNP.

Scalable manufacturing and batch-to-batch consistency create additional hurdles for clinical translation. When transferring LNP production from laboratory-scale preparation to GMP-compliant manufacturing, variables such as mixing geometry, flow rate, raw-material quality and buffer conditions can modify particle size, polydispersity, mRNA encapsulation yield and formulation stability [16, 17]. For microfluidic-based production, process scaling-up cannot be realized by simply enlarging processing volume; consistent mixing efficiency and mass-transfer conditions must be preserved. Correlations between critical-process parameters and critical-quality attributes should be defined, and robust process-control strategies ought to be implemented.

Collectively, clinical translation of mRNA-LNP relies not only on novel lipid materials and innovative delivery strategies, but also on synchronized advances in long-term safety assessment, scalable-production workflows and quality-control standards. Pre-clinical evaluation protocols should be standardized to address toxicological differences originating from diverse lipid chemistries and formulations. Optimized raw-material management, mixing-process control and well-defined quality metrics are required to enhance manufacturing stability and batch consistency, supporting continuous development and clinical deployment of mRNA-LNP therapeutics.

5. Conclusion and outlook

In summary, lipid nanoparticles exploit synergistic effects among ionizable lipids, helper phospholipids, cholesterol and PEG-lipids to accomplish mRNA encapsulation, protection, cellular

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uptake and cytosolic release, serving as a leading technical platform for mRNA delivery. Optimizations covering ionizable-lipid design, formulation adjustment, endosomal-escape promotion and tissue-targeting modification have improved LNP delivery performance and broadened its scope from infectious-disease vaccines to tumour therapy, protein-replacement treatment and gene-editing.

Major obstacles persist, including suboptimal endosomal-escape efficiency, limited extra-hepatic delivery, safety risks upon repeated administration, patient-to-patient variability, and batch inconsistency in scaled-up production. Future research should further refine lipid structures and formulations to strengthen cytosolic release and tissue selectivity. Meanwhile, enhanced long-term safety evaluation, strict critical-process-parameter management and improved quality standards are needed to advance stability, controllability and clinical utility of mRNA-LNP delivery systems.

References

[1] Wolff, J. A., Malone, R. W., Williams, P., et al. (1990). Direct gene transfer into mouse muscle in vivo. *Science, 247*(4949), 1465-1468.

[2] Hamar, P., Wittrup, A. (2021). Live-cell imaging of siRNA cytosolic release from lipid nanoparticles reveals limited endosomal escape and autophagic clearance of damaged endosomes. *Molecular Therapy, 29*(4), 1412-1426.

[3] Walsh, E. E., Frenck, R. W., Falsey, A. R., et al. (2020). Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. *The New England Journal of Medicine, 383*(25), 2439-2450.

[4] Jayaraman, M., Ansell, S. M., Mui, B. L., et al. (2012). Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angewandte Chemie International Edition, 51*(34), 8529-8533.

[5] Maier, M. A., Jayaraman, M., Matsuda, S., et al. (2013). Biodegradable lipids enabling rapidly eliminated lipid nanoparticles for systemic delivery of RNAi therapeutics. *Molecular Therapy, 21*(8), 1570-1578.

[6] Kulkarni, J. A., Cullis, P. R., van der Meel, R. (2019). Lipid nanoparticles enabling gene-siRNA and mRNA delivery. *Advanced Drug Delivery Reviews, 144*, 146-161.

[7] Herrera, M., Kim, J., Eygeris, Y., et al. (2021). Illuminating endosomal escape of polymorphic lipid nanoparticles that boost mRNA delivery. *Biomaterials Science, 9*(7), 2488-2499.

[8] Chatterjee, S., Kon, E., Sharma, P., et al. (2024). Endosomal escape: A bottleneck for LNP-mediated therapeutics. *Proceedings of the National Academy of Sciences, 121*(11), e2307800120.

[9] Paramasivam, P., Franke, C., Stöter, M., et al. (2022). Endosomal escape of delivered mRNA from endosomal recycling tubules visualized at the nanoscale. *The Journal of Cell Biology, 221*(2), e202110137.

[10] Geisler, H. C., Battistini, E., Thatte, A. S., et al. (2025). Preparation of targeted lipid nanoparticles for precision nucleic acid delivery. *Nature Protocols, 20*(7), 2143-2178.

[11] Baden, L. R., El-Sahly, H. M., Essink, B., et al. (2021). Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *New England Journal of Medicine, 384*(5), 403-416.

[12] Liu, Y., Li, Y., Zhang, Y., et al. (2023). mRNA-LNP vaccination-based immunotherapy augments CD8 T cell responses against HPV-positive oropharyngeal cancer. *Molecular Therapy-Nucleic Acids, 34*, 211-224.

[13] Nakamura, S., Kuwabara, T., Fujii, Y., et al. (2021). Low immunogenicity of LNP allows repeated administrations of CRISPR-Cas9 mRNA into skeletal muscle in mice. *Nature Communications, 12*(1), 7181.

[14] Chaudhary, N., Weissman, D., & Whitehead, K. A. (2021). mRNA vaccines for infectious diseases: Principles, delivery and clinical translation. *Nature Reviews Drug Discovery, 20*(11), 817-838.

[15] Kozma, G. T., Shimizu, T., Ishida, T., et al. (2023). Role of anti-polyethylene glycol (PEG) antibodies in the allergic reactions to PEG-containing COVID-19 vaccines: Evidence for immunogenicity of PEG. *Frontiers in Immunology, 14*, 1210731.

[16] Kulkarni, J. A., Darjuan, M. M., Pecaric-Petkovic, J., et al. (2023). Lipid nanoparticle technology for nucleic acid therapeutics. *Nature Nanotechnology, 18*(4), 347-364.

[17] Heukers, R., van der Maaden, K., van der Pol, L., et al. (2023). Lipid-based nanoparticles for drug/gene delivery: An overview of the production techniques and difficulties encountered in their industrial development. *ACS Materials Au, 3*(5), 2023-2042.