



Advancements in Lipid Nanoparticle Technologies for Precision Targeting in Therapeutic Delivery

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Abstract

Lipid nanoparticles (LNPs) are at the forefront of targeted therapeutic delivery, serving as an innovative platform for precisely delivering therapeutic agents directly to specific cells or tissues. Therapeutic delivery seeks to enhance drug efficacy and safety by controlling the distribution of drugs within the body, thereby minimizing off-target effects while maximizing therapeutic impact. This review delves into advancements and innovations in LNP design to achieve unprecedented targeting precision, surmount biological barriers, and diminish off-target interactions. It encompasses exploring targeting strategies, including passive targeting that leverages the enhanced permeability and retention effect, active targeting via receptor-ligand interactions, and stimuli-responsive methodologies for the controlled release of therapeutics. With a focus on novel targeting techniques, including endogenous targeting and strategic de-targeting, to enhance LNP delivery specificity and therapeutic efficacy. This review articulates the critical role of lipid composition, nanoparticle characterization, and surface engineering in tailoring LNPs for therapeutic applications. Through evaluating the forefront of LNP technology in drug delivery and gene therapy, this review forecasts the trajectory of nanoparticle-based therapeutics, envisioning their pivotal role in the advent of personalized medicine, thereby providing a foundation for future explorations aimed at refining and optimizing LNP systems for clinical applications.

Keywords: lipid nanoparticles, nucleic acid delivery, selective targeting, SORT, surface modification

Abbreviations:

DCs: Dendritic Cells

SORT: Selective Organ Targeting

ApoE: Apolipoprotein E

DOPE: 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine

DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine

LDLR: Low-Density Lipoprotein Receptor

RES: Reticuloendothelial System

TBI: Traumatic Brain Injury

NTA: Nanoparticle Tracking Analysis

pSar: polysarcosine

RGD: Arg-Gly-Asp

LDV: Leu-Asp-Val

VLA-4: Very-Late Antigen-4

HSPC: Hematopoietic Stem & Progenitor Cells

AIS: Acute Ischemic Stroke

CAMs: Cell Adhesion Molecules

VCAMs: Vascular Cell Adhesion Molecule-1

CA: caffeic acid

MCL: Micheliolide

LSCs: Leukemic Stem Cells

SQL: Sesquiterpene Lactone

MM: Multiple Myeloma

CKAP5: Cytoskeleton-Associated Protein 5

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DOTAP: 1,2-dioleoyl-3-trimethylammonium-propane

Purpose, Rationale, and Limitations

Advancements in LNP targeting technologies offer significant potential for precision targeting in drug delivery, addressing the limitations of traditional therapeutic delivery vehicles. Traditional vehicles often lack the specificity required for effective targeted delivery, leading to systemic side effects and reduced efficacy. Structural integrity, stability, and customizable surface properties make LNPs ideal for targeted therapeutic delivery.

This review aims to highlight innovative strategies that improve the precision of LNP targeting by employing various methods to significantly enhance therapeutic outcomes by ensuring drug substances reach specific cells or tissues with high accuracy. However, several limitations remain, including the challenge of navigating complex biological environments and overcoming biological barriers. Future research is needed to optimize LNP systems for clinical applications, focusing on biocompatibility, reduced immunogenicity, and scalable manufacturing processes.

Introduction

Lipid nanoparticle (LNP)-mediated delivery techniques are revolutionary instruments in biomedical engineering that have significantly altered the paradigm of therapeutics.^[1] These tactics incorporate lipid-based nanocarriers to deliver bioactive substances efficiently and precisely, including drugs, proteins, peptides, and nucleic acids, to specific cells or tissues. Utilizing LNPs as delivery systems offers numerous benefits. Specifically, LNPs shield bioactive elements from degradation, enhance their biodistribution and cellular uptake, and facilitate the controlled release of therapeutic agents.^[2] LNPs' biodegradable and biocompatible properties present them as a safer substitute for drug delivery, reducing unfavorable reactions. Developments such as the mRNA-based COVID-19 vaccines by Pfizer-BioNTech and Moderna are prominent examples of how LNP technology can be harnessed.^[3] Numerous mRNA-LNP-based medicines are currently under clinical investigation to treat various illnesses like cancer and several genetic disorders, indicating

their potential in personalized medicine and targeted therapeutics.

Yet, the LNP-mediated delivery technique, although promising, is subject to several challenges. Existing issues include low delivery efficiency, sensitive immune responses, and specifically targeting difficulties.^[4] While LNPs protect therapeutic substances from degradation and enhance cellular uptake, biological barriers and rapid removal from the bloodstream can limit their delivery efficiency.^[5] Immune responses to foreign entities, particularly after repeated dosage, can reduce efficacy and potential adverse events.^[6] However, the substantial challenge lies in the accurate targeting of LNPs. It is crucial to ensure therapeutic substances reach the intended cells or tissues. Surmounting this difficulty will notably alter the strategy for treating a wide array of diseases - from cancer to genetic disorders, leading the way to truly personalized medicine.

Various methods have been suggested to achieve precise targeting in LNP-mediated delivery systems, each with distinct mechanisms and advantages (see Figure 1).

Passive targeting focuses on LNPs' size, surface charge, and other physicochemical characteristics to facilitate their accumulation in pathological tissue, primarily due to variances in tissue structure, blood supply, lymphatic drainage, and molecular content between healthy and pathological tissue.^[7] Endogenous targeting leverages the body's biological functions by using naturally occurring elements like proteins or antibodies to direct LNPs to the targeted site. De-targeting modifies the LNPs to evade uptake by non-target cells, concentrating the therapy on the intended target cells.^[8]

Finally, active targeting conjugates LNPs with ligands or via other surface modifications that can interact with target cell receptors, enhancing delivery specificity and efficiency.^[9] Each of these strategies addresses the challenge of targeting in LNP-mediated therapy, adding to the creation of more precise and effective treatments.

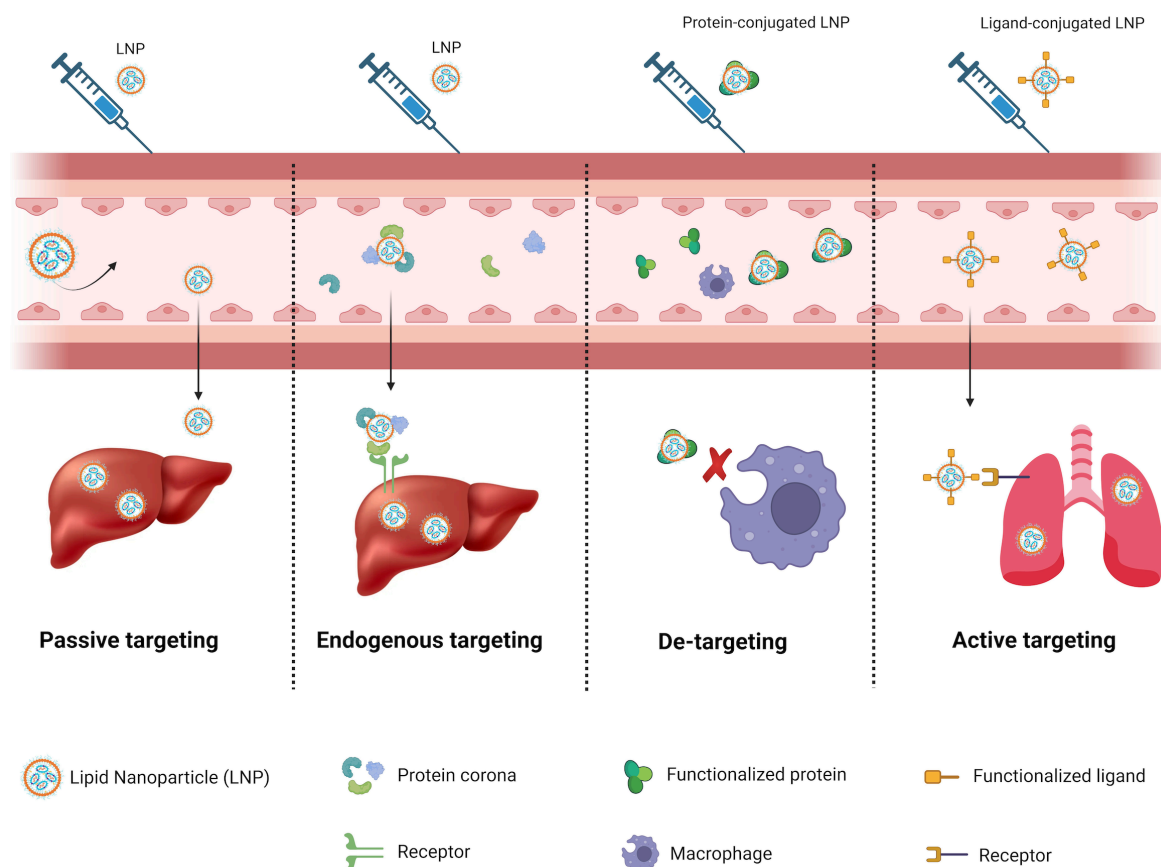


Figure 1. Targeting strategies for lipid nanoparticle (LNP)-mediated delivery. Passive targeting leverages the body's natural distribution mechanisms, allowing LNPs to accumulate in areas with compromised vasculature, exploiting the enhanced permeability and retention effect. Endogenous targeting guides LNPs to harness the body's transport systems, directing them to specific organs such as the liver. In contrast, de-targeting enables LNPs to evade immune detection and uptake by the mononuclear phagocyte system, preventing localization in non-targeted organs. Meanwhile, active targeting affixes ligands to the LNP surface to bind selectively to receptors on target cells, increasing LNP concentration at the desired site and improving therapeutic efficacy while minimizing side effects.

However, despite rapid advancements, the field of LNP targeting strategies still has a considerable journey to undertake. Challenges remain in executing precise and efficient targeting, particularly in complex biological landscapes. Off-target effects, limited tissue penetration, and immune responses pose persistent issues, making continuous research and innovation vital.^[10] Therefore, despite progress, optimizing LNP targeting strategies for improved therapeutic results warrants sustained efforts.

Discussion

Passive targeting

Passive targeting is a strategy used in LNP delivery that utilizes the natural physiological characteristics of the body, particularly the

properties of blood vessels, to steer nanoparticle delivery to the intended location within the body.^[8,11] This approach adjusts nanoparticle physical attributes to complement these physiological traits, with particle size and surface charge being key factors.^[12] Blood vessel diameters differ throughout the body, with organs like the liver and spleen possessing more porous or discontinuous blood vessels that allow larger particles to penetrate and accumulate. By managing the size of nanoparticles, their destination within the body can be influenced. Smaller particles will likely remain in the bloodstream for an extended period, while the liver or spleen may sift out larger particles.

The shape and rigidity of nanoparticles can affect their movement and interactions within the bloodstream, influencing their adhesion to vessel walls and ability to traverse the circulatory

system.^[13] Surface charge also impacts their interactions with surrounding biological molecules and cells, influencing their uptake and distribution.^[14-15] Thus, by altering the properties of the nanoparticles, passive targeting can improve drug delivery specificity without the necessity for additional targeting ligands or molecules. LNPs are designed to naturally concentrate in the desired tissues or organs due to their inherent characteristics, potentially enhancing the efficiency of drug delivery and reducing side effects.

In a study by Xu et al., the bio-distribution and gene expression dynamics of mRNA-LNPs based on their delivery route and particle size are explored.^[16] The study reports that LNPs injected intramuscularly circulate within the body, resulting in accumulation in the liver and spleen, particularly when the LNP sizes are comparatively small. Additionally, gene expression levels are influenced by the LNP bio-distribution and pharmacokinetics, with varying transfection efficiency in different organs.^[17] Another study by Brito's group investigates LNP size's impact on mRNA vaccines' immunogenicity.^[18] This includes a retrospective study of the relationship between LNP particle size and immunogenicity in mice without changing lipid composition. The study found that smaller LNPs induced less immunogenic response in mice, while all particle sizes tested garnered a robust immune response in non-human primates. Harashima's team, on the other hand, focused on optimizing mRNA-loaded LNPs that target dendritic cells for cancer immunotherapy.^[19] It is shown that regulating the size of RNA-loaded LNPs to over 200 nm with salt addition during their is vital. Larger LNPs are found to deliver RNA more effectively to splenic dendritic cells (DCs) compared to their smaller counterparts.

Meanwhile, a collaborative study by Xu and Saw's groups created three star-shaped LNPs featuring different lipid backbone lengths.^[20] These LNPs demonstrated enhanced cellular uptake and in vivo tumor extravasation compared to their spherical counterparts. Moreover, the arm-like structures around the surface of the star-shaped LNPs increased their surface area, resulting in augmented membrane fusion, possibly sidestepping the steric effect caused by proteins on the cell surface. Siegwart's group developed Selective Organ Targeting (SORT) nanoparticles aiming to achieve tissue-specific

mRNA delivery and CRISPR/Cas gene editing.^[21-22] They engineered LNPs to selectively edit extrahepatic tissues by adding a supplemental SORT molecule. The tactics developed could mediate tissue-specific delivery by adjusting the surface charge and the protein corona composition.

LNP properties such as particle size, shape, stiffness, and surface charge are meticulously engineered to align LNPs with natural physiological pathways in a passive targeting strategy.^[23] Advanced LNP manufacturing techniques, including microfluidic mixing, impingement, turbulent mixing, and sophisticated buffer exchange methods like dialysis, desalting, and tangential flow filtration, play significant roles in controlling LNP characterization.^[24] Although the targeting effects are not directly determined, the manufacturing process of LNPs could be critical to the delivery efficiency via passive targeting. Meticulous process development is crucial to creating LNPs with superior stability, reduced immunogenicity, and enhanced biocompatibility. Thus, a well-defined and controlled manufacturing process is indispensable for harnessing the full potential of LNPs.

Endogenous Targeting

Endogenous targeting is utilized in LNP delivery, where the body's inherent pathways are leveraged to transport therapeutics to their intended cells or tissues. In this context, endogenous ligands or molecules naturally recognized and internalized by target cells play crucial roles in receptor-mediated endocytosis.^[8,25] While passive targeting relies on the body's unique features, particularly the enhanced permeability and retention (EPR) effect in solid tumors, endogenous targeting actively engages with and enters specific cells. This results in increased drug concentration within these cells, potentially enhancing therapeutic impacts while diminishing side effects by reducing the drug exposure of healthy cells.^[26] However, the complexity of endogenous targeting necessitates a comprehensive understanding of biological processes and intricate LNP design.

Mitchell and colleagues explored the effects of helper lipid structure on the absorption of apolipoprotein E (ApoE) and the delivery of LNPs to the spleen and liver.^[27-29] Their findings suggested that LNPs formulated with

DOPE had stronger interactions with ApoE and were found in greater amounts in the livers than those LNPs formulated with DSPC. Similarly, Anderson's group focused on developing LNPs for mRNA delivery to the liver, emphasizing the roles of albumin and ApoE in cellular uptake and intracellular trafficking.^[30] They demonstrated that albumin plays a pivotal role in the cellular uptake of specific LNPs. LNPs coated with serum albumin may expedite the delivery of mRNA to the liver via an ApoE-independent pathway.

In a separate study, Siegwart's team discussed the role of phospholipids in LNPs for the delivery of mRNA and the development of SORT-LNPs.^[31] The report highlighted the importance of phospholipid chemistry in enhancing both mRNA delivery and organ targeting. The choice of phospholipids intracellularly enhances endosomal escape and drives organ tropism at the *in vivo* scale.^[32] Taraballi et al. also developed and characterized a novel nanoparticle system known as "aposomes," their research focused on targeted drug delivery to atherosclerotic plaques.^[33-34] The study used a microfluidic approach to synthesize aposomes from low-density lipoproteins isolated from blood plasma.

Overall, endogenous targeting aspires to reach higher drug concentrations within target cells, potentially improving therapeutic outcomes while reducing side effects.^[35] A profound understanding of biological processes and careful LNP design is essential for this approach. Advances in the field have explored various facets of endogenous targeting, including the influence of helper lipid structure on protein adsorption, organ-specific delivery, and the role of liver-targeting proteins such as albumin and ApoE.^[27,30] Additionally, optimizing phospholipid chemistry is essential for efficient mRNA delivery and organ-specific targeting through SORT-LNPs.^[31] Furthermore, the promise shown by innovative approaches such as the utilization of "aposomes" in targeting atherosclerotic plaques with high specificity and low immunogenicity underlines the potential of endogenous targeting in leading to more effective and well-targeted therapeutic delivery systems.^[33]

De-targeting

The de-targeting strategy in LNP delivery entails intentional adjustments of nanoparticles to elude identification by the immune system or specific cellular targets.^[36] This technique is designed to enhance the stealth ability of LNPs, increasing their circulation period in the bloodstream and improving their capability to transport therapeutic payloads to the required tissues or organs.^[37] The concept of de-targeting revolves around refining the pharmacokinetics and bio-distribution of LNPs, intending to diminish off-target impacts and immune clearance. By camouflaging LNPs to avoid detection by the immune system or specific cells, de-targeting tactics aim to decrease clearance by primarily the liver and spleen. This prolongs their circulation in the bloodstream, thereby increasing the chances of LNPs reaching their designated target tissues or organs.

The emphasis on the stealth effect is a critical element of the de-targeting strategy. Such particles can evade opsonization by altering the surface properties of LNPs using hydrophilic polymers like poly (ethylene glycol [PEG]). In this process, serum proteins bind themselves to foreign particles, earmarking them for clearance by the phagocytic cells.^[38] PEGylation shrouds LNPs, making them "invisible" to the immune system, leading to an extended circulation period in the bloodstream.^[39] Further, de-targeting may also mean minimizing relationships with specific cell surface receptors that could lead to faster clearance or off-target effects. If LNPs were initially designed to focus on a particular receptor found on tumor cells, de-targeting could involve modifying the surface ligands to lower binding affinity towards those receptors.^[40] This change can lower the chance of immune clearance and increase the prospect of LNPs reaching their destination without being caught by non-target cells.

In a research study focused on the creation of stealth LNPs for the delivery of hydrophobic photodynamic therapy (PDT) drugs, Puri and the team found that the "invisible" properties could be achieved by using a non-bilayer-forming polymeric lipid, DC8,9PC, along with the incorporation of DSPE-PEG2000 lipid.^[41] The study highlights the benefit of stealth properties in improving the effectiveness and potency of drug delivery systems, especially concerning

cancer nanomedicine. Researchers at the University of California also investigated the impact of the length of the PEG-lipid anchor on the pharmacokinetics and activity of LNPs tailored with mRNA in a mouse model of traumatic brain injury.^[42] The study found that PEG-lipid anchor length has a considerable impact on the accumulation and activity of LNPs in both injured and uninjured brain tissue and off-target organs.^[43]

Building on these findings, Piel's group studied the impact of different PEG-lipid compositions, alongside ionizable lipids, on the behavior and efficacy of LNPs in a serum-containing medium. C14 PEG-lipids showed potential toxicity, especially with HepG2 cells. However, C18 PEG-lipids LNPs exhibited a more significant stealth effect.^[44-45] Langguth's team explored the use of polysarcosine (pSar) as a stealth component in LNPs for mRNA delivery and found that pSar-LNPs showed better transfection efficiency and greater cell-binding affinity as compared to their PEG-shielded counterparts.^[46] On another front, Siegwart's group analyzed two LNPs composed of 5A2-SC8 and 3A5-SC14 in a liver cancer model. The LNPs showed distinct activity in hepatocyte delivery and therapeutic effectiveness, possibly attributed to the stealth effect of protein corona components.^[47-50] Inspired by the potential of albumin for de-targeting, Lavasanifar's team reviewed albumin as a multifaceted nano-delivery system.^[51] The attributes of albumin, namely its high biocompatibility, biodegradability, and non-immunogenicity, together with its ability to interact with receptors that are overexpressed in diseased tissues, make it a strong candidate for de-targeting strategies in LNP drug transport.^[52]

To summarize, de-targeting strategies in LNP delivery aim to boost circulation time, enhance stealth attributes, and improve the delivery of therapeutic payloads to target tissues with as minimal interaction with the immune system and off-target elements as possible.^[53] In addition to PEG-lipid modifications, alternative methods involving using pSar as a stealth moiety or utilizing the unique properties of albumin for extended circulation and targeted delivery are also being explored. The influence of protein corona composition in determining cellular uptake and therapeutic outcomes based on spe-

cific LNP formulations is an important consideration.^[42,46,51] Recent developments in de-targeting strategies underline nanoparticle design's essential role in maximizing returns on the delivery and effectiveness of LNP-based therapeutics.

Active targeting

Active targeting strategy in LNP delivery systems aims to enhance the efficiency and specificity of drug delivery. Employing this strategy involves the modification of the LNP surface with specific ligands such as antibodies, peptides, proteins, or small molecules that recognize and bind to receptors on target cells.^[54] Once administered, these specialized LNPs can circulate through the bloodstream until they reach the target tissue and actively interact with the receptor to prompt cellular uptake. As a result, the target cells can efficiently recognize the drug delivery vehicle to maximize the anticipated effect while limiting potential harm to non-target cells. This precisely targeted delivery method permits the use of lower drug doses, reducing possible side effects, and it can reach targets that are often difficult to access using traditional delivery methods. However, this active targeting strategy's design and development process can be complex and requires extensive research.

In one study, the group led by Vaughan focused on developing and applying an LNP delivery system aimed explicitly at macrophages. The novel approach used the F4/80 antibody as an active targeting ligand.^[55] By employing a method known as click chemistry, the research team integrated the DBCO-activated F4/80 antibody with Azide-functionalized LNPs. This culminated in an advanced LNP formulation called "MacLNP," which significantly enhances delivery efficiency to targeted macrophages. The important aspect emerging from this study is the successful targeting of lung macrophages using the F4/80-LNP strategy, with more benefits derived from the *in situ* delivery of LNPs rather than the systemic delivery.

Parhiz's group developed and tested anti-CD4/mRNA-LNPs for their efficiency and specificity for *in vitro* and *in vivo* delivery targeted to CD4⁺ T cells.^[56] These targeted mRNA-LNPs were created by conjugating anti-CD4 antibodies to LNPs. Before conjugation

with anti-mouse CD4 or control IgG, the resulting LNPs were directly labeled with 125I.^[57] The research confirmed that the CD4-targeted mRNA-LNP platform offered substantial advantages in successful genetic editing using a Cre/loxP reporter system in vivo. In exploring innovative strategies aimed at T cells, another study led by Ferrara focused on developing and evaluating anti-CD3-targeted LNPs (aCD3-LNPs) for in situ transfection of T cells.^[58] The fascinating aspect of this study was how the active targeting effect was achieved. The LNPs were coated with anti-CD3 F(ab')₂ fragments for mouse T cells or a full-length anti-human CD3 antibody for human T cells. The research also established the importance of carefully controlling the amount of aCD3 ligand on the surface of the LNPs.^[59]

A study conducted by Coll and his colleagues had a different approach. They created LNPs loaded with IR780 iodide and modified them with cyclic RGD peptides to target integrin $\alpha v \beta 3$ in tumors.^[60] The experiment aimed to demonstrate the binding capacity and internalization of cRGD-LNPs to create tumor-targeted nanoparticles. Though in vitro experiments were promising, in vivo evaluation indicated that cRGD did not significantly affect LNP accumulation in tumors compared to non-targeted LNPs, highlighting the importance of further research and investigation of this method.^[61-64] Then came a new approach developed by Heidenreich's team, where they created LDV-functionalized LNPs to target the VLA-4 receptor in bone marrow.^[65] This permitted improved retention of LNPs in the bone marrow and avoided lysosomal degradation. The method suggested potential therapeutic implications for leukemia. Lastly, to devise an effective drug delivery method to the brain for the treatment of acute ischemic stroke (AIS), a study pursued by Marcos-Contreras and his colleagues focused on the development of nanocarriers targeted to the blood-brain barrier (BBB) using antibodies that bind to various cell adhesion molecules.^[66-67] They employed VCAM as a target and found VCAM-targeted LNPs loaded with small drugs or mRNA were successful.

Thus, active targeting in LNP delivery presents a highly precise, efficient, and specific method for therapeutic delivery. With the application of specific ligands such as antibodies, peptides, or small molecules that can actively

target and bind to certain receptors on cells, this approach holds much promise across various medical conditions.^[56,60,64,68] Despite the need for extensive research to successfully implement each strategy, advancements in this field can potentially revolutionize therapy delivery systems and medication efficiency. Active targeting can be seen to progress the fields of personalized medicine and targeted therapy, promoting precision in the intervention of disease mechanisms.

Challenging targets

Although existing strategies such as passive, endogenous, active targeting, and de-targeting approaches have improved the efficiency of LNP targeting, achieving tropism to specific organs such as the brain, bone, and pancreas remains a formidable challenge.^[69] Navigating the complex biological landscapes of these organs for therapeutic delivery poses significant challenges due to the unique physiological and anatomical barriers inherent to each organ.

Brain:

The brain, shielded by the BBB, presents a formidable challenge for targeted drug delivery. The BBB blocks nearly all large-molecule therapeutics and over 98% of small-molecule drugs. Modulating the BBB's permeability is a significant hurdle in delivering LNP to the brain.^[70-72] Strategies under development to navigate this barrier include direct injection, chemical modification of drugs, and carrier-mediated transport.^[73] Another critical concern is preventing lysosomal degradation during LNP's intracellular trafficking to ensure therapeutic efficacy. Moreover, precise targeting is crucial to mitigate neurotoxicity arising from nonspecific binding of therapeutic agents to off-target sites within the brain.^[74]

A study by Uchida's group evaluates LNPs for delivering mRNA to brain capillary endothelial cells, which is crucial for the BBB.^[75] Utilizing LNPs composed of a pH-activated and reductive environment-responsive lipid-like material (ssPalm), the study achieves high transfection efficiency, with nearly all cells expressing the marker gene green fluorescent protein and exhibiting low toxicity even at elevated concentrations. Moreover, proteomic analysis reveals the ssPalm-LNP minimally affects global cell signaling pathways compared to conventional transfection reagents, even with

higher mRNA concentrations. In another study, Loureiro et al. explore the potential of caffeic acid (CA) loaded into engineered LNPs for Alzheimer's disease (AD) therapy, capitalizing on CA's anti-amyloidogenic, anti-inflammatory, and antioxidant properties.^[76] The team generates CA-liposomes using various techniques, conjugating transferrin (Tf) to the surface to direct the nanoparticles to the BBB. The lipid vesicles' physicochemical properties indicate suitable sizes for brain delivery and a neutral surface charge. The optimized delivery system's anti-amyloidogenic efficacy suggests its potential to prevent and treat AD by preventing A β aggregation and fibril formation.

Bone

The bone matrix, which has a dense and mineralized structure mainly comprised of collagen and hydroxyapatite, has a dual function.^[77-78] It provides structural robustness and a barrier against nanoparticle penetration. This calls for the design of LNPs specifically crafted to bypass or infiltrate this barrier without affecting their therapeutic content.^[79] Within this dense matrix, nestled away, is the bone marrow, which is an important site for hematopoiesis. Here, an intricate interplay of stem, progenitor, and immune cells occurs within specialized niches, further complicating targeted delivery.^[80] The bone's unique vascular adaptation, directed by endothelial cells and the bone-lining osteoblasts, adds another layer of obstacles to LNPs' access to the inner bone marrow spaces.^[81]

A breakthrough study by Benoit's group focuses on launching novel Micheliolide (MCL) analogs to target leukemic stem cells (LSCs) for delivery through bone-targeted polymeric nanoparticles.^[82] They made significant strides in this area by zeroing in on plant-derived sesquiterpene lactone natural products, particularly MCL, given their selective cytotoxicity towards LSCs, and went on to develop analogs with enhanced antileukemic activity.^[83] Another study by Peer's group spotlights the administration of therapeutic substances toward the bone marrow as part of multiple myeloma treatments via targeted LNPs.^[84] This particular type of cancer, originating in differentiated plasma cells housed in the bone marrow, stubbornly remains incurable despite advances in therapeutic techniques.^[85]

Pancreas

The pancreas, particularly in relation to conditions such as pancreatitis or pancreatic cancer, is defined by its densely fibrotic stroma.^[86] This fibrous tissue is not only a significant physical obstacle that restricts the effective diffusion and penetration of therapeutic agents, like LNPs, but it also engages in complex bio-chemical dialogues with pancreatic cells, potentially influencing therapeutic delivery and actions.^[87] The pancreas's critical roles in digestion and metabolism mean it has an extensive blood supply and variable blood flow, which can accelerate the clearance of administered agents.^[88] Furthermore, the action of several enzymes, particularly those involved in lipid metabolism, could compromise the integrity and stability of LNPs, possibly leading to their degradation before reaching the target cells within the pancreas.

Plus, considering the pancreas's dual functionality in endocrine and exocrine operations, precision in delivery to specific cells, such as insulin-producing β cells for diabetes treatment - without negatively impacting neighboring cells and tissues - is crucial.^[89] This multi-layered complexity adds intricacy to achieving effective and precise LNP-mediated therapeutic delivery to the pancreas. In a pioneering study, Whitehead's team used ionizable LNPs to target the pancreas's macrophages.^[90] By adjusting and refining nanoparticle chemistry and administration routes, these optimized LNPs are promised to facilitate mRNA-induced protein expression uniquely within the pancreas. By employing cationic helper lipids, such as 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), these LNPs display robust and specific protein expression in the pancreas following an intraperitoneal delivery.^[91]

Chithrani's team, in another study, assessed the efficacy of a lipid-encapsulated docetaxel (DTX) prodrug with a specific focus on targeting pancreatic cancer.^[92] Comparing the bio-distribution of gold nanoparticles (GNPs) alongside LNPDTX-P against free DTX in pancreatic cancer models, both in vitro and in vivo, the research aimed to determine the optimal timing for achieving maximum GNP uptake in cancer cells before the combined treatment. The results showcased that pancreatic tumor samples treated with LNPDTX-P recorded a two-fold increase in GNP uptake, demonstrating the

enhanced targeting capabilities of LNPDTX-P towards pancreatic tumors.

Conclusions

Traditional drug delivery methods such as liposomes, polymeric nanoparticles, and micelles are effective for a broad range of treatments. However, they often lack the targeted delivery capabilities of advanced nanoparticle systems.^[93] LNPs offer significant advantages over these nanocarriers by ensuring targeted delivery that minimizes systemic side effects and maximizes drug accumulation at the disease site. The structural integrity and customizable surface properties of LNPs provide superior stability and tailored bio-distribution, which are often challenging with liposomes. Compared to polymeric nanoparticles, LNPs typically show a more favorable safety profile and a less complex synthetic route, which is crucial for clinical translation and scalability. While beneficial for certain applications, Micelles usually have a lower loading capacity and stability than LNPs.^[94]

The advancement of LNPs as carriers for drug delivery marks a substantial progression in targeted therapy. Numerous strategies have been implemented to fortify the potency and precision of LNP-facilitated delivery to intended cells or tissues. The capacity of LNPs to be refined with complete control over elements like size, charge, and surface traits paves the way for creating highly specialized delivery systems.^[95] These systems overcome biological barriers and confine off-target effects. Passive targeting,

leveraging the EPR, active targeting through ligand-receptor engagement, and stimuli-mediated strategies for controlled drug release offer promising improvements in therapeutic results.^[11,25,36]

However, challenges remain. These include achieving heightened targeting precision, avoiding swift clearance and detection by the immune system, and addressing the variability of disease states. Future progress in LNP technology will presumably incorporate multi-targeting tactics that combine passive, active, and stimuli-responsive elements, ensuring the optimal delivery for enhanced therapeutic effectiveness.^[96]

In addition to LNP properties, the administration route can also impact LNPs' targeting and efficacy. Intravenous administration ensures rapid and widespread distribution and is often used for liver, spleen, and lymph node targeting through passive and endogenous strategies, though it risks off-target effects and immunogenicity.^[97] Intramuscular and subcutaneous routes are common for vaccines, promoting localized immune response and facilitating active targeting through ligand-receptor interactions. Intradermal and microneedle delivery offer minimally invasive options that enhance immune cell targeting by leveraging the skin's immune landscape.^[98] Each route's characteristics must align with the specific LNP targeting strategy, whether passive, endogenous, or active, to optimize therapeutic outcomes and minimize systemic side effects.

Conclusions

The sustained development of LNPs, focusing on improved biocompatibility, reduced immunogenicity, and scalable manufacturing processes, is crucial for their successful transition from research laboratories to clinical use. Given the rapid advancements in nanomedicine, LNPs are expected to play a pivotal role in delivering an array of therapeutic agents, ranging from conventional pharmaceuticals to cutting-edge nucleic acid therapies. Insights gained from current research and development will undeniably steer the formation of innovative LNP compositions capable of addressing the multidimensional requirements of personalized medicine, thereby providing new treatment possibilities for various diseases that currently pose a challenge to conventional therapeutic approaches.

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