REVIEW OPEN ACCESS

Lipid Nanoparticles for Nucleic Acid Delivery and Gene Therapy: A Comprehensive Review

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Abstract

Introduction: Conventional drug delivery systems face significant limitations for nucleic acid therapeutics, including poor bioavailability, instability, and inefficient cellular uptake. Lipid nanoparticles have emerged as a transformative platform for nucleic acid delivery and gene therapy. These systems provide essential protection from degradation while enabling efficient cellular internalization and endosomal escape.

Methods: A systematic analysis of 45 publications (2004-2025) from National Institutes of Health and PubMed databases was conducted. This analysis evaluated lipid nanoparticle technologies for nucleic acid delivery, focusing on fundamental design principles, optimization strategies, and targeting mechanisms for gene therapy applications.

Results: This review identifies critical advances in lipid nanoparticle technology across three domains: fundamental design principles, optimization strategies, and targeting approaches. Lipid nanoparticles utilize pH-dependent endosomal escape mechanisms through ionizable lipids with optimized pKa values (6.2-6.5 for liver targeting, 6.6-6.9 for vaccines). Branchedtail lipidoid architectures demonstrate tenfold improved potency over linear analogs through enhanced endosomal ionization. Microfluidic manufacturing achieves >90% encapsulation efficiency with polydispersity indices ≤0.25. Clinical validation includes COVID-19 mRNA vaccines achieving >94% efficacy. Gene editing applications like VERVE-101 demonstrate sustained genetic modifications.

Discussion: Despite significant technological advances, challenges persist in manufacturing scalability, targeting specificity, and methodological standardization across diverse ionizable lipid libraries. Safety considerations require careful evaluation of dose-dependent toxicity profiles, while regulatory frameworks must address manufacturing scalability and quality control for gene therapy applications.

Conclusion: Lipid nanoparticle technology has revolutionized nucleic acid delivery and gene therapy through innovations in ionizable lipid design, manufacturing optimization, and targeting strategies. Critical challenges remain in targeting efficiency and endosomal escape mechanisms. Future developments should prioritize enhanced targeting specificity, improved biocompatibility, and standardized evaluation protocols to advance precision genetic therapeutics.

Keywords: lipid nanoparticles; nucleic acid delivery; gene therapy; mRNA; siRNA; ionizable lipids

Introduction

Conventional Drug Delivery Systems and Their Limitations

Conventional drug delivery systems encompass traditional pharmaceutical formulations including oral tablets, capsules, syrups, and ointments. These drug delivery systems suffer from poor bioavailability, rapid systemic clearance, and fluctuating plasma drug levels that often fall outside the therapeutic window, either below effective concentrations or above toxic thresholds [1]. These challenges are particularly pronounced in oral formulations due to incomplete gastrointestinal absorption and extensive first-pass hepatic metabolism, both of which significantly reduce drug efficacy [1]. Beyond bioavailability concerns, conventional drug delivery systems face fundamental challenges in nucleic acid therapies, where biological barriers

and instability have historically limited clinical translation. Nucleic acids face additional challenges including inherent instability requiring protection from degradation. They also experience immune recognition leading to antibody formation, accelerated clearance, and inefficient cellular internalization with poor endosomal escape [2, 3]. Clinical experience with specialized nucleic acid delivery systems has demonstrated poor gene expression, short circulation lifetimes, and limited therapeutic effectiveness [2]. These conventional formulations are designed without controlling release kinetics, whereas nucleic acid therapeutics such as messenger RNA (mRNA), small interfering RNA (siRNA), and gene editing components require specialized delivery systems. These systems must protect these inherently unstable molecules, enable efficient cellular delivery, and facilitate endosomal escape. This led to the development of advanced formulations such as lipid nanoparticles [3].

Lipid Nanoparticles: A Transformative Solution for Nucleic Acid Delivery

The fundamental limitations of conventional delivery approaches have driven the development of advanced lipid nanoparticle (LNP)-based systems which address many of these core delivery challenges. LNPs are widely used for the delivery of nucleic acids and have become particularly important in personalized genetic therapy applications due to their simple synthesis, small size, and serum stability. These structures can encapsulate and transport diverse therapeutic agents, including mRNA, siRNA, and small molecules [4]. LNPs provide several critical advantages including biocompatibility, biodegradability, and adaptable surface modifications for targeted delivery that enhance efficacy while minimizing off-target interactions [5]. Their ability to encapsulate cargo is particularly advantageous for nucleic acid therapeutics, which require protection from enzymatic degradation and facilitated cellular uptake mechanisms [5].

LNPs enable targeted nucleic acid delivery through surface engineering with specific ligands that facilitate receptor-mediated cellular uptake and organ-selective distribution for therapeutic RNA and DNA applications [6]. These delivery platforms are comprised of four key components: ionizable cationic lipids, helper phospholipids, cholesterol, and polyethylene glycol-lipids in precisely controlled ratios. Besides lipid composition, critical physicochemical parameters including particle size and surface charge significantly influence LNP biodistribution and cellular uptake [7]. As a result, carefully optimized compositions facilitate cellular uptake and endosomal escape while maintaining nucleic acid cargo and reducing recognition by immune system cells making LNPs particularly effective for nucleic acid therapeutics [7].

Clinical Validation and Current Challenges

The transformative potential of LNP-mediated nucleic acid delivery has been validated through breakthrough clinical successes, exemplified by Coronavirus Disease 2019 (COVID-19) mRNA vaccines achieving >94% efficacy [8]. A paradigm shift from conventional pharmacological interventions to precision genetic medicines has also been demonstrated by VERVE-101, an investigational CRISPR base-editing therapy targeting proprotein convertase subtilisin/kexin type 9 (PCSK9). Preclinical studies showed that a single intravenous infusion achieved 70% hepatic gene editing. This resulted in sustained 83% reduction in PCSK9 protein and 69% lowdensity lipoprotein cholesterol reduction maintained for up to 476 days [9].

Despite these clinical successes, substantial challenges remain in this field [10]. These include intricate fourcomponent preparation processes, highly inflammatory and cytotoxic adverse events from cationic lipid interactions, low gene transfection efficiency, and unintended tissue targeting [10]. These challenges largely stem from the excess cationic charge density required for mRNA compression through electrostatic interaction, which causes adverse events. Additional challenges include complex delivery requirements such as protection from degradation, cellular uptake, endosomal escape, and intracellular targeting [10].

This review examines LNP technology as a transformative platform for nucleic acid delivery and gene therapies. It analyzes technological innovations in molecular design, targeting mechanisms, and manufacturing approaches that have revolutionized nucleic acid delivery efficacy and precision, representing a clear evolution beyond the limitations of conventional pharmaceutical formulations in addressing the unique requirements of genetic medicine.

Methods

A systematic literature review was conducted using National Institutes of Health and PubMed databases from 2004-2025. Search keywords included "lipid nanoparticles," "nucleic acid delivery," "gene therapy," "mRNA delivery," "siRNA delivery," "ionizable lipids," and "endosomal escape." Inclusion criteria comprised English-language peerreviewed articles relevant to LNP design, targeting mechanisms, optimization strategies, and clinical applications for nucleic acid delivery. Exclusion criteria included non-English publications and studies focusing solely on small molecule delivery. The final analysis incorporated 45 publications.

Results

Fundamental LNP Design Principles

Contemporary LNP formulations are typically composed of four key components: ionizable lipids facilitating RNA encapsulation and endosomal escape, phospholipids

providing structural integrity, cholesterol enhancing membrane stability, and polyethylene glycol modification (PEGylated) lipids conferring circulation longevity [8]. Mechanistic studies reveal that these ionizable lipids undergo progressive lyotropic phase transitions as pH decreases, shifting from inverse micellar to cubic micellar and ultimately to inverse hexagonal structures. Notably, the cubic micellar transition, which occurs at ~pH 6, strongly correlates with endosomal escape efficiency [11].

Ionizable lipids function through pH-sensitive amphiphilicity. They remain neutral at physiological pH (7.4) for safe systemic delivery. However, in acidic endosomes (pH 5.4-6.5), they undergo protonation to destabilize endosomal membranes [12]. Optimal amino lipids demonstrate pKa values around 6.5, as lipids with pKa ≤5.4 exhibit significantly reduced efficiency. When the packing parameter of the lipids exceeds unity, they adopt inverted hexagonal phases that favor endosomal membrane destabilization crucial for nucleic acid delivery [12]. Following cellular endocytosis, LNPs traverse endosomallysosomal pathways. In these pathways, cargo is digested or exocytosed, making endosomal escape the critical bottleneck for effective gene delivery [12].

Advances in ionizable lipid design have significantly improved LNP strategies for gene therapy and nucleic acid delivery. The evolution of lipid structures and functions has progressed from early-stage simple formulations to multi-component LNP and multifunctional ionizable lipids [12].

Optimization Strategies

Precise pKa control is critical for optimizing lipid performance in nucleic acid delivery systems. This parameter significantly influences the efficacy of LNP delivery systems for RNA therapeutics [13, 14]. Simultaneously, optimal lipids within the composition can also enhance endosomal escape mechanisms through controlled protonation during endosomal acidification [14]. Research demonstrates that ionizable lipid pKa modulates RNA complexation, endosomal escape, and cytoplasmic release. This influences both efficacy and toxicity profiles of gene therapy applications [14]. Furthermore, pKa values drive organ-specific targeting patterns. This targeting behaviour represents a fundamental characteristic of the LNP platform itself, independent of the therapeutic payload being delivered [13]. The optimal pKa ranges that have been established for nucleic acid delivery include 6.2-6.5 for liver-targeted siRNA delivery and 6.6-6.9 for intramuscular mRNA vaccine delivery [15, 16].

Next, structural architecture optimization leverages branched macromolecules, particularly dendrimers, to maximize electrostatic interactions with the negatively charged nucleic acid phosphate backbone through enhanced cationic charge density and proton sponge behaviour. Synergistic integration of chemical modifications including 2'-sugar modifications, phosphorothioate linkages, and base alteration further optimizes cellular uptake and endosomal escape while reducing off-target effects [17].

Branched-tail lipidoids demonstrate superior mRNA delivery efficacy compared to linear analogs due to enhanced ionization at endosomal pH. One study demonstrated branched-tail lipidoid (306Oi10) exhibiting a tenfold improved potency over its straight-chain counterpart of identical molecular weights. This improvement stems from enhanced surface ionization at late endosomal pH of 5.0 [18]. This represents a previously unappreciated parameter that strongly correlates with delivery efficacy and is crucial for therapeutic mRNA applications including protein replacement therapy, vaccination, and gene editing, directly addressing the shortage of efficacious delivery systems that has impeded clinical translation of mRNA drugs [18].

Optimization of LNPs can also be accomplished using surface modifications. PEGylation significantly impacts their pharmacokinetic profile, reducing clearance and extending systemic circulation time from 0.89 hours for uncoated nanoparticles to 15.5-19.5 h for PEGylated formulations [19]. PEGylation creates a hydrated steric barrier that inhibits protein adsorption and phagocytic recognition, with effectiveness determined by molecular weight and surface density, yet presents a dilemma wherein extended circulation time compromises cellular uptake and endosomal escape [19, 20].

Clinical LNP formulations typically use a 50:10:38.5:1.5 molar ratio of ionizable lipid: DSPC:cholesterol:PEG-lipid, a composition that achieves over 90% nucleic acid encapsulation efficiency across modalities [21]. Ionizable lipids are central to this success, enabling efficient encapsulation and pH-responsive membrane destabilization in acidic endosomes while remaining neutral at physiological pH, thereby minimizing cytotoxicity [12]. In this ratio DSPC enhances structural stability, cholesterol modulates membrane fluidity, and PEG-lipids confer colloidal stability and extend circulation time by providing steric hindrance [21]. Comparative optimization studies have demonstrated that this formulation yields a six-fold increase in transfection efficiency in non-human primates versus older formulations such as the 40:10:40:10 ratio [12].

These structural optimization strategies demonstrate how architectural modifications can produce substantial improvements in therapeutic potency, exemplifying how systematic design changes yield order-of-magnitude therapeutic enhancements through optimized structurefunction relationships [18].

Targeting Approaches

Bispecific antibodies offer an advanced approach for targeted RNA delivery using LNPs. This method utilizes

antibodies that simultaneously bind to PEG components within the LNP structure and specific cell surface proteins. This eliminates complex chemical conjugation requirements while improving manufacturing reproducibility [23]. Despite these advancements, enhanced specificity remains essential for selective cellular uptake and reduction of off-target effects [23].

While antibody-based approaches show promise, peptide-mediated targeting offers manufacturing advantages. RGD (Arg-Gly-Asp) peptide-based lipids represent an advanced targeted delivery strategy for targeted mRNA delivery in LNPs. RGD peptide-based lipids selectively bind to integrin receptors (particularly $\alpha v\beta 3$ and $\alpha 5\beta 1$) that are overexpressed on specific cell populations [24]. Research has demonstrated that an optimized formulation containing 20% RGD peptide-based lipid achieved significantly enhanced transfection efficiency compared to non-targeted LNP controls [24]. Target specificity was confirmed through competitive inhibition studies, where pre-treatment with soluble RGD peptide effectively reversed the enhanced cellular uptake, validating the mechanism for mRNA delivery applications [24].

Two distinct peptide-based approaches have been investigated for enhancing cellular uptake: cellpenetrating peptides (CPPs) and fusogenic coiled-coil peptides. CPPs include cationic, hydrophobic, and amphipathic variants that facilitate cellular internalization through membrane interactions. Unfortunately, their effectiveness for gene delivery is limited by predominant uptake via endocytosis, resulting in suboptimal cytosolic delivery due to inefficient endosomal escape [25]. Conversely, fusogenic coiled-coil peptide systems enable direct membrane fusion that bypasses the endolysosomal pathway, thereby substantially improving intracellular nucleic acid delivery efficiency [25].

Building upon targeting approaches, aptamers are synthetic single-stranded DNA or RNA molecules that have significantly advanced LNP targeting for nucleic acid delivery through their high binding affinity and specificity, flexible structure, low immunogenicity, and easily modifiable chemical structure [26]. Generated through Systematic Evolution of Ligands by Exponential Enrichment (SELEX) methodologies, aptamers can be isolated to recognize diverse targets. This is accomplished by chemically modifying their structure to enhance stability and extend circulation time [27]. Aptamer-functionalized LNPs have also demonstrated effectiveness in delivering various therapeutic agents, including siRNA and accelerated development (3-7 weeks versus ~6 months) for nucleic acid delivery applications [26, 27].

LNP technology combined with gene editing tools such as CRISPR-Cas9 advances genetic medicine. The lipid layers protect CRISPR components from degradation enabling cellular entry by efficiently delivering editing components to target cells. These systems provide targeted delivery, scalability, and high modification efficiency with minimal immunogenicity [28]. As a result, LNPs' selfassembly, biocompatibility, and versatile characteristics have made them the leading FDA-approved nanomedicines [28].

The diverse targeting strategies for LNP delivery systems each offer distinct advantages and limitations in achieving selective cellular uptake and therapeutic efficacy, as summarized in Table 1.

Table 1: Comparison of Targeting Approaches for LNP Delivery

Targeting Strategy	Mechanisms	Key Advantages
Bispecific Antibodies	Simultaneously bind to PEG components within LNP structure and specific cell surface proteins.	Eliminates complex chemical conjugation requirements, and improves manufacturing reproducibility.
RGD Peptide-based	Selectively bind to integrin receptors (particularly αvβ3 and α5β1) overexpressed on specific cell populations.	Manufacturing advantages over antibodybased approaches and target specificity confirmed through competitive inhibition studies.
Cell-Penetrating Peptides (CPPs)	Facilitate cellular internalization through membrane interactions (cationic, hydrophobic, and amphipathic variants).	Facilitate cellular internalization.
Fusogenic Coiled-Coil Peptides	Enable direct membrane fusion that bypasses the endolysosomal pathway.	Substantially improves intracellular nucleic acid delivery efficiency.
Aptamers	Synthetic single-stranded DNA or RNA molecules generated through SELEX methodologies.	High binding affinity and specificity, flexible structure, low immunogenicity, easily modifiable chemical structure, and accelerated development.

Discussion

LNP technology has addressed traditional limitations of drug delivery systems such as poor solubility, low bioavailability, and targeting challenges, demonstrating versatility and efficacy across therapeutic domains [29]. Despite these advances, LNPs' synthetic nature continues to present challenges affecting therapeutic efficacy and safety [30].

Stability Enhancement and Manufacturing Advancements

Recent mRNA vaccine technology has addressed storage and distribution challenges through innovative strategies. Research has demonstrated that optimal size selection represents a critical factor in mRNA vaccine stability. Specifically, studies show that LNPs sized 80-100 nm exhibit superior performance during six-month storage at 4°C and −20°C compared to other particle sizes [31]. These LNPs maintained consistent physicochemical characteristics, mRNA integrity, and prevented thermal degradation pathways [31].

Additional research exploring freeze-drying techniques has enabled storage at higher temperatures using continuous spin-freezing with optimized C12-200 lipid formulations that preserved transfection properties across varying temperatures (4°C, 22°C, and 37°C) for 12 weeks, and maintained encapsulation efficiency for 8 weeks [32]. These innovations represent a transformative advance that enhances the stability of mRNA LNPs allowing their storage at higher temperatures for a prolonged period.

Nucleic Acid Protection Mechanisms

LNPs provide essential protection against multiple degradation pathways that compromise naked nucleic acids. Encapsulation shields RNA from extracellular ribonucleases and prevents water-mediated hydrolysis, for formulations requiring >80% encapsulation efficiency for therapeutic efficacy [31]. Size-dependent protection is evident, as LNPs

sized 80-100 nm maintained approximately 70-75% mRNA purity compared to 48% degradation in larger formulations over 6 months, attributed to reduced nucleic acid exposure to degradative water fractions within the particle core [31].

Microfluidic Manufacturing and Process Control

Microfluidic manufacturing parameters significantly influence LNP production quality through both formulation composition and operational conditions. Flow rate ratio and total flow rate modulate physicochemical properties, with increasing total flow rate reducing particle size and higher flow rate ratios yielding smaller particles [33]. Additionally, lipid composition, buffer selection, and nucleic acid payload type affect LNP properties. Researchers have achieved consistently high nucleic acid loading efficiency (>90%) while maintaining particle sizes below 100 nm with low polydispersity indices (≤0.25). This demonstrates scalability and reproducibility of microfluidic manufacturing for good manufacturing practice-scale LNP production [33]. These techniques have significantly advanced LNP manufacturing, providing precisely controlled, scalable processes with improved batch-to-batch reproducibility over previous macroscopic mixing methods [34].

Methodological Limitations and Standardization Challenges

Significant methodological challenges impede advancement in LNP design and optimization, particularly regarding the correlation between theoretical predictions and experimental observations. Theoretical ionizable lipid pKa values frequently deviate from their apparent pKa in LNP formulations by 2-3 pH units, demonstrating that isolated lipid characteristics inadequately predict in vivo performance [21]. For liver-targeted siRNA delivery, optimal pKa ranges of 6.2-6.5 have been identified, though the apparent pKa varies with ionic interactions and environmental parameters [14].

Additionally, apparent pKa measurements of nanoparticles are strongly influenced by various environmental parameters including ionic strength, dielectric constant, hydrophobic interactions, and neighbouring charges. Specific measurement techniques having inherent limitations that affect the accuracy of pKa determination in nucleic acid delivery systems [14]. As a result, researchers advocate that each formulation should be evaluated using a standardized procedure to accurately measure differences in characterization and performance, highlighting the importance of methodological consistency in advancing gene therapy applications [35]. Robust multistep screening methodologies can efficiently identify optimal formulations from numerous candidate libraries, potentially improving the development of delivery systems for gene therapy applications. [36].

While structure-activity relationship studies have established general correlations between ionizable lipid design and transfection efficiency, research groups employ divergent empirical approaches throughout the LNP development pipeline for gene therapy. Some investigations rely on high-throughput lipid library screening while others use in vivo testing across multiple organ systems [13, 17]. This methodological divergence creates evidence bases that resist integration and complicates efficacy improvements due to inconsistently applied evaluation metrics [17].

Current Limitations in LNP Targeting and Formulation Stability

LNP targeting strategies face several key limitations. Specifically, antibody-mediated approaches offer high specificity but face manufacturing and potential immunogenicity challenges [23, 37]. Peptide-modified systems provide simpler production with lower targeting specificity [23, 37]. Aptamer-based targeting balances easier synthesis with stability, though clinical translation lags [26, 38].

Formulations also encounter significant stability issues, experiencing degradation during storage [31]. Freezedrying approaches have shown improved temperature stability for mRNA LNPs but require specific formulation parameters, including appropriate buffer types and sufficient lipid-to-mRNA ratios [32, 39]. Despite the promise of various targeting modalities, the development of clinically viable LNP systems requires careful consideration of the inherent trade-offs between targeting specificity, manufacturing complexity, and formulation stability to achieve optimal therapeutic efficacy.

Safety Considerations and Regulatory Challenges

Lipid-based nanomedicines face significant regulatory challenges due to inadequate nanomedicine-specific guidelines, necessitating preclinical and clinical evaluations for comprehensive safety assessment [40]. Manufacturing scalability presents obstacles as laboratory-optimized processes often lack reproducibility at the industrial scale [41]. There are also high costs associated with raw materials and specialized equipment which contributes to the financial burden of LNP commercialization [42].

LNP formulations' safety profiles are contingent upon the selected ionizable lipid. Newer compounds like SM-102 offer enhanced biodegradability and tolerability compared to earlier-generation lipids such as DLin-MC3-DMA [43]. Structure-function relationships also significantly influence toxicity profiles through mechanisms affecting RNA binding efficiency, endosomal escape, and cytotoxicity. This emphasizes the importance of rational design when developing effective mRNA delivery systems [44].

LNP siRNA delivery presents several dose-dependent safety concerns. These include increased lethality, hepatotoxicity, systemic inflammation (elevated IFN-γ, IL6, TNF-α, MCP-1), thrombocytopenia, coagulopathy, and hematuria [45]. This has led to regulatory frameworks mandating standardized protocols and long-term toxicity studies as manufacturing inconsistencies affecting pharmacokinetics and biodistribution require stringent quality control [30].

Future Directions and Therapeutic Expansion

The convergence of LNP technology with gene editing tools represents a critical advancement pathway for genetic medicine; however, the advancement of LNP therapeutic potential requires resolving several interconnected challenges. First, enhancing targeting specificity through cell-specific promoters and receptor-binding ligands is essential to minimize off-target effects [30]. Additionally, the development of cost-effective microfluidic evaluation platforms offers superior controllability compared to traditional in vivo models [37].

Manufacturing processes must also be refined to facilitate precise ligand conjugation while maintaining functional integrity [30]. Biocompatibility should be improved through novel molecular structures like zwitterionic materials and enhanced endosomal escape mechanisms to address cytotoxicity concerns [30]. Finally, streamlined regulatory frameworks are needed to standardize processes across the expanding landscape of ionizable lipids [30].

Conclusions

Lipid nanoparticles have transformed gene therapy through innovations in lipid design and manufacturing. Advancements in branched-tail architectures, precise pKa tuning, and PEGylation have significantly improved gene delivery while overcoming biological barriers. Microfluidic mixing methodologies now achieve low polydispersity indices, with encapsulation efficiencies exceeding 90%. Gene editing applications have also demonstrated significant therapeutic potential, exemplified by VERVE101 achieving sustained genetic modifications with singledose administration.

This review identifies targeting efficiency and endosomal escape as the critical challenges in gene therapy applications. Future investigations should prioritize cell-specific targeting ligands, zwitterionic lipid structures, and standardized manufacturing protocols for gene therapy delivery. Addressing these fundamental challenges will advance personalized medicine approaches and elevate patient care through precision genetic therapeutics.

List of Abbreviations

COVID-19: Coronavirus disease 2019
CPPs: cell-penetrating peptides
CRISPR: clustered regularly interspaced short palindromic repeats
DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine
IFN-γ: interferon gamma
IL-6: interleukin-6
LNPs: lipid nanoparticles
MCP-1: monocyte chemoattractant protein-1 mRNA: messenger RNA
PCSK9: proprotein convertase subtilisin/kexin type 9
PEGylation: polyethylene glycol modification
RGD peptide: Arg-Gly-Asp
SELEX: systematic evolution of ligands by exponential enrichment siRNA: small interfering RNA
TNF-α: tumor necrosis factor alpha

Conflicts of Interest

The author declares that they have no conflict of interests.

Ethics Approval and/or Participant Consent

Given the nature of this work as a literature review, no institutional research ethics board (REB) approval or participant consent was required. This review is based on publicly available data and follows ethical guidelines for the synthesis of published research.

Authors' Contributions

SL: Conceptualized and designed the study, selected and reviewed the relevant literature, synthesized the information, critically analyzed the findings, and wrote the manuscript.

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References

[1] Adepu S, Ramakrishna S. Controlled drug delivery systems: current status and future directions. Molecules. 2021 Sep;26(19):5905. Available from: https://doi.org/10.3390/molecules26195905

[2] Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. Science. 2004 Mar 5;303(5665):1818-

1822. Available from: https://doi.org/10.1126/science.1095833

[3] Park H, Otte A, Park K. Evolution of drug delivery systems: from 1950 to 2020 and beyond. J Control Release. 2022 Feb;342:53-65. Available from: https://doi.org/10.1016/j.jconrel.2021.12.030

[4] Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov. 2021 Feb;20(2):101-124. Available from: https://doi.org/10.1038/s41573-020-0090-8

[5] Yang L, Gong L, Wang P, Zhao X, Zhao F, Zhang Z, et al. Recent advances in lipid nanoparticles for delivery of mRNA. Pharmaceutics. 2022 Dec;14(12):2682. Available from: https://doi.org/10.3390/pharmaceutics14122682

[6] Lin Y, Cheng Q, Wei T. Surface engineering of lipid nanoparticles: targeted nucleic acid delivery and beyond.
Biophys Rep. 2023 Oct;9(5):255-278. Available from: https://doi.org/10.52601/bpr.2023.230022

[7] Mendonça MCP, Kont A, Kowalski PS, O'Driscoll CM. Design of lipid-based nanoparticles for delivery of therapeutic nucleic acids. Drug Discov Today. 2023 Mar;28(3):103505. Available from: https://doi.org/10.1016/j.drudis.2023.103505

[8] Jung HN, Lee SY, Lee S, Youn H, Im HJ. Lipid nanoparticles for delivery of RNA therapeutics: current status and the role of in vivo imaging. Theranostics. 2022 Oct;12(17):7509-7531. Available from: https://doi.org/10.7150/thno.77259

[9] Lee RG, Mazzola AM, Braun MC, Platt C, Vafai SB, Kathiresan S, et al. Efficacy and safety of an investigational single-course CRISPR base-editing therapy targeting PCSK9 in nonhuman primate and mouse models. Circulation. 2023 Jan;147(3):242-253. Available from: https://doi.org/10.1161/CIRCULATIONAHA.122.062132

[10] Wang C, Zhao C, Wang W, Liu X, Deng H. Biomimetic noncationic lipid nanoparticles for mRNA delivery. Proc Natl Acad Sci U S A. 2023 Dec;120(51):e2311276120.
Available from: https://doi.org/10.1073/pnas.2311276120

[11] Philipp J, Dabkowska A, Reiser A, Frank K, Krzysztoń R, Brummer C, et al. pH-dependent structural transitions in cationic ionizable lipid mesophases are critical for lipid nanoparticle function.
Proc Natl Acad Sci U S A. 2023 Dec;120(50):e2310491120. Available from: https://doi.org/10.1073/pnas.2310491120

[12] Sun D, Lu ZR. Structure and function of cationic and ionizable lipids for nucleic acid delivery. Pharm Res. 2023 Jan;40(1):27-46. Available from: https://doi.org/10.1007/s11095-022-03460-2

[13] Zhang T, Yin H, Li Y, Yang H, Ge K, Zhang J, et al. Optimized lipid nanoparticles (LNPs) for organselective nucleic acids delivery in vivo. iScience. 2024 Apr;27(6):109804. Available from: https://doi.org/10.1016/j.isci.2024.109804

[14] Patel P, Ibrahim NM, Cheng K. The importance of apparent pKa in the development of nanoparticles encapsulating siRNA and mRNA. Trends Pharmacol Sci. 2021 Jun;42(6):448-460. Available from: https://doi.org/10.1016/j.tips.2021.03.002

[15] Jayaraman M, Ansell SM, Mui BL, Tam YK, Chen J, Du X, et al. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. Angew Chem Int Ed Engl. 2012 Aug;51(34):85298533. Available from: https://doi.org/10.1002/anie.201203263

[16] Hassett KJ, Benenato KE, Jacquinet E, Lee A, Woods A, Yuzhakov O, et al. Optimization of lipid nanoparticles for intramuscular administration of mRNA vaccines. Mol Ther Nucleic Acids. 2019 Apr;15:1-11. Available from: https://doi.org/10.1016/j.omtn.2019.01.013

[17] Liu M, Wang Y, Zhang Y, Hu D, Tang L, Zhou B, et al. Landscape of small nucleic acid therapeutics: moving from the bench to the clinic as next-generation medicines. Signal Transduct Target Ther. 2025 Mar;10(1):73. Available from: https://doi.org/10.1038/s41392-024-02112-8

[18] Hajj KA, Ball RL, Deluty SB, Singh SR, Strelkova D, Knapp CM, et al. Branched-tail lipid nanoparticles potently deliver mRNA in vivo due to enhanced ionization at endosomal pH. Small. 2019 Feb;15(6):e1805097. Available from: https://doi.org/10.1002/smll.201805097

[19] Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticlebased drug and gene delivery. Adv Drug Deliv Rev. 2016 Apr;99(Pt A):28-51. Available from: https:// doi.org/10.1016/j.addr.2015.09.012

[20] Fang Y, Xue J, Gao S, Lu A, Yang D, Jiang H, He Y, Shi K. Cleavable PEGylation: a strategy for overcoming the "PEG dilemma" in efficient drug delivery. Drug Deliv. 2017 Dec;24(Suppl 1):22-32. Available from: https://doi.org/10.1080/10717544.2017.1388451

[21] Hald Albertsen C, Kulkarni JA, Witzigmann D, Lind M, Petersson K, Simonsen JB. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. Adv Drug Deliv Rev. 2022 Sep;188:114416. Available from: https://doi.org/10.1016/j.addr.2022.114416

[22] Kapoor DU, Sharma JB, Gandhi SM, Prajapati BG, Thanawuth K, Limmatvapirat S, et al. AI-driven design

and optimization of nanoparticle-based drug delivery systems. Sci Eng Health Stud. 2024 Dec;18:24010003. Available from: https://doi.org/10.69598/sehs.18.24010003

[23] Marques AC, Costa PC, Velho S, Amaral MH. Lipid nanoparticles functionalized with antibodies for anticancer drug therapy. Pharmaceutics. 2023 Jan;15(1):216. Available from: https://doi.org/10.3390/pharmaceutics15010216

[24] Qin J, Xue L, Gong N, Zhang H, Shepherd SJ, Haley RM, et al. RGD peptide-based lipids for targeted mRNA delivery and gene editing applications. R Soc Chem Adv. 2022 Sep;12(39):25397–25404. Available from: https://doi.org/10.1039/d2ra02771b

[25] Aschmann D, Knol RA, Kros A. Lipid-based nanoparticle functionalization with coiled-coil peptides for in vitro and in vivo drug delivery. Acc Chem Res. 2024 Apr;57(8):1098-1110. Available from: https://doi.org/10.1021/acs.accounts.3c00769

[26] Zhuo Z, Yu Y, Wang M, Li J, Zhang Z, Liu J, et al. Recent advances in SELEX technology and aptamer applications in biomedicine. Int J Mol Sci. 2017 Oct;18(10):2142. Available from: https://doi.org/10.3390/ijms18102142

[27] Zhou J, Rossi J. Aptamers as targeted therapeutics: current potential and challenges. Nat Rev Drug Discov. 2017 Mar;16(3):181-202. Available from: https://doi.org/10.1038/nrd.2016.199

[28] Mohammadian Farsani A, Mokhtari N, Nooraei S, Bahrulolum H, Akbari A, Farsani ZM, et al. Lipid nanoparticles: The game-changer in CRISPR-Cas9 genome editing. Heliyon. 2024 Jan;10(2):e24606. Available from: https://doi.org/10.1016/j.heliyon.2024.e24606

[29] Ashfaq R, Rasul A, Asghar S, Kovács A, Berkó S, Budai-Szűcs M. Lipid nanoparticles: an effective tool to improve the bioavailability of nutraceuticals. Int J Mol Sci. 2023 Oct;24(21):15764. Available from: https://doi.org/10.3390/ijms242115764

[30] Wang J, Ding Y, Chong K, Cui M, Cao Z, Tang C, et al. Recent advances in lipid nanoparticles and their safety concerns for mRNA delivery. Vaccines (Basel). 2024 Oct;12(10):1148. Available from: https://doi.org/10.3390/vaccines12101148

[31] Shi R, Liu X, Wang Y, Pan M, Wang S, Shi L, et al. Long-term stability and immunogenicity of lipid nanoparticle COVID-19 mRNA vaccine is affected by particle size. Hum Vaccin Immunother. 2024 Dec;20(1):2342592. Available from: https://doi.org/10.1080/21645515.2024.2342592

[32] Meulewaeter S, Nuytten G, Cheng MHY, De Smedt SC, Cullis PR, De Beer T, et al. Continuous freezedrying of messenger RNA lipid nanoparticles enables storage at higher temperatures. J Control Release. 2023 May;357:149-160. Available from: https://doi.org/10.1016/j.jconrel.2023.03.039

[33] Roces CB, Lou G, Jain N, Abraham S, Thomas A, Halbert GW, et al. Manufacturing considerations for the development of lipid nanoparticles using microfluidics. Pharmaceutics. 2020 Nov;12(11):1095. Available from: https://doi.org/10.3390/pharmaceutics12111095

[34] Belliveau NM, Huft J, Lin PJ, Chen S, Leung AK, Leaver TJ, et al. Microfluidic synthesis of highly potent limit-size lipid nanoparticles for in vivo delivery of siRNA. Mol Ther Nucleic Acids. 2012 Aug;1(8):e37. Available from: https://doi.org/10.1038/mtna.2012.28

[35] El-Mayta R, Padilla MS, Billingsley MM, Han X, Mitchell MJ. Testing the in vitro and in vivo efficiency of mRNA-lipid nanoparticles formulated by microfluidic mixing. J Vis Exp. 2023 Jan;(191). Available from: https://doi.org/10.3791/64810

[36] Zhu Y, Shen R, Vuong I, Reynolds RA, Shears MJ, Yao ZC, et al. Multi-step screening of DNA/lipid nanoparticles and co-delivery with siRNA to enhance and prolong gene expression. Nat Commun. 2022 Jul;13(1):4282. Available from: https://doi.org/10.1038/s41467-022-31993-y

[37] Amabile A, Phelan M, Yu Z, Silva P, Marks A, MorlaFolch J, et al. Bispecific antibody targeting of lipid nanoparticles. bioRxiv [Preprint]. 2024 Dec. Available from: https://doi.org/10.1101/2024.12.20.629467

[38] Liang C, Guo B, Wu H, Shao N, Li D, Liu J, et al. Aptamer-functionalized lipid nanoparticles targeting osteoblasts as a novel RNA interference-based bone anabolic strategy. Nat Med. 2015 Mar;21(3):288-294. Available from: https://doi.org/10.1038/nm.3791

[39] Kommineni N, Butreddy A, Sainaga Jyothi VGS, Angsantikul P. Freeze-drying for the preservation of immunoengineering products. iScience. 2022 Sep;25(10):105127. Available from: https://doi.org/10.1016/j.isci.2022.105127

[40] Hua S, de Matos MBC, Metselaar JM, Storm G. Current trends and challenges in the clinical translation of nanoparticulate nanomedicines: pathways for translational development and commercialization. Front Pharmacol. 2018 Jul;9:790. Available from: https://doi.org/10.3389/fphar.2018.00790

[41] Burroughs L, Burgess DJ. How could the affordable, reliable and continuous production of lipid nanoparticles revolutionize healthcare? Nanomedicine. 2025 Aug;20(15):1829-1833. Available from: https://doi.org/10.1080/17435889.2025.2500911

[42] Escalona-Rayo O, Zeng Y, Knol RA, Kock TJF, Aschmann D, Slütter B, Kros A. In vitro and in vivo

evaluation of clinically-approved ionizable cationic lipids shows divergent results between mRNA transfection and vaccine efficacy. Biomed Pharmacother. 2023 Sep;165:115065. Available from: https://doi.org/10.1016/j.biopha.2023.115065

[43] Xu Y, Golubovic A, Xu S, Pan A, Li B. Rational design and combinatorial chemistry of ionizable lipids for RNA delivery. J Mater Chem B. 2023 Jun;11:65276539. Available from: https://doi.org/10.1039/D3TB00649B

[44] Tao W, Mao X, Davide JP, Ng B, Cai M, Burke PA, et al. Mechanistically probing lipid-siRNA nanoparticleassociated toxicities identifies Jak inhibitors effective in mitigating multifaceted toxic responses. Mol Ther. 2011 Mar;19(3):567-575. Available from: https:// doi.org/10.1038/mt.2010.282

[45] Bruno BJ, Miller GD, Lim CS. Basics and recent advances in peptide and protein drug delivery. Ther Deliv. 2013 Nov;4(11):1443-1467. Available from: https://doi.org/10.4155/tde.13.104

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