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Nanoplatform-Based Delivery Systems for Enhanced Stability and Efficacy of Nucleic Acid Vaccines

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ABSTRACT

The rapid development of nucleic acid-based vaccines represents a major advancement in immunization strategies, characterized by their reliance on host cellular machinery to produce antigens. These third-generation platforms, including DNA and mRNA vaccines, offer key advantages such as rapid scalability, simplified manufacturing, and versatility in targeting a broad spectrum of diseases caused by infectious pathogens or conditions like cancer. While they avoid the use of live pathogens and thereby minimize many infection-related risks associated with pathogen reversion, potential side effects such as immune overactivation or off-target responses remain key safety considerations. Nanotechnology, particularly lipid nanoparticle (LNP) systems, has emerged as a critical enabler in addressing these limitations. LNPs enhance nucleic acid stability, promote cellular uptake, and facilitate targeted delivery, thereby improving both immune activation and overall safety profiles. Innovations in ionizable lipid design, PEGylation, and size-controlled formulations have been pivotal to the success of mRNA vaccines in response to the COVID-19 pandemic. This review focuses primarily on LNPs as delivery platforms, while also discussing emerging nanotechnologies under investigation in both preclinical and clinical settings. By examining advances in nanoparticle engineering, delivery strategies, and disease-specific applications, this review aims to provide a comprehensive overview of how nanotechnology is reshaping the future of nucleic acid vaccine development. This underscores the transformative potential of nanoscale delivery systems in overcoming current barriers and accelerating the innovation of next-generation vaccines.

INTRODUCTION

Since the late 18th century, vaccines have played a pivotal role in reducing worldwide morbidity and mortality from infectious diseases [1]. Traditional vaccines, which include live attenuated, inactivated, toxoid, and subunit types, have achieved significant success in the eradication or near-eradication of diseases such as smallpox and polio, and in the effective control of others like diphtheria and measles [2]. Despite their efficacy, conventional vaccination approaches face several inherent challenges. For instance, while oral polio vaccines (OPV) and other live attenuated vaccines have been effective, they carry a small but notable risk of reversion to a virulent form, especially in immunocompromised individuals or under-vaccinated populations. Additionally, live attenuated vaccines require precise conditions, such as controlled storage, to ensure safety and effectiveness. Inactivated vaccines often elicit limited immune responses, necessitating the use of adjuvants to improve efficacy. On the other hand, the production of these vaccines involves complex processes due to biosafety requirements and the risk of contamination [3]. Similarly, subunit and recombinant protein-based vaccines exhibit low immunogenicity and rely on delivery systems to achieve effective protection [4].

The ongoing emergence of new pathogens and the resurgence of known ones emphasizes the urgent need for innovative vaccine technologies that are not only highly effective but also facilitate rapid development [5]. Nucleic acid-based vaccines—including both DNA and mRNA

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vaccines—have emerged as promising alternatives to traditional approaches, especially in response to the COVID-19 pandemic. The success of mRNA vaccines such as BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) has highlighted the potential of this technology to enable rapid design, scalable production, and effective immune responses [6]. These vaccines offer unique advantages, such as rapid adaptability to emerging pathogens, simplified manufacturing, and the potential for inducing robust cellular and humoral immunity. Moreover, they avoid the risks associated with live pathogen use, making them suitable for a wide population, including immunocompromised individuals and other vulnerable groups. However, they are not entirely risk-free, as rare adverse events (*e.g.*, anaphylaxis or myocarditis) or excessive immune responses may still occur. Despite these advantages, nucleic acid vaccines face critical hurdles—including poor *in vivo* stability, inefficient cellular delivery, low immunogenicity without proper formulation, and potential safety concerns such as immune overstimulation or rare inflammatory reactions [7].

This is where nanotechnology, particularly the application of lipid nanoparticles (LNPs), becomes essential. LNPs play a crucial role in protecting nucleic acids from enzymatic degradation, facilitating cellular uptake via endocytosis, enabling endosomal escape, and ensuring tissue-specific biodistribution. Without such delivery systems, the clinical efficacy of mRNA and DNA vaccines would be severely limited, as evidenced by challenges in stability and uptake.

Accordingly, this review focuses on both DNA and mRNA vaccines by exploring their immunological principles, current challenges, and clinical progress across a broad range of applications, including infectious diseases, cancer, and other non-infectious conditions in diverse global contexts, as well as preclinical developments [6, 7]. We place additional emphasis on lipid nanoparticle-based delivery systems, which have been key to recent vaccine success during the COVID-19 era in global health contexts, as well as emerging nanotechnologies such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) that are under development to address remaining ongoing delivery and stability issues, such as enzymatic degradation, thereby illustrating their advantages over conventional platforms, with an eye toward future innovations.

In summary, by reviewing the recent evolution of vaccine platforms, the distinct key benefits of nucleic acid vaccines over conventional approaches, and the central role of nanotechnology in advancing these platforms, this review provides a detailed overview of the field and its future directions, with implications for global health.

Ultimately, the authors aim to highlight both the ongoing limitations and future prospects of nucleic acid vaccines, and illustrate through examples how continued innovation in nanotechnology can advance vaccine development for infectious and non-infectious diseases equally, including safety enhancements.

Vaccine generations: A background

Researchers have advanced vaccine development through three generations. The first generation includes live-attenuated and inactivated vaccines, such as the live-attenuated smallpox vaccine, live-attenuated measles vaccine, and inactivated polio vaccine. The second generation includes protein subunit, toxoid, and conjugate vaccines—for example, the recombinant hepatitis B, diphtheria, and *Haemophilus influenzae* type B (Hib) vaccines [4]. These platforms significantly reduce the risk of adverse effects associated with live or inactivated vaccines. The third generation encompasses advanced platforms such as nucleic acids (DNA or mRNA), and virus-like particles (VLPs). These vaccines offer distinct advantages—such as rapid development and adaptability—but they also present critical challenges, including the need for efficient delivery and stability, which must be addressed to ensure optimal clinical outcomes [8, 9]. Nucleic acid-based vaccines consist of circular plasmid DNA or mRNA that encodes the desired antigen(s). Unlike conventional vaccines that use whole pathogens or subunit forms, nucleic acid-based vaccines rely on host cellular machinery to express the antigen [7]. They provide benefits like rapid development but require solutions for delivery and stability challenges to optimize clinical outcomes.

Advantages of nucleic acid-based vaccines

Simplified manufacturing and stability

DNA vaccines and mRNA vaccines both present innovative approaches to vaccine development, but they differ significantly in terms of manufacturing complexity and stability. DNA vaccines are relatively straightforward to construct compared to protein-based ones using molecular techniques such as polymerase chain reaction (PCR) or synthetic methods (*e.g.*, gene synthesis). In DNA vaccine construction, researchers insert the antigen-encoding gene into bacterial plasmids, which they subsequently amplified within bacterial hosts such as *Escherichia coli* (*E. coli*). The standardized plasmid-based manufacturing processes allow cost-effective large-scale production in bacterial bioreactors [10, 11]. Unlike protein-based vaccines, which require complex storage systems, DNA vaccines are relatively more stable (*e.g.*, tolerating room temperature for limited periods), making them easier to store and transport (*e.g.*, without strict cold chain) in various climates. This offers a practical advantage for global distribution [10]. DNA vaccine stability offers a practical advantage for global distribution, especially in low-resource settings, facilitating equitable access. In contrast, mRNA vaccines' production involves *in vitro* transcription processes that can be scaled efficiently using cell-free systems through enzymatic methods. Improvements in codon optimization

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and mRNA design algorithms, *e.g.*, LinearDesign and RNAstructure, enhance translational efficiency by aligning codon usage with host tRNA availability. They also minimize mRNA by optimizing secondary structure, thereby increasing production yield and stability. These codon and structure optimizations can improve protein expression in host cells up to 10-fold [12-14].

Virtually any protein antigen can be encoded, allowing vaccines to be personalized and tailored for infectious diseases and cancer immunotherapy [15]. Furthermore, the mRNA platform enables rapid updates against emerging pathogens by encoding antigens targeting various viral strains, such as those used in SARS-CoV-2 vaccines [16] and beyond (*e.g.*, variant-specific ones).

Safety and non-pathogenicity

Compared to live attenuated vaccines, which carry the risk of reversion to virulent forms in under-vaccinated populations (see Table 1), DNA vaccines are non-replicating and non-transmissible, thereby greatly reducing the potential for secondary infections [17] (see Figure 1) in diverse populations, including pregnant individuals. Similar to DNA vaccines, mRNA vaccines also avoid the risk of reversion to virulent pathogenic forms, ensuring a generally strong safety profile despite rare events particularly advantageous for immunocompromised individuals and the elderly [18], as confirmed by post-approval studies. Preclinical and clinical studies confirm the positive overall favorable safety profile of DNA vaccines, demonstrating minimal toxicity and no induction of anti-DNA antibodies, thereby enabling repeated administrations as needed for booster doses without major immunogenicity concerns [19, 20] in animal models and humans. In contrast to DNA vaccines, mRNA vaccines, which do not integrate into the genome, induce transient immune responses and degrade quickly *in vivo* (within hours to days), reinforcing their safety and versatility in various therapeutic applications [21].

Table 1. Comparison of vaccine generations

Vaccine generation	Vaccine type	Advantages	Disadvantages
First generation	Live attenuated/inactivated vaccines	Strong immunogenicity	Risk of reversion to virulent form (live attenuated)/weaker immunogenicity and requirement for multiple doses (inactivated vaccines)
Second generation	Protein subunit vaccines, toxoid vaccines, conjugate vaccines	High safety, no risk of infection	Requires adjuvants for enhanced efficacy
Third generation	DNA vaccines/mRNA vaccines	Rapid production, induction of cell-mediated and humoral immunity	Lower immunogenicity and inefficient delivery (DNA vaccines)/challenges with stability, storage, and immune overstimulation (mRNA vaccines)

Table 2. Composition of LNPs

Component	Role	Example
Ionizable lipids	RNA encapsulation and endosomal escape	DLin-MC3-DMA, ALC-0315
Phospholipids	Structural integrity	DSPC
Cholesterol	Stability and transfection efficiency	Cholesterol
PEG-lipids	Prolonged circulation and reduced aggregation	DMG-PEG or ALC-0159

Abbreviations: LNPs: lipid nanoparticles, RNA: ribonucleic acid, DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine, PEG: polyethylene glycol, PEG-lipids: polyethylene glycol–conjugated lipids, DMG-PEG (also written PEG2000-DMG): 1,2-dimyristoyl-rac-glycerol–polyethylene glycol 2000, DLin-MC3-DMA: ionizable lipid commonly referred to as "MC3", ALC-0315: proprietary ionizable lipid (code name), ALC-0159: proprietary PEG-lipid (code name)

Induction of both humoral and cellular immune responses

A key advantage of DNA vaccines lies in their ability to induce both humoral (antibody-mediated) and cellular (T-cell-mediated) immune responses in diverse populations. Antigen expression occurs intracellularly in host cells following transcription and translation, which are presented through both major histocompatibility complex (MHC) Class I and II pathways. This process activates $CD4^+$ helper T cells, $CD8^+$ effector cytotoxic T cells, and B cells, collectively generating humoral and cellular immunity via cross-presentation [17] (Figure 1). This dual immune activation is particularly important for diseases requiring strong cellular immunity (*e.g.*, via $CD8^+$ responses), such as cancer, autoimmune conditions, and chronic viral infections, including emerging infections [8, 22]. Similarly to DNA vaccines, mRNA vaccines also trigger both humoral and cellular immunity effectively, with modifications to the mRNA sequence, *e.g.*, 5' cap base methylation, as well as the inclusion of stabilizing agents further enhancing these responses [23]. DNA vaccines, however, are uniquely versatile, capable of incorporating multiple genes within a single plasmid to encode various antigens or immune-modulatory molecules, such as cytokines (*e.g.*, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF)) and co-stimulatory proteins (*e.g.*, CD40L, IgG-Fc), to potentiate immune responses [24]. Additionally, codon optimization improves antigen expression levels, while synthetic unmethylated CpG motifs on the plasmid backbone stimulate Toll-like receptor 9 (TLR9) pathways, activating innate immunity

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and counteracting general tumor immune suppression mediated by regulatory T (Treg) cells [22].

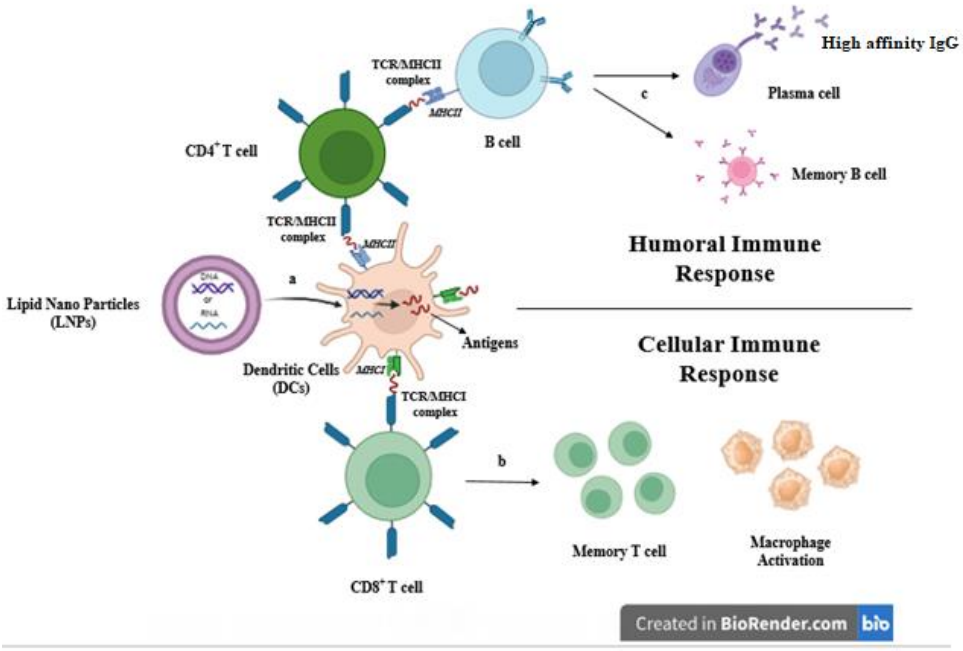

Fig. 1. A schematic illustration of the humoral and cell-mediated immune responses induced by nucleic acid vaccines: a) LNPs encapsulating nucleic acids (DNA or RNA) directly transfect dendritic cells (DCs), leading to the translation of nucleic acids into antigens, which are then presented to T cells through T cell receptors (TCRs) via both MHC-I and MHC-II molecules. b) MHC-I pathway: Cell-mediated immune responses, including the activation and proliferation of $CD8^+$ T cells and macrophages. c) MHC-II pathway: Humoral immune responses, involving the activation of $CD4^+$ helper T cells, which subsequently stimulate B cell activation, proliferation, and antibody secretion.

Challenges of nucleic acid-based vaccines

Low immunogenicity

Nucleic acid-based vaccines, particularly DNA vaccines, reportedly display reduced immunogenicity in humans across diverse populations compared to traditional vaccines. The reason is that only a very small fraction (*e.g.*, <1%) of the injected DNA or mRNA reaches target cells, consequently reducing antigen production and subsequent immune responses [25, 26]. Efficient antigen expression requires nuclear delivery for DNA vaccines, which poses significant technical challenges, such as overcoming cellular membranes and other biological barriers (*e.g.*, endosomal escape) [17]. While DNA vaccines demonstrate robust immunogenicity in animal models, similar outcomes are not consistently observed in humans due to differences in immune system components and DNA uptake efficiency across species. These limitations significantly reduce the clinical efficacy of DNA vaccines, particularly in larger mammals and humans [26, 27].

In contrast, mRNA vaccines are generally highly immunogenic, but they too face challenges, such as RNA degradation, stability issues, or waning immunity, which can diminish their effectiveness. However, ongoing advancements in vaccine formulation and the development of booster regimens are helping to address these issues, offering promising solutions to enhance their clinical utility [28].

Delivery efficiency to target cells

Ensuring the effective intracellular delivery of nucleic acid molecules to target cells continues to be a significant challenge. Nucleic acids like DNA, due to their large size and negative charge, are unable to efficiently cross the cell membrane without external assistance. Similarly, mRNA is inherently unstable and highly susceptible to degradation by nucleases, necessitating chemical modifications or protective delivery systems, such as lipid nanoparticles, to enhance its stability and delivery efficiency [17].

For optimal therapeutic outcomes, delivery systems must be capable of targeting specific tissues or cells, such as dendritic cells, to maximize immune responses while minimizing potential side effects. Non-viral delivery systems, including lipid nanoparticles and polymer formulations, show great promise but still require additional advancements to improve their stability and precision in targeted delivery [25]. This targeting can be achieved through several strategies, including ligand-receptor interactions that guide nanoparticles to specific cell types, tissue-specific promoters that drive gene expression only in selected cells, and surface

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modifications of delivery vehicles to improve cell-type recognition and uptake.

Safety concerns

Although DNA vaccines are generally considered safe, theoretical concerns remain regarding their potential integration into the host genome, which could lead to insertional mutagenesis or disruption of critical genes, such as tumor suppressor genes [27]. Circular plasmids pose a lower integration risk compared to linear DNA; however, ongoing safety monitoring is essential to mitigate any potential long-term risks. Additionally, the prolonged expression of foreign antigens or the presence of unmethylated CpG motifs in DNA could inadvertently activate autoimmune pathways. Despite these concerns, existing evidence indicates that the risks are minimal when DNA vaccines are appropriately designed and administered [26].

Conversely, mRNA vaccines may cause excessive immune stimulation, which can lead to inflammation or cytokine storms, underscoring the importance of careful dose optimization and monitoring [29]. Delivery vehicles, such as lipid nanoparticles, may also induce local or systemic reactions, including rare cases of inflammation or anaphylaxis. Innovations in delivery systems, codon optimization, and immunostimulatory components are critical to addressing these challenges and maximizing the clinical potential of nucleic acid vaccines [25].

Impact of nanotechnology in addressing nucleic acid vaccine challenges

Nanotechnology has transformed medicine, particularly in drug delivery and vaccine development. The unique characteristics of nanoparticles, such as their small size and high surface area-to-volume ratio, have enhanced drug solubility, improved bioavailability, and reduced toxicity. These advancements enable selective drug delivery, prolonged therapeutic effects, and reduced off-target effects, driving innovation in fields like genetic medicine, oncology, and infectious diseases [30]. In nucleic acid vaccine delivery, emerging nanocarrier systems offer significant benefits. They protect DNA and mRNA from nuclease degradation, enhance cellular uptake, and maintain stability during circulation. Moreover, these carriers often function as self-adjuvants, stimulating cytokine production and ensuring targeted antigen delivery to specific immune cells or tissues, thereby improving vaccine specificity and effectiveness. Such nanomaterials are particularly promising for co-delivering DNA vaccines with immunostimulatory molecules, amplifying immune activation and overall vaccine efficacy [8, 31].

Lipid-based nanoparticles in vaccine development

Lipid nanoparticles (LNPs) are highly efficient carriers for nucleic acid therapeutics, including siRNA, saRNA, and mRNA. By encapsulating nucleic acids, LNPs protect these molecules from enzymatic degradation, enhance their stability, and enable precise delivery to target tissues, such as the liver (hepatocytes) and immune cells like dendritic cells and macrophages [32]. The clinical approval of Onpattro® in 2018 was a groundbreaking achievement for LNP-based therapies, showcasing their effectiveness in delivering siRNA to treat hereditary transthyretin-mediated amyloidosis [33-35].

In addition to their roles in protecting nucleic acids and enhancing cellular uptake, LNPs can also act as adjuvants, contributing to immune activation. During the COVID-19 pandemic, LNPs played a crucial role in the success of mRNA vaccines (Pfizer/BioNTech and Moderna), offering high efficacy rates, scalability, and the facilitation of rapid development timelines [36].

Historical development of LNPs

Liposomes: the first generation of lipid-based nanoparticles

Liposomes, the pioneering generation of lipid nanoparticles, were discovered in 1961 by Alec Bangham. These bilayer lipid vesicles were the first nanoparticles to achieve clinical application, receiving FDA approval in the 1990s for Doxil®, a doxorubicin formulation used to treat ovarian and breast cancer [37]. Additionally, liposomes have been successfully utilized in anticancer, anti-inflammatory, and antifungal treatments, in gene therapy, and in vaccines such as Inflexal® V for influenza and Epaxal® for hepatitis A [38].

Liposome-based delivery systems offer controlled drug and vaccine release while minimizing off-target effects. However, challenges remain, particularly in complex manufacturing processes and scalability, which require ongoing innovation and optimization [39].

SLNs and NLCs: the second generation of lipid-based nanoparticles

Solid lipid nanoparticles (SLNs), composed of solid lipids, exhibit superior physical stability compared to liposomes. In contrast, nanostructured lipid carriers (NLCs), which combine solid and liquid lipids, enhance drug-loading efficiency and bioavailability. Both SLNs and NLCs offer scalability, improved stability, and sustained drug release, effectively addressing the major limitations of earlier liposomal systems [40].

Niosomes: nonionic surfactant-based alternatives

Niosomes, nonionic surfactant-based vesicles, have been developed as cost-effective and stable alternatives to liposomes. They have demonstrated potential in delivering plasmid DNA and viral antigens, making them promising candidates for vaccine applications against diseases such as influenza and hepatitis B. While niosomes are effective for oral, transdermal, and parenteral drug delivery, their use in mucosal routes, such as intranasal and pulmonary delivery, remains underexplored and warrants further investigation [41].

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Composition and functionality of LNPs

LNPs are composed of four key lipid components, each contributing uniquely to their functionality (see Table 2): Ionizable lipids play a critical role in RNA delivery by facilitating RNA encapsulation at acidic pH (~4.0) through electrostatic interactions, while neutralizing at physiological pH (7.4) to reduce toxicity and enhance biocompatibility. Notable examples include DLin-MC3-DMA, utilized in Onpattro®, and proprietary lipids such as ALC-0315 (Pfizer) and SM-102 (Moderna). They enable delivery of an estimated approximately 50-100 mRNA molecules per LNP [42, 43]. These lipids also exhibit charge changes in acidic environments, such as endosomes, enabling endosomal escape and efficient release of RNA into the cytosol.

Additional components critical for RNA delivery include:

Phospholipids: Provide structural integrity and support the lipid bilayer (*e.g*., DSPC) [44]. Cholesterol: Stabilizes the lipid structure and enhances transfection efficiency [45].

PEG-lipids: Prevent particle aggregation, prolong systemic circulation, and minimize immune system recognition [46].

Optimal LNP performance requires precise tuning of particle size (~80–100 nm) and molar ratios of these components to maximize delivery efficiency, optimize biodistribution, and minimize immunogenicity. Fine-tuning the lipid composition is essential for balancing RNA release efficiency while minimizing toxicity [47].

Influence of lipid nanoparticle size on delivery efficiency and immunogenicity

The size of LNPs is a critical factor in modulating the immune response. Particle size significantly impacts cellular uptake, interactions with immune cells, and the efficiency of payload delivery (*e.g*., nucleic acids). The influence of particle size on immune responses is outlined below:

Uptake by immune cells

LNPs are primarily designed to deliver their payload to antigen-presenting cells (APCs), such as dendritic cells (DCs), which are key in initiating immune responses. The size of LNPs affects their uptake efficiency and the type of immune response they trigger:

- Smaller LNPs (~20-100 nm): These nanoparticles are more readily taken up by lymphatic tissues and can efficiently enter dendritic cells and macrophages through endocytosis. This size range optimizes LNP drainage to lymph nodes to support immune activation. Small LNPs (around 60 nm) tend to show better accumulation in these nodes, enhancing the activation of T cells and promoting the development of adaptive immunity [48, 49].
- Larger LNPs (~150-200 nm): Larger particles may be less efficient at entering cells through endocytosis, but they are more likely to be phagocytosed by macrophages, which can help initiate an immune response through different mechanisms. Larger LNPs may also trigger a more robust innate immune response due to increased interactions with pattern recognition receptors (PRRs) on immune cells. However, their size can limit their ability to reach the lymph nodes as quickly as smaller particles [50, 51].

Immune activation

Particle size also impacts the nature of immune activation:

- Smaller particles (under 100 nm) often induce a more potent adaptive immunity because they are more efficiently delivered to the appropriate cells in lymph nodes, where they can activate T and B cells. This size is ideal for mRNA vaccines, as the mRNA is efficiently delivered into the cytoplasm of cells, leading to antigen expression and the activation of both $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells. This results in long-lasting immunity [36].
- Larger particles may elicit more innate immune responses, as they are more likely to interact with Toll-like receptors (TLRs) or complement receptors on macrophages and dendritic cells. This could potentially enhance the inflammatory response or induce cytokine production, which might be beneficial for stimulating certain types of immunity, like the activation of helper T cells and antibody production. However, a robust innate response might also cause unwanted side effects like excessive inflammation [52].

Humoral versus cell-mediated immunity

The immune response can be classified into humoral immunity (antibody production by B cells) and cell-mediated immunity (T cell activation):

- Smaller LNPs: Their ability to efficiently target dendritic cells and other APCs in lymph nodes leads to stronger humoral immunity, enhancing antibody production. This is particularly important for vaccines, where the goal is often to produce neutralizing antibodies that can protect against future infections [50].
- Larger LNPs: Larger LNPs are more likely to trigger a stronger innate immune response, which can enhance cell-mediated immunity, including the activation of cytotoxic T cells. While this may be useful for targeting specific intracellular pathogens or tumors, it may not be as effective for vaccines aimed at inducing antibody responses alone [53].

Complement activation

Larger LNPs are more likely to activate the complement system, a component of the innate immune response that helps to clear pathogens. This can lead to the deposition of complement proteins on the LNPs, which can facilitate their recognition by phagocytic cells,

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thereby enhancing the immune response. However, excessive complement activation could lead to inflammation or unwanted tissue damage [54].

Particle size and antigen presentation

The particle size can also influence how well the LNPs present antigens to immune cells:

- Smaller LNPS tend to induce more efficient antigen presentation. Their smaller size allows them to be more readily internalized by dendritic cells, where the encapsulated mRNA (or other antigens) can be processed and presented on MHC class I or class II molecules. This leads to a more robust adaptive immune response [55].
- Larger LNPs might have a slower or less efficient antigen presentation, but they can stimulate a stronger immune response through different pathways, like increasing antigen uptake by macrophages or stimulating different types of T cells [54].

Safety considerations

While smaller LNPs may exhibit greater immune response efficiency, they can also carry the risk of increased immunogenicity and potential side effects, such as allergic reactions or inflammation. Larger LNPs, on the other hand, may have a greater risk of triggering unwanted innate immune activation and inflammation, which could lead to tissue damage or more severe side effects [56].

Biodistribution, clearance, and safety of LNPs
Biodistribution

The distribution of LNPs depends on the route of administration. In intravenous administration, LNPs tend to accumulate in the liver, as seen with the siRNA drug Patisiran, where 97% of the dose was found in the liver within hours [57-59]. For intramuscular administration, LNPs, such as those used in the Pfizer-BioNTech COVID-19 vaccine, localize primarily at the injection site and then drain to nearby lymph nodes, initiating immune responses [59]. Subcutaneous administration, especially of smaller LNPs (<100 nm), results primarily in accumulation within draining lymph nodes, with minimal systemic distribution to organs such as the liver and spleen [36, 60].

Clearance and biodegradability

The design of LNPs incorporates biodegradable ionizable lipids, which facilitate rapid clearance after fulfilling their role. For example, ionizable lipids like SM-102 and ALC-0315 used in vaccines undergo hydrolysis *in vivo*, ensuring rapid elimination and reduced accumulation [61]. Biodegradable LNPs are generally better tolerated, with fewer adverse reactions at the injection site and a lower risk of long-term toxicity [62].

Cytotoxicity and safety

While LNPs are largely safe, certain components, such as cationic lipids and PEGylated lipids, can trigger adverse effects like cytotoxicity and immune responses. PEGylation, for instance, can lead to antibody formation against PEG, limiting repeated dosing [61]. Strategies to mitigate these effects include optimizing lipid composition and minimizing toxic components [59].

CONCLUSIONS

Nucleic acid-based vaccines, encompassing DNA and mRNA platforms, represent a transformative leap in vaccine technology, offering simplified manufacturing processes, rapid scalability, and the ability to induce robust humoral and cell-mediated immune responses. The integration of advanced delivery systems, particularly LNPs, has enhanced their clinical success, as evidenced during the COVID-19 pandemic.

Despite these advancements, several challenges remain. DNA vaccines face hurdles related to low immunogenicity and inefficient nuclear delivery, while mRNA vaccines require improvements in stability and mitigation of excessive immune activation. Additionally, safety concerns, including potential risks of DNA integration and inflammation from delivery systems, necessitate rigorous monitoring and optimization. Emerging nanotechnologies, such as LNPs, SLNs, and NLCs, are addressing these issues by improving delivery efficiency, protecting nucleic acids from degradation, and fine-tuning immune activation.

The convergence of nanotechnology and nucleic acid vaccine platforms underscores the potential to overcome existing limitations and unlock new therapeutic possibilities. Future research focusing on enhancing delivery systems, refining vaccine formulations, and addressing immunogenicity gaps will be pivotal for broadening their applicability and ensuring their long-term safety. As this field continues to evolve, nucleic acid-based vaccines are poised to redefine the landscape of preventive and therapeutic medicine, offering hope for combating a wide array of diseases with unprecedented precision and efficacy.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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DATA AVAILABILITY

This is a review article; all referenced data are available in the cited sources.

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AUTHORS' CONTRIBUTIONS

All authors contributed to the writing of the manuscript. MG conceptualized the review. SM and KB reviewed the literature, and prepared the initial draft of the manuscript.

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