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REVIEW

Toward Safe and Effective Gene Therapy: Non-Viral Nanostructured Delivery Systems

Fatemeh Madani[1], Elaheh Izadi[2], Maral Motamedi[1], Mazdak Ganjalikhani Hakemi[3], Zohreh Saltanatpour[2], Thomas J Webster[4–7], Seyed Farzad Mohammadi[2]

[1]Department of Medical Nanotechnology, Tehran University of Medical Sciences, Tehran, Iran; [2]Translational Ophthalmology Research Center, Tehran University of Medical Sciences, Tehran, Iran; [3]Regenerative and Restorative Medicine Research Center (REMER), Research Institute for Health Sciences and Technologies (SABITA), Istanbul Medipol University, Istanbul, Turkey; [4]Division of Pre-College and Undergraduate Studies, Brown University, Providence, RI, USA; [5]School of Biomedical Engineering and Health Sciences, Hebei University of Technology, Tianjin, People's Republic of China; [6]School of Engineering, Saveetha University, Chennai, India; [7]Department of Pharmacy, University of the Basque Country, Vitoria, Spain

Correspondence: Zohreh Saltanatpour; Thomas J Webster, Email zohre_saltanatpour@yahoo.com; thomas_webster@brown.edu

Abstract: Gene therapy has emerged as a transformative strategy for treating a wide range of genetic and acquired disorders. Despite its potential, clinical translation is hindered by the limitations of viral vectors, including immunogenicity, insertional mutagenesis, limited cargo capacity, and production challenges. Consequently, non-viral gene delivery systems have gained increasing attention as safer, more versatile alternatives. These platforms, including lipid-based nanoparticles, polymers, dendrimers, inorganic nanocarriers, and hybrid systems, offer customizable physicochemical properties, scalable manufacturing, and reduced risk of adverse immune responses. This review systematically expresses recent advances in non-viral vectors, focusing on the key parameters that influence cellular uptake, endosomal escape, nuclear localization, and overall transfection efficiency in various disease, especially cancers. Additionally, we evaluate recent preclinical and clinical studies, highlighting promising translational outcomes and therapeutic applications. By comparing different non-viral strategies and discussing their mechanistic underpinnings, this review underscores the potential of non-viral vectors to overcome the inherent limitations of viral delivery and to drive the development of next-generation gene therapy approaches that are safer, more adaptable, and clinically relevant.

Keywords: nanomedicine, gene therapy, gene delivery, non-viral vectors, nanoparticles

Introduction

Over the past three decades, significant technological advancements have transformed gene delivery systems from the direct delivery of naked nucleic acids to more sophisticated platforms employing biomaterials as non-viral vectors.[1,2] Gene therapy offers great capability to address cancers, inherited disorders, and viral infections, by addressing their genetic origins through gene replacement, inactivation, or the introduction of therapeutic proteins at the target site.[3,4] Multiple forms of genetic material such as plasmid DNA (pDNA), messenger RNA (mRNA), small interfering RNA (siRNA), microRNA, antisense oligonucleotides, and genome-editing complexes like CRISPR/Cas9 have been utilized in preclinical settings with considerable success.[5,6] However, the clinical translation of these therapies remains limited, primarily due to delivery challenges. Naked nucleic acids are prone to enzymatic degradation and rapid systemic clearance, while their size and negative charge hinder efficient cellular uptake. Additional intracellular barriers, particularly the nuclear membrane, further restrict effective gene expression.[7–9] These limitations highlight the need for delivery platforms that can protect genetic material and facilitate targeted intracellular transport.[10]

Non-viral vectors have emerged as attractive candidates due to their favorable safety profiles, high loading capacity, and design flexibility. These systems include physical methods and synthetic carriers such as polymers and lipid-based nanostructures, although many still face challenges related to delivery efficiency and biological barriers.[5–7] Recent advances in nanotechnology have enabled the development of more effective and biocompatible delivery platforms. Despite encouraging preclinical outcomes, the clinical translation of non-viral systems remains challenging and, in some

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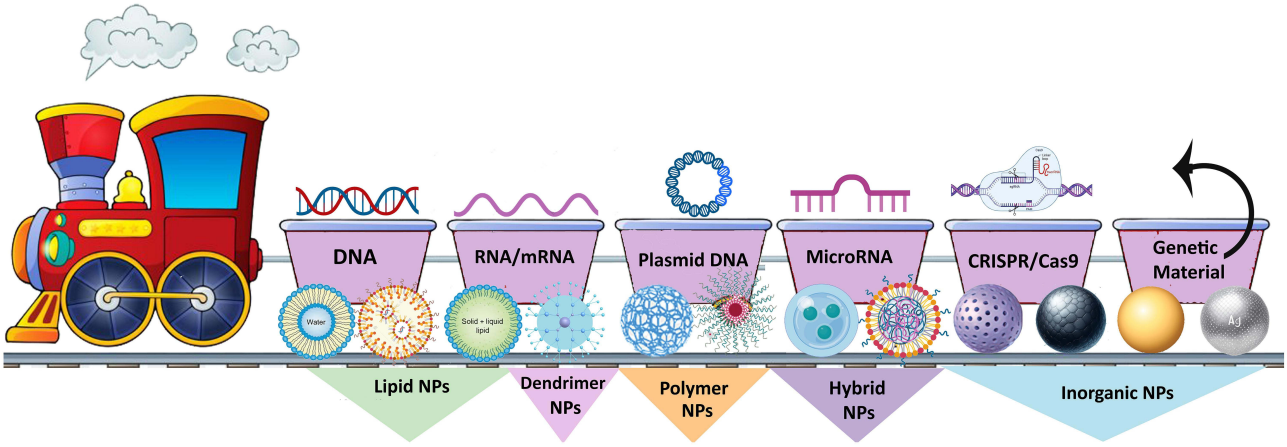
Graphical Abstract



cases, controversial.[11] This review focuses on recent advances in non-viral gene delivery systems for the treatment of various disease, especially cancers. Furthermore in vitro, in vivo, and clinical trials are highlighted, with particular emphasis on current clinical trial outcomes and the challenges limiting their successful translation.

Principles of Non-Viral Gene Delivery

Non-viral vectors have emerged as promising alternatives for gene delivery due to their lower immunogenicity, design flexibility, and capacity to carry large genetic payloads.[1,12] Unlike viral systems, they lack intrinsic mechanisms for efficient cellular entry and intracellular trafficking; therefore, their successful application depends on rational design strategies that enable them to overcome multiple biological barriers, including cellular internalization, endosomal escape, and sustained gene expression. Advances in nanotechnology, polymer chemistry, and biomaterials science have significantly contributed to the development of non-viral platforms with improved targeting ability, controlled release, and biocompatibility.[13–16]

Non-viral gene delivery systems can be broadly categorized into physical, chemical, and hybrid approaches. Physical methods, such as electroporation, microinjection, and ultrasound-mediated delivery, rely on external forces to introduce genetic material into cells. In contrast, chemical systems including polymers, cationic lipids, and dendrimers form complexes with nucleic acids (polyplexes or lipoplexes), facilitating cellular uptake through endocytosis or membrane fusion. Hybrid systems combine these strategies to further enhance delivery efficiency.[17–19]

From a material perspective, non-viral vectors are commonly designed as nanoparticle (NP)-based systems. NPs, typically ranging from 1 to 1000 nm in size, exhibit unique physicochemical properties that distinguish them from bulk materials and make them suitable for gene delivery applications.[18,20] These systems protect nucleic acids from enzymatic degradation, improve cellular uptake, and enable controlled intracellular release. Their performance is strongly influenced by key parameters such as size, zeta potential, and polydispersity index (PDI). For biomedical applications, NP size is generally maintained between 1 and 200 nm to enhance tissue penetration and cellular uptake. Surface charge plays a critical role in stability and membrane interaction, where moderate positive charge improves transfection efficiency, while excessive charge may induce cytotoxicity. A low PDI (<0.3) ensures uniformity and reproducibility of delivery behavior.[21–25] Based on composition, NP-based non-viral vectors can be classified into several groups, including lipid-based, polymer-based, inorganic, dendrimer-based, carbon-based, and hybrid systems.[14,26–28] Each category employs distinct mechanisms to encapsulate, protect, and deliver genetic material.

Lipid NPs (LNPs) are among the most widely used non-viral delivery systems. Due to their structural similarity to biological membranes, they exhibit excellent biocompatibility and can encapsulate both hydrophilic and hydrophobic

molecules. This property enables efficient delivery of diverse nucleic acids, including DNA, RNA, and siRNA. Following cellular uptake, LNPs facilitate the release of genetic material into the cytoplasm. However, their stability in biological fluids remains a challenge, as interactions with serum proteins may lead to destabilization and reduced delivery efficiency.[29,30]

Polymer-based NPs (PNPs) represent another versatile class of non-viral vectors with high loading capacity and tunable properties. Cationic polymers interact electrostatically with negatively charged nucleic acids, forming compact structures that protect genetic cargo and promote cellular uptake via endocytosis.[31] Their chemical structure can be modified to enhance biocompatibility and reduce toxicity.[32] However, issues such as cytotoxicity at high concentrations and non-specific interactions remain concerns. Parameters such as the nitrogen-to-phosphate (N/P) ratio play a critical role in determining complex stability, transfection efficiency, and overall biological performance.[33–35] Figure 1 provides a schematic view of how the N/P ratio affects the efficiency of gene delivery systems. Breakthroughs in polymer chemistry have contributed to the development of biodegradable and biocompatible polymers, which can enhance the safety profile of these vectors while preserving efficacy.[33–35]

Inorganic NPs, including gold NPs (Au NPs) and iron oxide NPs (IONPs), offer advantages such as large surface area, ease of synthesis, and high reproducibility.[36] The surface functionalization of IONPs is another key feature, enabling the attachment of targeting ligands or other molecules that can enhance cellular uptake and specificity to the targeted tissue. The stability of these NPs facilitates their efficient accumulation within cells, where they can exert an influence on cellular behavior, such as promoting gene expression or altering cell growth.[4] Additionally, their resistance under various environmental conditions further supports their potential for gene delivery applications; though challenges related to their potential toxicity and aggregation still need to be considered.[37–39]

To improve delivery performance, non-viral vectors are often functionalized with specific chemical groups. These modifications serve various purposes:

- Functional groups like peptides or antibodies can be attached to the vector backbone to confer specificity. This ensures that the gene delivery system selectively targets the intended cells, mitigating off-target risks and advancing the therapeutic outcome.[40,41]
- The backbone can also be modified to strengthen the complex's integrity while circulating in vivo, such as by adding hydrophilic groups like polyethylene glycol (PEG), which prevents the aggregation of the vector and protects it from immune reactions.[42]

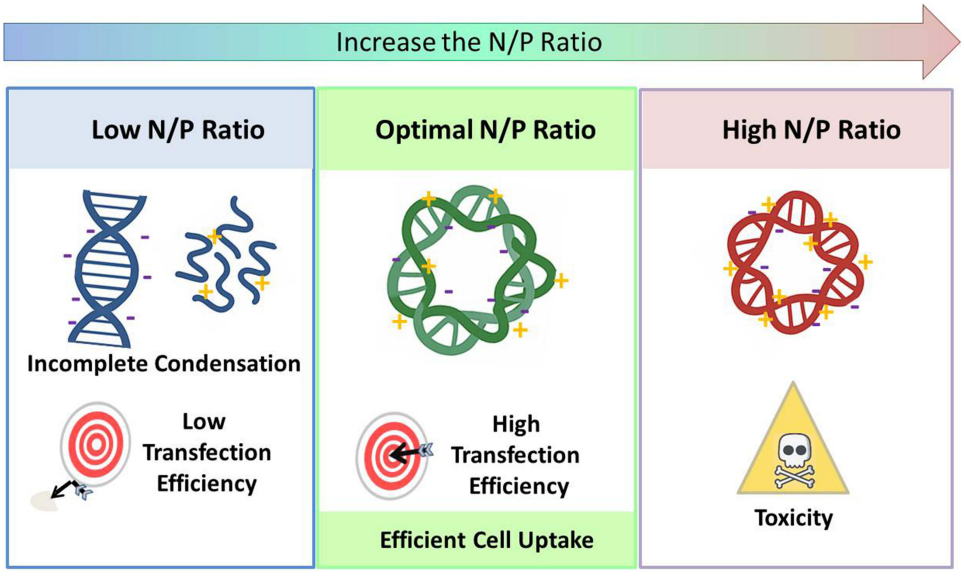


Figure 1 Effect of the nitrogen to phosphate ratio on the functionality of genetic material delivery platforms.

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- pH-sensitive functional groups can be incorporated to make the vector responsive to environmental changes, helping the vector escape the endosome after cellular uptake. At acidic pH levels in the endosome, these groups can trigger a conformational change in the vector that destabilizes endosomes, releasing the pDNA into the cytoplasm.[43,44]

Another factor that should be considered is the interactions of the gene delivery system within biological compartments, particularly serum proteins, immune cells, and the extracellular matrix. Effective gene delivery depends on the vector's ability to evade serum protein binding (which can prevent cellular uptake) and resist degradation by nucleases. Modifying the vector with specific functional groups, such as PEGylation, can enhance the vector's ability to extend systemic circulation time by shielding it from serum proteins and immune surveillance. Additionally, these modifications can help the vector and circumvent quick elimination by the mononuclear phagocyte system (MPS), which is responsible for removing foreign particles from the body.[45–47]

After cellular uptake, the genetic materials must be transported within the cell to reach its target site, typically the nucleus, for gene expression. The process of intracellular trafficking involves several steps (Figure 2):

- Endocytosis: After binding to the cell membrane, the vector is internalized by the cell through endocytosis. However, once inside the cell, the genetic materials are trapped within an endosome, and the proton-rich environment inside endosomes causing the degradation of genetic substances if it is not released in time.
- Endosomal Escape: Ensuring successful endosomal escape is a critical challenge for non-viral gene delivery approaches. To impede this phenomenon, functional groups can be included to promote the rupture of the endosomal membrane resulting from the decrease in pH within the endosome, allowing the genetic materials to escape into the cytoplasm. Once in the cytoplasm, the genetic materials can be transported to the nucleus.
- Nuclear Localization: Genetic materials must reach the nucleus for gene expression. Some vectors incorporate nuclear localization signals (NLS) or other functional groups which assist in the penetration of the genetic materials into the nucleus, where it can be transcribed and translated into proteins. This step is crucial, in particular, when the aim is to modify the expression of specific genes within the target cells.[48–51]

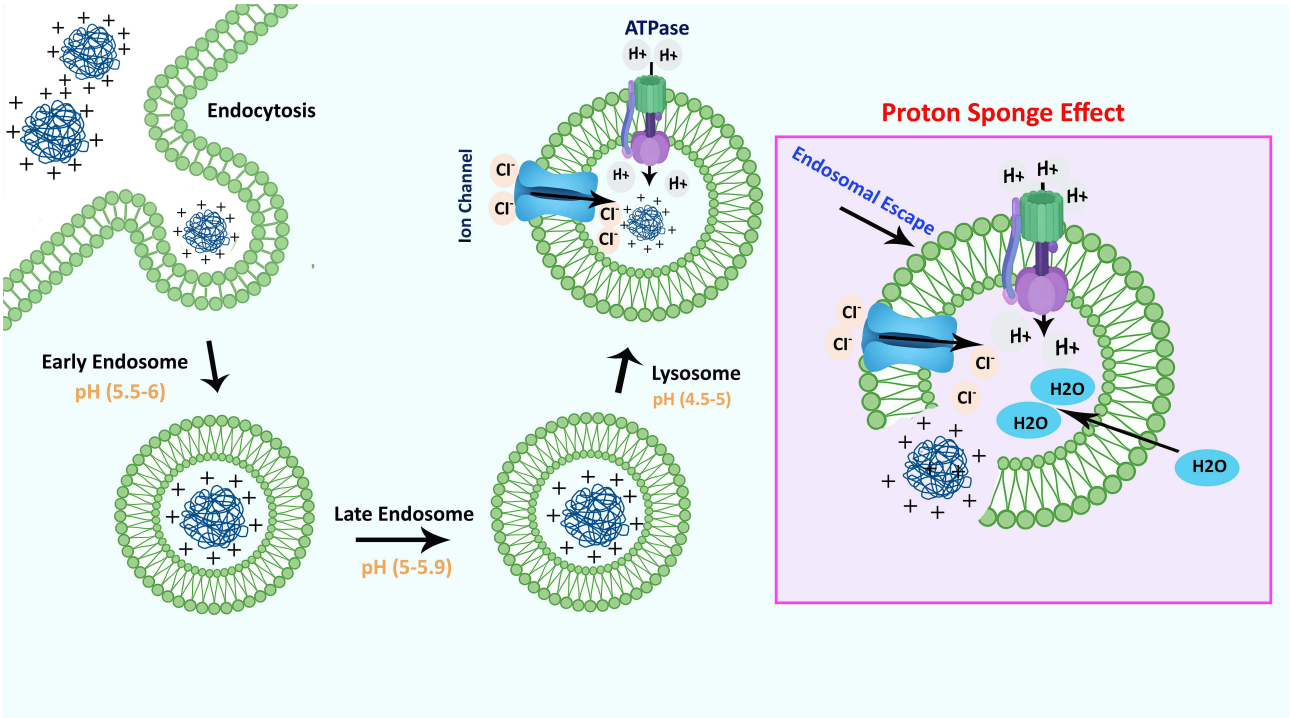

Figure 2 The process of successful intracellular trafficking of genetic materials.

Non-viral platforms are missing the built-in capacity of viruses to effectively traverse cellular barriers. Therefore, during their engineering process, it is necessary to optimize them in order to promote endocytosis and endosomal escape, resulting in the cytosolic delivery of nucleic acids. The achievement of endosomal escape can be facilitated through the utilization of several techniques inherent in the vector, such as the proton-sponge effect aided by NPs.[52]

Non-Viral Gene Delivery Nanoparticles

In the context of gene delivery, NPs can encapsulate genetic material offering protection from enzymatic degradation while facilitating its delivery. The size, zeta potential and functional groups of NPs are critical factors influencing their cellular uptake, interaction with cellular membranes, and ability to escape endosomal compartments for efficient gene release. Furthermore, the ability of NPs to cross biological barriers, such as the blood-brain barrier (BBB) or tumor vasculature, enhances their potential for targeted gene therapy.[18,20]

The physicochemical properties of NPs, including size, zeta potential, and PDI, are essential to their performance in gene delivery. The size of NPs typically ranges from 1 to 200 nm for biomedical applications, with smaller NPs facilitating better cellular uptake and tissue penetration, which are crucial for effective gene delivery. Zeta potential, which reflects the surface charge of NPs, influences their stability in suspension and their interaction with cell membranes. A positively charged surface enhances binding to the negatively charged cell membrane, improving transfection efficiency, while excessively high charges may cause cytotoxicity. The PDI, which measures the uniformity of NP size distribution, is ideally kept below 0.3 for consistent and reliable gene delivery, as a narrow PDI ensures uniform behavior across the NP population. Together, these factors govern the effectiveness and safety of NPs as delivery vehicles for genetic materials.[21–25] NPs can be categorized based on their composition into five primary groups: organic, inorganic, dendrimer, carbon-based and hybrids.[26,27] Figure 3 depicts various types of NPs in gene delivery applications.

Lipid NPs (LNPs)

LNPs are very effective delivery systems for various nucleic acids containing DNA, mRNA, siRNA and pDNA.[53,54] Administering bare, chemically unaltered nucleic acids directly, is inefficient since they are easily broken down by nucleases and can be potentially harmful because of the immunological response of the host. The utilization of LNPs, which incorporate ionizable or cationic lipid molecules to promote nucleic acid condensation, presents an effective and straightforward approach to address these challenges and effectively transport nucleic acid to tissue.[55]

Cationic lipids possess a broad spectrum of sizes, a significant capacity for DNA loading, and excellent storage stability.[56] The cationic lipids used in liposomes are amphiphilic and composed of a cationic amine group connected to a hydrocarbon chain. One crucial characteristic is their capacity for electrostatic interaction between their positively charged head group and the negatively charged nucleic acids enabling encapsulation of the nucleic acids.[57] There is a wide range of commercially available LNPs and lipofectamine is a commonly referenced example.[58] Nevertheless, the application of LNPs is limited due to their short-term expression levels and cytotoxicity.[59] Various attempts have been made to improve LNPs formulations while improving various factors that can make them suitable for clinical use. These factors include: (i) being easy to replicate and scale up,[60] (ii) effectively encapsulating genetic materials,[60] (iii) controlling their physical and chemical characteristics such as size, charge and hydrophobicity,[61] (iv) assessing their pharmacokinetic properties such as preventing fast clearance and accumulating in specific tissues,[62] (v) studying the processes of internalization; and (vi) understanding the principles of nucleic acid release.[63]

Liposomes

Liposomes are a kind of LNP and as the oldest version of LNP, are structures consisting of either unilamellar or multilamellar phospholipid bilayers that surround an aqueous core, allowing for the encapsulation of genetic materials. The preparation of liposomes is induced by the interaction between polar head groups (hydrophilic) and nonpolar tail groups (hydrophobic) of phospholipids.[54] Widely used in numerous therapeutic applications, liposomes serve as carriers for drugs and genes owing to intrinsic potential in biodegradation, effectiveness, low toxicity, and convenient preparation.[57,64] One remarkable example of gene delivery via LNPs includes Onpattro®, an invaluable tool for gene

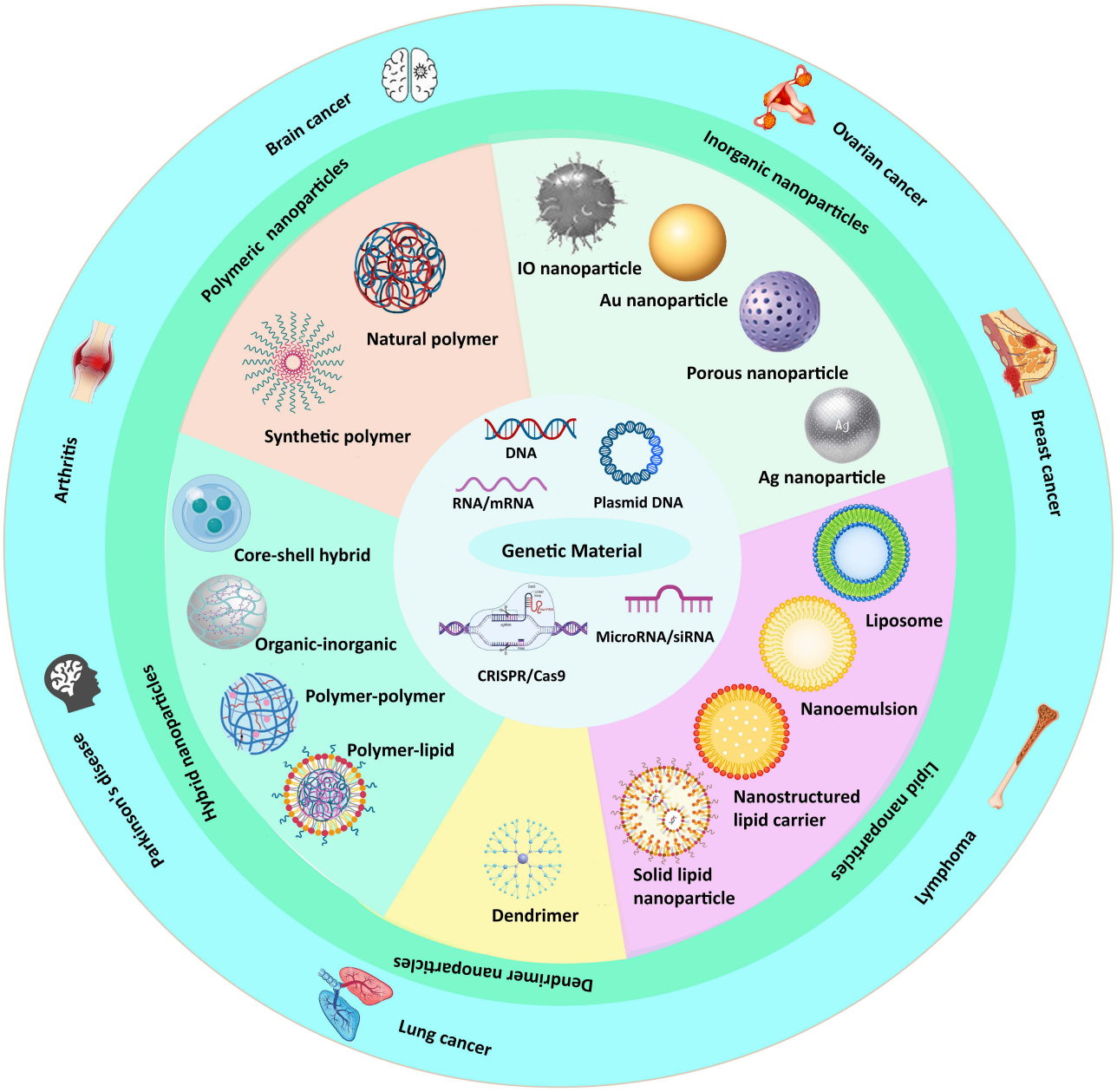

Figure 3 Various types of NPs used in gene delivery.

regulation in the liver.[65] Several other nucleic acid drugs based on LNPs have been subjected to clinical evaluation for the management of genetic diseases, cancers, or infectious disease.[66]

Micelles

Micelles are nanostructures formed through the self-assembly of amphiphilic structures (polymers or lipids) characterized by hydrophilic and hydrophobic parts, such as surfactants or block copolymers. These molecules spontaneously organize into a spherical or cylindrical shape in an aqueous environment (10 to 100 nm).[67] Micelles are endowed with properties that enable them to be suitable options for gene delivery systems. Their structure enables the encapsulation of both aqueous and lipid-soluble drugs within the hydrophilic shell and hydrophobic core, respectively. This property enables the encapsulation of a broad range of genetic materials, such as pDNA, siRNA, and mRNA.[68,69] Another advantage of using micelles in the gene delivery field involves enhancing the bioavailability of compounds with limited solubility, which is applicable for

hydrophobic nucleic acids. The small size of micelles promotes efficient cellular uptake through endocytosis, a vital process in gene delivery. Following internalization, micelles can evade entrapment within endosomes, which is crucial for releasing the encapsulated genetic material into the cytoplasm for effective gene expression.[68,70,71]

Solid Lipid NPs (SLNs)

SLNs are a type of nanostructure constructed from solid lipids that remain solid at both room and body temperature which have received great recognition in the realm of advanced delivery platforms (50 to 1000 nm). Their solid lipid core enhances stability by protecting encapsulated compounds from degradation.[72–74]

Controlled release, along with surface modification using targeting ligands, enhance the efficiency of SLNs in gene delivery and reduce off-target effects. Moreover, SLNs have greater potential for gene therapy because of their capability to breach cellular and tissue barriers, such as the BBB, and their non-toxic profile. Additionally the concept of endosomal escape in gene delivery systems, such as SLNs, pertains to the ability of these nanostructures to release genetic material from the endosome into the cytoplasm.[75]

Nanostructured Lipid Carriers (NLCs)

NLCs (50 to 500 nm) are advanced nanostructures that have revolutionized delivery system technology by intelligently combining solid and liquid lipids. The hybrid structure of the NLC matrix provides a significant improvement in performance such as: stability, flexibility, and loading capacity compared to SLNs. High shear homogenization, micro-emulsion, or solvent evaporation are common preparation methods.[76,77] The incorporation of a semi-crystalline matrix in the formulation enhances its colloidal stability, which is contingent upon the quantity of solid lipid employed. However, it is stated that NLCs are more readily synthesized and sterilized compared to other SLNs, and they exhibit less toxicity.[78]

A study by Erasmus et al successfully generated an NLC formulation and paired it with self-amplifying mRNA producing Zika virus antigens. Zika virus-rvRNA was combined with NLC at N:P ratios of 50 and 15, respectively. The NPs had mean diameter of 40–100 nm with zeta potential from 15 to 28 mV. This combination resulted in complete defense against the Zika virus in C57BL/6 mice, immunized with a single intramuscular (I.M.) dose (10 ng).[79]

Despite their potential, NLCs face key limitations in gene delivery applications, including suboptimal nucleic acid loading capacity, instability of lipid–nucleic acid complexes, potential cytotoxicity, and manufacturing complexities. These challenges have hindered their progression into clinical development.[80] As of 2023, no NLC-based formulations for mRNA delivery or vaccines have received approval from the FDA.[80,81] It is predicted that by optimizing formulation methods and conducting long term studies, clinical applications of these nanostructures will be improved over the next decade.[82]

Polymer NPs (PNPs) and Polymeric Micelles

In the last twenty years, there has been significant utilization of biodegradable polymers in biomedical applications.[83,84] Biodegradable polymers may be broadly grouped into two distinct classes namely natural and synthetic polymers.[32,85] Both of them have distinct merits and drawbacks. One notable characteristic of natural polymers is their exceptional biocompatibility, bioactivity, and ability to undergo cell-activated proteolytic breakdown.[32] Nevertheless, the exact purification, characterization, and identification of the chemical composition and content of natural polymers pose significant challenges, leading to an issue of batch-to-batch variance.[84,86]

The formulation optimization of PNPs and micelles for gene delivery has attracted considerable interest recently because of the numerous advantages they offer. Micelles possess characteristics that make them well suited for use in gene delivery systems. Their architecture allows them to carry both water-soluble and lipid-soluble compounds, with hydrophilic substances accommodated in the outer shell and hydrophobic ones in the core. As a result, they can encapsulate a wide variety of genetic materials, including pDNA, siRNA, and mRNA.[68,69]

Despite their considerable therapeutic potential, several critical challenges must be addressed to fully realize their clinical utility. Among these are the requirements of biocompatibility, biodegradability, and effective encapsulation of nucleic acids, enabling controlled and targeted delivery, tailoring physicochemical properties, enhancing cellular uptake and endosomal escape, prolonging systemic circulation while minimizing clearance, and ensuring scalability and

reproducibility of production processes.[32,87] Collectively, these considerations underscore the necessity for the continued optimization of PNPs as non-viral gene delivery vectors to improve their safety, efficacy, and translational potential.[32,88]

Natural Polymers

Natural polymer NPs with a size range of about 50–200 nm[89] have significant advantages including biocompatibility, biodegradability, and functional versatility. Alginate, gelatin, albumin, polysaccharides, chitosan and cyclodextrins, are widely used to fabricate natural PNPs for gene delivery. For example, chitosan is a positively charged polysaccharide, that is widely used for encapsulating nucleic acids and drugs because of its property of adhering to mucus and its ability to form NPs with controlled release profiles.[90] Alginate is an important material in the development of biomedical technologies extracted from brown seaweed. It is mainly used to develop hydrogels and NP formulations and biological scaffolds. It should be mentioned that the excellent biocompatibility and gelation properties under physiological conditions make hydrogels a very appropriate option in biomedicine.[91] Alginate NP sizes range from nanometer to sub-micron (~50–300 nm depending on their preparation method).[92] Moreover, chemical modification of biopolymers has the potential to increase their stability and responsiveness to environmental stimuli and efficiency. Natural polymers with their inherent biodegradability and low toxicity result in the creation of safer and more effective therapeutic platforms.[93]

Gelatin and albumin are the predominant proteins utilized in gene-delivery vectors. Gelatin polymers are extensively applied for the fabrication of vectors because of their notable attributes of minimal antigenicity and excellent biocompatibility.[94] Gelatin is derived from animal collagen. This polymer has amphiphilic properties, characterized by the presence of cationic and anionic charges, together with hydrophobic groups, at a predominant 1:1:1 ratio.[95] It possesses several reactive groups on its surface, which serve to connect and alter various molecules, thus, endowing the polymer with a diverse range of functions. Gelatin possesses amino acid sequences in its structure, namely the cell adhesive Arg-Gly-Asp (RGD) peptide, which sets it apart from other polymers.[96–99]

Polysaccharide-based gene-delivery vectors have predominantly utilized chitosan, which is made up of irregularly scattered β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine, owing to its inherent positive charge. Chitosan expresses meager solubility in physiological conditions and because of its low pKa, it has inefficient complexation potential.[100] Chitosan is a polysaccharide with positive charges, which is derived from chitin (an abundant biopolymer found primarily in fungus and arthropods).[101] Chitosan is biocompatible and biodegradable, making it an attractive material for gene delivery. It can form NPs by electrostatically binding, facilitating the intracellular transfection of genetic material, because of its cationic nature.[101–105]

Cyclodextrins (cyclic oligosaccharides) exist naturally and are classified as natural polymers[106] linked by (α-1,4) bonds, arranged in a basket-like pattern exhibiting an amphipathic nature with an "endo-exo" structure. The stability of structures formed through the interaction of unmodified cyclodextrins with pDNA is limited, and their impact on transfection efficiency is relatively modest. The cavity size is precisely defined at the sub-nanometer scale and varies according to the type of cyclodextrin, with approximate internal diameters of ~0.5 nm for α-cyclodextrin, ~0.6–0.7 nm for β-cyclodextrin, and ~0.7–0.8 nm for γ-cyclodextrin.[107]

Synthetic Polymers

Synthetic polymers have been known as famous substances for the development of nanostructures and their size ranges can be tuned from nanometers to micrometers.[32,108] In contrast to natural polymers, synthetic polymers offer the benefit of exact control over molecular weight, chemical composition, and structural characteristics, enabling the precise adjustment of their performance and functionality.[109] PNPs expressed promising results for controlled release systems and, furthermore, they can be easily modified with targeting ligands or genetic materials; providing multiple functions simultaneously.[110]

Aliphatic polyesters are known for biodegradation[84,111] and various forms of polyesters, which differ based on the monomer, have been widely utilized for gene-delivery in recent years. Polyester-based gene-delivery polymers that are frequently employed in various applications encompass polyethylenimine (PEI) poly(beta-amino ester) (PBAE), PEG, poly-L-lysine (PLL), polycaprolactone (PCL) and poly(lactic-co-glycolic acid) (PLGA) which have been chosen as potential vectors.[112] In this review manuscript, we will describe the structures that have been studied more extensively than others.

PEI demonstrates the proton sponge effect, which is a phenomenon where positively charged polymers or NPs typically in the nanometer range (~50–300 nm), once inside acidic endosomal compartments accumulate protons, and this accumulation leads to an increase in osmotic pressure. The osmotic imbalance disrupts the integrity of the endosomal membrane allowing the encapsulated genetic material to escape into the cytoplasm.[113,114] The buffering property of the substance induces swelling and rupture of endosomes, leading to polyplexes release. Endosome escape is believed to be caused by several causes, apart from the proton sponge effect. Protonation of PEI leads to the elongation of the polymer chain because of electrostatic repulsion, resulting in the swelling of the polymer.[95,115–118] To achieve cellular absorption through ligand-mediated mechanisms, PEI can be combined with PEG to protect the surface charges of PEI before attaching a ligand like the anti-HER1 antibody for precise delivery.[119,120]

PBAE is a cationic polymer which demonstrates pH-responsive properties, providing useful properties for nucleic acid delivery.[87,121–124] The ability to modify PBAE structures to reach sustained release makes them suitable for numerous gene delivery platforms.[96]

PEG is a hydrophilic, biocompatible polymer broadly exploited in gene delivery. PEG chains can be linked to NPs or biomolecules; this procedure increases the stability and pharmacokinetic properties of gene delivery systems by reducing opsonization, lowering renal clearance, and prolonging circulation time in the bloodstream. In gene delivery, PEGylated NPs, such as LNPs and PNPs (eg, PEG-PLGA), facilitate systemic administration, and promote targeted cellular uptake that improve transfection efficiency. Nevertheless, cellular internalization and endosomal escape are affected by extreme PEGylation.[45]

Biswal et al designed a pDNA expressing dihydrofolate reductase (DHFR) siRNA that was condensed with folate-PEG-PEI and introduced into KB cells and A549 cells. This complex formed self-assembled polyelectrolyte complexes with acidic genetic materials and cationic polymers. The study demonstrated that siRNA was successfully transported into KB cells, resulting in the suppression of DHFR. Conversely, A549 cells had an inability to internalize the siRNA nanostructure. It was determined that the complexation enabled targeted delivery by FR positive cells, thereby establishing them as a delivery system with an excellent level of specificity.[125]

Zhang et al designed a system focused on the controlled delivery of Pt (IV) for platinum-resistant ovarian cancer (PROC). The findings from an in vivo study on BALB/c nude mice showed that the NPs had an ability to selectively target tumor sites, resulting in enhanced gene silencing and antitumor effectiveness via subcutaneous (S.C.) administration.[126]

PLL is a positively charged polymer composed of repeating lysine units; this structure allows PLL to form electrostatic bonds with negatively charged nucleic acids, thereby, enhancing their encapsulation and facilitating their delivery. The biocompatibility and ability of PLL to facilitate cellular uptake make it a popular choice for gene delivery systems. This application enhances the efficiency of transfection preserving the genetic material from degradation.[127]

Li et al designed a PNP system that exhibited long-lasting gene transport vector. This system was biodegradable and demonstrated effective gene transfer when employing the pDNA expressing GFP (pGFP). The NPs consisted of three parts: (i) a PEG outer layer, (ii) a shell of PLGA, and (iii) and a core of PBAE/pGFP. The average particle diameter was about 165 nm with a poly dispersity index (PDI) of ~0.1 indicating that the particles were very homogeneous in size at about 130 nm. Furthermore, it was observed that adding the cationic polymer PBAE to PLGA-PEG NPs significantly increased pGFP entrapment efficiency (from 3% to 97%).[6]

Another investigation by Pretzer et al, was performed to synthesize a biotin-conjugated block copolymer micelle. The NPs were 50–60 nm and the zeta-potential was 4 mV. In this study at a N/P ratio= 20, the transfection efficiency for pDNA was variable in various formulations, however by enhancing the N/P ratio $\geq$ 100, polyplexes expressed similar transfection efficiencies about 72% and 85% for N/P =100 and 300, respectively.[128]

Inorganic NPs (NPs)

Controlling the size, morphology and surface charge of inorganic NPs allows for the optimization of cellular uptake and endosome escape: two key factors in efficient gene delivery. These features, combined with magnetic fields or light as external stimuli, make inorganic NPs a promising tool as further described below.[129]

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AuNPs

AuNPs are considered ideal candidates in this realm because of biocompatibility, chemical stability and extensive surface modification capabilities. Through thiol bonds or electrostatic interactions, AuNPs can be modified. These NPs are considered promising alternatives to conventional gene delivery methods by providing a favorable balance between cytotoxicity and delivery efficiency.[130]

Silver NPs (AgNPs)

AgNPs are prominent candidates for targeted gene therapy for infection which results from intrinsic antimicrobial properties and surface modification capabilities. By surface modification with cationic agents, the efficiency of gene transfer will be improved. These NPs exert both antimicrobial effects and enhance genetic material delivery.[131]

Iron Oxide NPs (IONPs)

Among all inorganic NPs, IONPs have been studied more and in recent studies, various forms of IONPs have been designed specifically to diagnose and treat cancer.[132] Coating these NPs with polymers or lipids improve their biocompatibility and facilitates the loading of nucleic acids, thereby increasing their potential as gene transfer vectors.[131] The size of these entities renders them highly suitable for surface functionalization. These alterations in the diameter of NPs serve as the foundation for various pharmacological and biological uses, including diagnosis, drug and gene delivery.[133] Two approaches for gene loading in IONPs are:

- The initial method involves the application of cationic chemicals on the surface of IONPs, followed by their binding to negatively charged genes via electrostatic absorption. PEI can be applied as a layer-by-layer coating on the surface of IONPs. The ultimate altered IONPs possess a positive charge to facilitate gene loading. In addition, PEI has the ability to directly alter the surface of IONPs, forming a core-shell platform.[134] According to the results of a study by Bi et al, the platform can bind effectively with siRNA, leading to the formation of evenly distributed NPs.[135] In addition to PEI, other polycations such as poly-arginine, PLL, and chitosan have the potential to be employed for creating surface modification on IONPs to enhance gene binding efficacy.[136,137]
- The second method is the utilization of gene/carrier complexes, wherein the surface charge does not play a decisive role.[135,138]

The prospective applications of IONPs in biological domains are attributed to their unique qualities, such as a greater surface-volume ratio, increased surface energy, and severe reactivity, which can be achieved by reducing their size to below 100 nm.[139]

Silica NPs

Silica-coated metal NPs significantly boost gene delivery system efficiency by creating a stable protective structure. These NPs consist of two main parts: a metal core (usually gold, silver or iron) and a porous silica shell that plays a key role in protecting and delivering genetic material. Surface functionalization of these NPs with amine groups, creates a positive charge that forms bonds with the negative charge of nucleic acids via electrostatic interactions which improves the stability of the gene-NPs complex.[140]

Dendrimers

Dendrimers are branched, tree-like macromolecules composed of a central core and repeating units. Their structure is defined at the nanoscale and their uniformity and their multiple end groups can be modified to bind to nucleic acids, which increases the loading capacity and stability of genetic material. Dendrimers also facilitate efficient cellular uptake and endosome escape, which are essential for successful gene delivery. In addition, their size and shape can be tuned to optimize biodistribution and reduce toxicity.[141,142] Polyamidoamine (PAMAM) dendrimers are a class of three-dimensional branched platforms characterized by active amine groups.[143]

Upon endocytosis of the PAMAM/nucleic acids complex, the amino groups undergo protonation, leading to a substantial inflow of chloride ions. The osmotic pressure within the endosome subsequently increases and ultimately

induces endosomal deterioration.[52] The second generation of PAMAM dendrimers have been developed with specific modifications to improve gene delivery to the brain. These dendrimers contain arginine (R) and histidine (H) residues, which demonstrate a key role in increasing efficiency by attaching the oligopeptide RRHRH to their surface. Cytotoxicity research showed that PAMAM dendrimers were non-toxic to various cell lines. The results of in vivo investigations demonstrated dendrimers had superior gene delivery efficiency.[144] In another study, the polyplex structure was formed by dendrimers via self-assembly at several N/P ratios including: 0.5, 1, 2, 5, 10 and 20. The results of that study demonstrated that this structure could be used as a new and environmentally friendly method for gene or drug delivery, while being less expensive and, unlike traditional carriers, biodegradable.[145]

Hybrid NPs

Hybrid NPs are composites made up of multiple organic and inorganic components. These NPs combine the beneficial properties of specific components to improve their performance in various applications. In this way, they can offer both types of materials in a single constituent unit. This combination helps improve the efficiency of NPs in gene delivery, making them suitable for medical and biological applications because it enhances properties such as stability, biocompatibility, drug loading, and release control. The organic components, like polymers and lipids, are biocompatible and biodegradable, due to their similarity to biological compounds. These structures allow for efficient gene loading and protection, but inorganic materials like metal or silica NPs improve stability, targeting, and controlled release. The key benefit of this combination is to create synergy between the properties of various organic or inorganic components. Functionalization with ligands (specific for cell targeting) and tuning surface features to improve cell entry and endosome escape are two key mechanisms which are implicated in the function of hybrid NPs for gene delivery. Hybrid NPs create a multifunctional platform for the delivery of various genetic materials such as pDNA, siRNA, and mRNA due to their combined features. This technology has been particularly successful for cancer treatment and genetic diseases.[146–148]

Hybrid NPs can solve the challenges associated with single substance NPs in gene delivery, such as the toxicity of NPs. By combining different properties of organic and inorganic materials, hybrid NPs can also offer additional advantages including: enhanced transfection efficiency, additional capabilities, and the capacity to modulate gene expression kinetics.[149] In a study, novel biodegradable PNPs for gene delivery were developed, which are a combination of two polymers: PLGA and PBAE. While PLGA alone has low gene delivery efficiency, its combination with PBAE increases the strength, stability and persistence of the particles. This combination also facilitates fine-tuning of the particle dimensions, which helps in passive targeting to different tissues or phagocytic antigen-presenting cells (APC). Cellular and animal investigations with nanoplatforms of 75–85% PLGA and 15–25% PBAE are the most efficient for gene delivery to APCs, while being relatively safe. These particles have a stimulatory influence on APCs contributing to their value in genetic vaccination strategies.[150]

When lipid shells are paired with a polymer core, they facilitate improved targeting as well as binding of particles to specific cells. This is especially beneficial for hard-to-transfect cells, such as immune cells.[151] Persano et al showed that PBAE/mRNA complexes (polyplexes) coated with a lipid layer with a diameter about 50 nm induced complete gene transfer in DC2.4 cells (similar to dendritic cells). In contrast, uncoated polyplexes showed no detectable gene transfer in DC2.4 cells.[152] Subsequently, polyplexes (coated with lipids) were employed for the purpose of delivering mRNA encoding ovalbumin to immune cells through subcutaneous administration. This process resulted in the stimulation of immune cells, as evidenced by measuring IFN-β levels in blood, as well as the activation of T cells and lymph node cells.[152]

Moreover, lipid coatings have the ability to provide protection to the cationic polymer core, hence mitigating the issue of excessive positive charge-induced toxicity. This becomes highly pertinent within the framework of in vivo applications.[153] Additionally, it has been reported that liposome-coated PBAE particles exhibit negligible toxicity in vivo.[153,154]

Achieving effective transfection in vivo is strongly governed by the balance between lipids and PEG-lipids. Therefore, it is of the utmost importance to optimize these ratios to achieve greater efficacy of transfection.[153] Researchers have succeeded in developing efficient IONPs for DNA delivery using low molecular weight linear PEI (Mw 423 and 1800). This investigation expressed that a N/P ratio of 1 effectively enhanced the DNA binding capacity in comparison to PEI alone.[155] Wang et al developed multifunctional NPs based on IONPs using PEI coatings which had the size range of 10–15 nm. These NPs were used for both MRI imaging and gene delivery to cancer cells. PEI as

a cationic polymer, adsorbs siRNA through electrostatic interactions and prevents in vitro enzymatic degradation. This system effectively restrains cell growth and promotes both apoptotic and autophagic pathways in glioblastoma (U251) cells. This novel technology has great potential for personalized cancer medicine.[156]

In another study conducted by Du et al, a magnetic mesoporous silica loaded microbubble (M-MSN@MBs) was designed specifically for the purpose of ultrasound-mediated imaging and gene transfection. The pDNA incorporated in M-MSNs and the lipid microbubbles were loaded with pDNA-carrying M-MSNs. These NPs had a mean diameter of 1120 ± 345 nm with a zeta potential of −17.65 ± 2.8 mV. The findings of this study suggested that the designed vector had favorable biocompatibility, stability for DNA binding, performance in ultrasonic imaging, and response to magnetic stimuli. The M-MSNs that were modified with PEI showed significant efficacy in preserving the loaded pDNA from enzyme breakdown.[157]

In research by Lin et al, a nanostructure for pDNA delivery to MSCs was fabricated. The nanostructures that were smaller than 400 nm, targets the CD44 receptor on MSCs via hyaluronic acid (HA). The lipid-polymer nanostructures have two parts of DOTAP for DNA compaction and PLGA-PEG-HA for stability and controlled release. With its high transgene efficiency and meager cytotoxicity, this system has great potential for numerus gene therapy applications.[158]

Wang et al investigated the simultaneous treatment of high gene transfection and magnetic resonance imaging (MRI) contrast to enable MRI-guided visual gene transfection.[159] In this study, polymers grafted onto the surface of IONPs@SiO_2 NPs achieved both enhanced transfection efficiency and real-time MRI by utilizing an external magnetic field. In this investigation, the transfection efficiency on C6 and HepG2 cell lines using pDNA pRL-CMV as the reporter gene exhibited a threefold increase when subjected to a magnetic field compared to the control culture. These structures had a size between 500–600 nm and had a one dimensional peapod-like shape that improved penetration into cells as it was revealed that under the influence of a magnetic field, the transfection efficiency could be further enhanced.[159] While there is a large number of hybrid NPs, only some of them are presented and summarized in Figure 4. The major classes of non-viral gene delivery systems, along with their mechanisms of nucleic acid encapsulation, in vivo distribution profiles, advantages, and safety considerations, are summarized in Table 1.

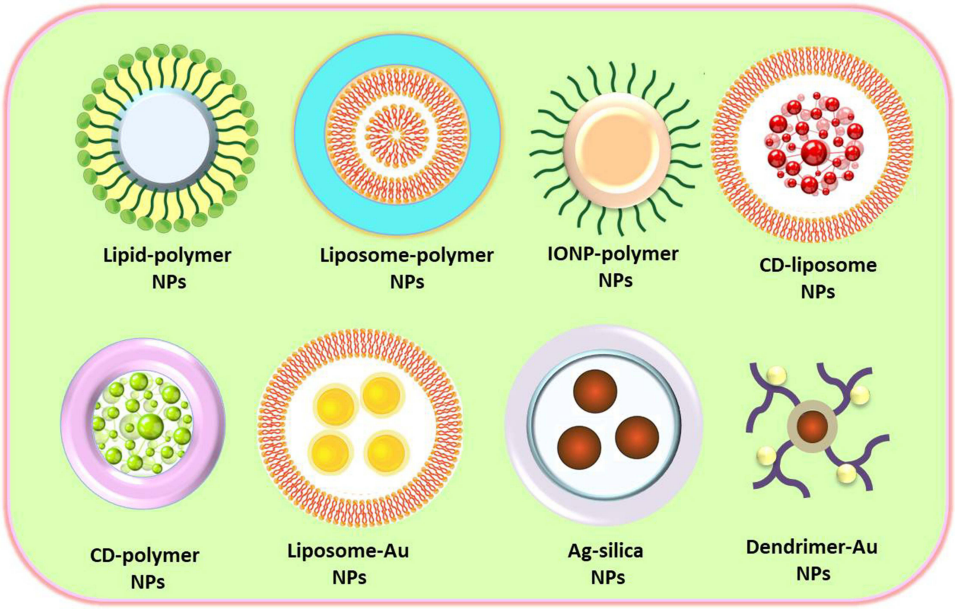

Figure 4 Schematic representation of some types of hybrid NPs formed from combinations of diverse material classes. These include organic–organic hybrids (lipid-polymer NPs and liposome-polymer NPs), organic–inorganic hybrids (IONP-polymer NPs, CD-liposome NPs, CD-polymer NPs and liposome-Au NPs), and inorganic–inorganic hybrids (Ag-silica NPs and dendrimer-Au NPs). The figure illustrates the structural diversity and enhanced multifunctional properties resulting from these hybrid configurations.

Table 1 Major Classes of Non-Viral Gene Delivery Systems and Their Characteristic Mechanisms of Nucleic Acid Encapsulation, Biodistribution Behavior, Advantages, and Safety Considerations

Carrier	Principle of Gene Encapsulation/ Stabilization	Biodistribution	Advantages	Ref
LNPs	Electrostatic complexation and encapsulation of nucleic acids or lipoplexes	Liver accumulation after systemic administration	Clinically validated platform with multiple approved therapeutics and scalable manufacturing	[2,160]
PNPs	Electrostatic condensation of nucleic acids via polymers forming polyplexes	Broad biodistribution depending on polymer chemistry, size, and surface charge	Highly tunable chemistry enabling controlled release and modular carrier design	[161,162]
Inorganic NPs	Physical adsorption or electrostatic binding enabling nucleic acid protection and functionalization	Predominant uptake by liver and spleen due to RES clearance	High stability and potential for theranostic applications	[160,163]
Dendrimers	Binding of nucleic acids via multivalent electrostatic interactions and surface functional groups	Efficient cellular uptake with organ-dependent accumulation influenced by generation size and surface modification	Precisely defined architecture with high loading capacity and multifunctional surface engineering	[164,165]
Hybrid NPs	Electrostatic complexation, encapsulation, or surface adsorption of nucleic acids within multi-component nanostructures	Tunable biodistribution depending on composition; often improved circulation stability	Enhanced stability, multifunctionality, and improved delivery efficiency	[166,167]

Therapeutic Applications of Non-Viral Gene Delivery Systems

Non-viral gene delivery systems have been widely explored across a broad range of disease settings, owing to their favorable safety profile, tunability, and capacity to deliver diverse genetic cargos. Their applications span cancer therapy as well as genetic, infectious, and neurological disorders, in addition to vaccination strategies.[168] However, a substantial proportion of studies remains limited to in vitro investigations. Notably, in vitro models often fail to accurately replicate the complexity of in vivo environments, leading to discrepancies in transfection efficiency, cellular uptake, and toxicity. Furthermore, variations in cell types, culture conditions, and analytical methods reduce reproducibility and hinder direct comparisons across studies.[169,170] The following sections summarize the major disease areas in which non-viral gene delivery systems have been investigated, along with representative recent studies.

Cancer Therapy

Cancer represents one of the most extensively investigated applications of non-viral gene delivery systems. These platforms enable targeted modulation of gene expression within tumor cells and the tumor microenvironment.[171] Common strategies include silencing oncogenes using siRNA,[172] restoring tumor suppressor genes through pDNA, and delivering mRNA to induce the expression of therapeutic proteins.[173] NPs have demonstrated the ability to preferentially accumulate in tumor tissues through the enhanced permeability and retention (EPR) effect. Surface functionalization with targeting ligands further improves selectivity toward cancer cells, minimizing off-target effects.[8] In addition, NPs have been employed to co-deliver multiple therapeutic agents, such as chemotherapeutics and nucleic acids, resulting in synergistic antitumor effects.[174] Beyond direct tumor targeting, non-viral systems are also being explored for cancer immunotherapy. For example, the delivery of mRNA encoding tumor-associated antigens can stimulate antigen presentation and activate cytotoxic T-cell responses.[175] Despite these advances, challenges such as heterogeneous tumor environments, limited penetration into solid tumors, and variable transfection efficiency continue to hinder clinical translation.[176]

Genetic Disorders

Non-viral gene delivery offers a promising approach for the treatment of inherited genetic diseases by enabling gene replacement, correction, or silencing. Delivery systems have been developed to transport functional copies of defective genes or genome-editing tools such as CRISPR/Cas9 to target cells. However, achieving efficient and sustained gene expression in target tissues remains a major limitation.[177] Recent advances in NPs engineering and targeted delivery strategies have improved the therapeutic potential of these systems. For example, lipid- and polymer-based carriers have demonstrated enhanced gene expression and partial functional recovery in preclinical models of cystic fibrosis[178] and muscular dystrophies.[179] Despite these encouraging results, challenges related to tissue-specific targeting and long-term expression continue to limit clinical translation.[30]

Infectious Diseases and Vaccination

Non-viral gene delivery systems have gained significant attention in the prevention and treatment of infectious diseases, particularly through nucleic acid-based vaccines. Recent advances have demonstrated that LNP-mediated delivery of mRNA enables rapid and efficient antigen expression, leading to robust humoral and cellular immune responses. Notably, the clinical success of mRNA vaccines against coronavirus disease 2019 (COVID-19) represents a major milestone, highlighting the translational potential of non-viral delivery platforms.[180] These systems function by protecting nucleic acids from degradation, facilitating cellular uptake, and promoting antigen expression in host cells, which subsequently triggers immune activation. In addition to mRNA vaccines, non-viral vectors have been utilized to deliver DNA vaccines, siRNA, and antisense oligonucleotides for the inhibition of viral replication and modulation of host immune responses.[181] Targeted delivery strategies have further enhanced vaccine efficacy. For example, NPs-based systems can be engineered to accumulate in lymphoid tissues, improving antigen presentation and immune activation. This lymph node-targeting capability is particularly important for inducing strong adaptive immune responses and has been widely explored in next-generation vaccine design.[182,183]

Neurological Disorders

The treatment of neurological diseases using gene therapy is particularly challenging due to the presence of the BBB, which restricts the delivery of therapeutic agents to the central nervous system.[184] NPs have been engineered to overcome this barrier through size optimization, surface modification, and ligand-mediated targeting.[184] These systems have shown potential in delivering therapeutic genes or RNA molecules for conditions such as Parkinson's[185] and multiple sclerosis.[186]

Cardiovascular and Inflammatory Diseases

Non-viral gene delivery has also been investigated for cardiovascular and inflammatory conditions. In cardiovascular diseases, gene delivery strategies aim to promote angiogenesis,[187] tissue regeneration, and repair following injury.[188] Similarly, in inflammatory and autoimmune disorders, nucleic acid-based approaches can modulate the expression of key cytokines and signaling pathways.[189]

Table 2 summarizes recent in vitro and in vivo studies on gene delivery nanostructures.

Clinical Trials of Non-Viral Gene Delivery Systems

In recent years, non-viral nanostructures representing a hopeful alternative to viral-based gene delivery have been studied clinically. With the advancement of nanotechnology, it is expected that non-viral nanostructures will be increasingly used to treat various disease like cancer and genetic diseases.[35,231] Figure 5 depicts clinical trials of gene-delivery structures, performed via the application of NPs. LNPs have been identified as an efficient delivery system for CRISPR/Cas9 components in gene editing therapy. They are used in the treatment of genetic disease such as hereditary transthyretin amyloidosis with polyneuropathy (ATTRv-PN) due to their bioavailability, safety, and immunogenicity. The first clinical trial using LNP for CRISPR/Cas9 delivery was performed by Intelaya Therapeutics. NTLA-2001 delivered via I. V. infusion for CRISPR/Cas9 gene therapy which targeted the TTR gene in hepatocytes for the treatment of ATTRv-

Table 2 Recent Cellular/Animal Applications of Non-Viral Gene Delivery Systems

Disease/ Disorder	NPs	Encapsulant	Cell	Animal	Ref
Cancer	PEI NPs conjugated with extracellular vesicles	siRNAs or anti-miRs	PC3 (prostate carcinoma), SKOV3 (ovarian carcinoma), HCT-116 (colon carcinoma) and Saos-2 (osteosarcoma) cell lines	Athymic nude mice bearing PC3 cells	[190]
	Hyaluronic acid–modified chitosan NPs	BCL2 siRNA	Human lung cancer cells (A549)	-	[191]
	Cationic Au NPs		Mesenchymal stem cells	-	[192]
	Au NPs	Antisense oligonucleotide against BCR-ABL mRNA	K562 cell (erythroleukemia)	-	[193]
	Chimaeric polymersomes (cNGQ/RCCPs)	Polo-like kinase1 specific siRNA (siPLK1)	A549 (lung cancer)	Orthotopic human lung cancer in nude mice	[194]
	HumFt-PAMAM NPs	miRNA-145-5p	NB4 cell line (acute promyelocytic leukemia (APL)	-	[195]
	Biodegradable PNPs	mRNA (Anti-CD19 CAR)	Endogenous T cells	Healthy C57BL/6	[196]
	Ag-based hybrid NPs	siRNA (eEF2K)	TNBC cell (triple-negative breast cancer)	-	[197]
	Angiopep-2 (Ang) functionalized intracellular-environment-responsive NPs	siRNA	U87MG (Glioblastoma)	Nude mice bearing U87MG xenografts	[198]
	LNPs	PDNA (pDNA-pCBGr99, pOX40L, p4-1BBL, pNC)	B16F10 cell (mouse- melanoma cell line)	TLR9 knockout and interferon signaling-deficient mice	[199]
	IONPs	Metformin conjugating DNA–Fe_3O_4 nanoconjugate	MCF-7 (human breast cancer cell)	-	[200]
	Lipid–PEG–folate NPs (LPF NPs)	siRNA (LIN28B)	A2780 (human ovarian cancer cells), ID8 (murine ovarian cancer cells), HCT116 (colorectal cancer cells)	Murine ovarian ascites model	[201]
	Core–shell lipid/PCL-PEI NPs	siRNA	PC-3 cells (human prostate cancer)	PC-3-xenografted in BALB/c nude mice model	[202]
	Chitosan coated lanthanum phosphate NPs	siRNA	HT-29 cells (human colorectal cancer)	Orthotopic colorectal cancer in C57BL/6-ApcMinC/Nju transgenic model	[203]
	Lipopolyplex	mRNA	MDA-MB-231 (breast adenocarcinoma), DC2. 4 (immortalized murine dendritic cells) and endothelial cells	C57BL/6 mice models of melanoma metastasis	[152]
	Layer-by-Layer-Assembled Se Nanocomplexes	siRNA	H1299 cells (human non-small cell lung carcinoma)	-	[204]

(*Continued*)

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Table 2 (Continued).

Disease/ Disorder	NPs	Encapsulant	Cell	Animal	Ref
	Stimuli-Responsive Polysaccharide Enveloped Liposome	Survivin-shRNA	MDA-MB-231 cells (breast cancer)	BALB/c nude mice breast tumor model	[205]
	Layer-by-layer NPs	siRNA	RS4(11) (human acute lymphoblastic leukemia) and Toledo (human non-Hodgkin's B-cell lymphoma) cell lines	Lymphoma cancer of SCID beige immune-compromized mice	[206]
	Layer by Layer Assembled Chitosan-Coated Au NPs	siRNA	H1299-eGFP cells (human large cell carcinoma cell line)	-	[207]
	Layer-by-layer assembled PLGA NPs	miR-34a	TNBC cell (triple-negative breast cancer cells)	-	[208]
	Calcium phosphate nanoparticle (CaP NPs)	CRISPR-Cas9)PDL1/Gag9)	B16F10 cell (mouse- melanoma cell line), CT26 cell (mouse-undifferentiated colon carcinoma cell line)	C57BL/6 mice bearing B16F10 melanoma	[209]
	Extracellular vesicles	MicroRNA (miR)-372-3p	HCC cells (hepatocellular carcinoma)	Hepato-cellular carcinoma in nude mice	[210]
	Au-NPs	Cas13d + guide RNAn (SARS-CoV -2)	Cas13-expressing Huh-7 cells (hepatoma line)	-	[211]
	Layer-by-layer elastin-like polypeptide nucleic acid NPs (LENN)	mRNA	T24 bladder carcinoma ($EGFR^{+}$)	-	[212]
	PNPs (polyplexes)	mRNA	MDA-MB-231 (MD Anderson - metastatic breast)	-	[213]
	Skeletoplexes (HAA-based polymer–DNA NPs	pDNA	A549 cells (human lung carcinoma cells), HeLa cells (human cervical cancer cells		[214]
Genetic	Epigallocatechin-3-gallate - lactoferrin self-assembling NPs (EGCG-LF NPs), poly (β-amino ester) based NPs (PBAE- NPs).	CRISPR/CasRx (JAK1), CasRx (JAK1)+siRNA	NIH/3T3 (mouse embryonic fibroblast), DC2.4 cells (mouse dendritic cell line)	BALB/c mice with DNCB-induced atopic dermatitis (AD)	[215]
	AU NPs	CRISPR β2-microglobulin (B2M)	HSPCs (primary human CD34+)	-	[216]

Neurological	LNPs	mRNA, (TGM1)	Human ARCI skin models (keratinocytes/skin tissue)		
	LNPs	Consensus S1 (GI-19, GI-13, GI-7, GVI-1) and consensus N	DF-1 (chicken embryo fibroblast) cells	SPF chickens and embryonated eggs	[217]
	PEG-PBAE NPs	PDNA (GBA1)	-	α-syn PFF C57BL/6J mice	[185]
	PLGA NPs	pDNA (TLR2 scFv)	BV2 microglia	5xFAD transgenic mice	[[218]
	LNPs	Cd14 siRNA-L	Murine macrophages	IME-induced neuroinflammation mouse model (intracortical microelectrode implantation)	[219]
	Functionalized Au nanorods (fGNRs)	pDNA (MCO2 opsin gene)	Spinal neurons (GABAergic neurons)	C57BL/6 mice	[220]
	LNPs	mRNA	Neuro2a cells (mouse neuroblastoma cell line)	CCI-TBI mice model	[221]
Cardiovascular and inflammatory	Poly (β-amino ester)s	ICAM-1 siRNA	RCMECs (rat cardiac microvascular endothelial cells)	Male Sprague-Dawley rats	[222]
	Poly-butylcyanoacrylate nanoparticles (PBCA-NPs)	Acetylcholine	Cardiac spheroids (iPSC-derived cardiomyocytes, endothelial cells, fibroblasts)	-	[223]
	Silica-IONPs (SoMag5-MNPs)	MMLV viral vector (Connexin-43)	NIH/3T3 (mouse fibroblasts), primary embryonic cardiac fibroblasts (eFB), C166 (mouse endothelial cells)	CD1 mice (cardiac cryo-injury)	[224]
Others	mPEG-PLGA-PLL NPs	MicroRNA-125a (miR-125a)	Splenic T cells	-	[225]
	Exosomes	Circular RNAs (circRNAs)	HDMECs (human dermal microvascular endothelial cells)	DBA/1J mice model of collagen-induced arthritis (CIA) mouse model	[226]
	Polymer-lipid hybrid NPs	mRNA vaccine	Splenic B cells	Healthy mouse	[227]
	Carbon dots (CDs)	siRNA	ATDC5 (murine chondroblast cell line)	-	[228]
	Hybrid nano polyplex	PDNA	hRPE cells (human retinal pigment epithelial cells)	-	[229]
	DNA nanostructure NPs	miR 143 3p	Primary condylar chondrocytes	UAC TMJOA C57BL/6 mice	[230]

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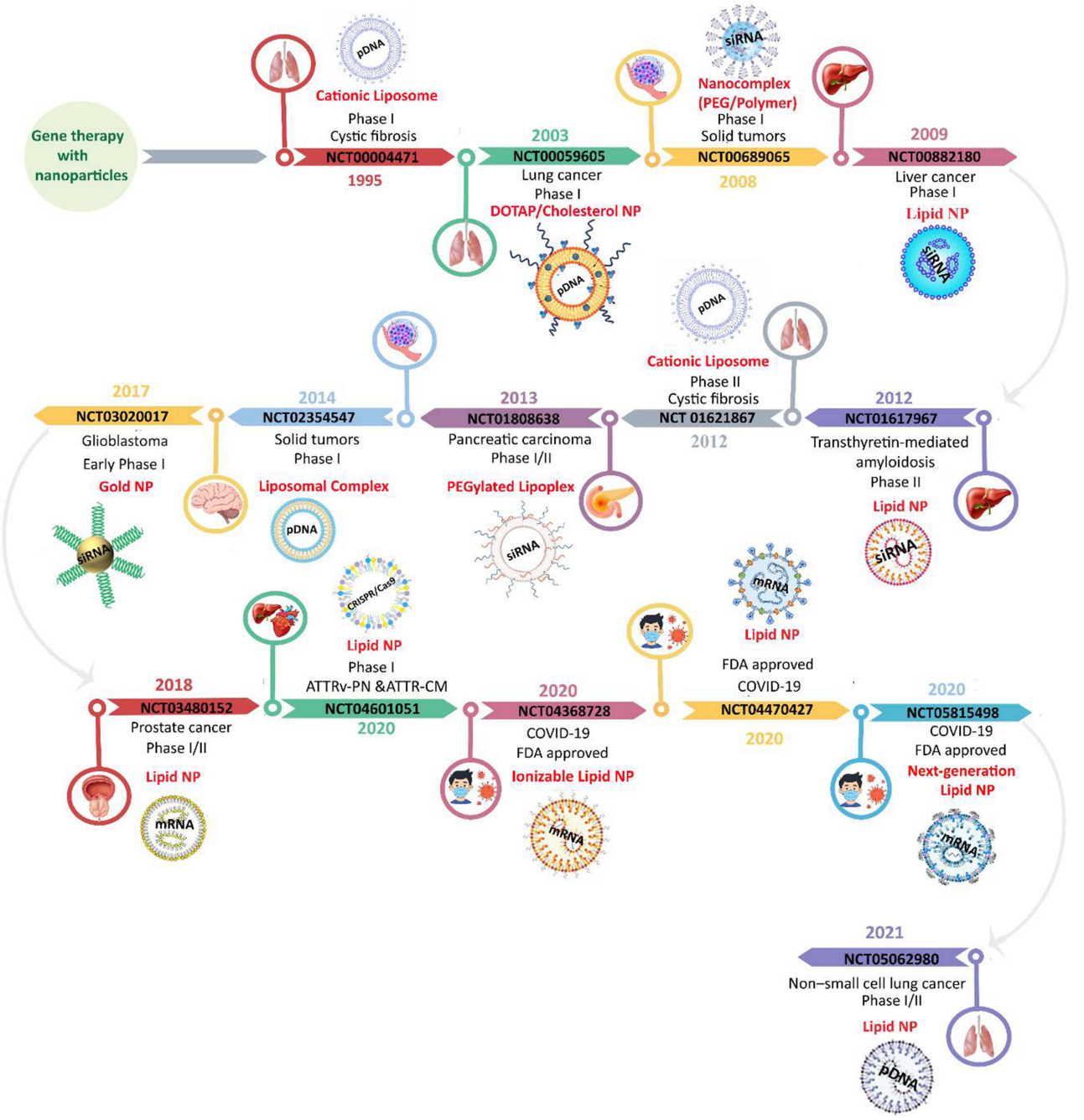

Figure 5 Clinical trials of nanostructures as gene delivery systems.

PN. In that study by Intelaya Therapeutics, NTLA-2001 was given to patients at different doses, and initial results showed significant reductions in TTR protein levels.

In another clinical trial (NCT01621867), researchers investigated the potency and safety aspects of a non-viral gene therapy approach via cationic liposomes for treating cystic fibrosis (CF). This Phase II, randomized, double-blind, placebo-controlled trial involved repeated nebulization of a pDNA encoding the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The goal was to restore functional CFTR protein expression in the airway epithelium, thereby improving lung function in CF patients. Unlike viral vectors, the non-viral delivery method used here aimed to minimize immune responses and allow for repeated dosing. The study concluded that the treatment showed a modest but beneficial effect on lung function,

and no considerable treatment-associated side effects were reported, reflecting good patient tolerance. These findings support the potential of non-viral CFTR gene therapy as a promising strategy for cystic fibrosis.

In another clinical trial, the efficacy of a non-viral delivery system was evaluated through the use of an mRNA-based cancer vaccine known as Autogene Cevumeran (RO7198457), in combination with the immune checkpoint inhibitor atezolizumab (NCT03480152). This Phase 1/2 study focused on participants with locally advanced or metastatic tumors and aimed to determine the safety, tolerability, and immune activation caused by the treatment. Autogene Cevumeran utilizes a non-viral platform to deliver mRNA encoding patient-specific neoantigens, designed to trigger a focused immune reaction directed at cancer cells. The non-viral nature of the LNPs for mRNA delivery allowed for flexibility in design, rapid manufacturing, and a reduced risk of insertional mutagenesis. The study was completed and demonstrated that the mRNA-based vaccine, when used in combination with atezolizumab, could generate a neoantigen-specific T cell response, supporting the promise of non-viral gene delivery in personalized cancer immunotherapy. Table 3 summarizes representative FDA-approved nucleic acid therapeutics and gene therapy products, highlighting their therapeutic modality, delivery strategy, and major clinical applications.

Future Directions of Non-Viral Gene Delivery Technologies Employing Nanostructures

The efficiency of gene therapy is highly contingent upon the vector's effectiveness in ensuring both secure and specific gene delivery. Poorly designed vectors might trigger non-specific interactions, low transfection efficiency, or even adverse phenotypic outcomes.[239] Although viral vectors such as retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses (AAVs) exhibit high gene transfer efficiency, their broader application is constrained by several technical and clinical limitations. These include immunogenicity and the risk of insertional mutagenesis, limited cargo capacity (eg, AAV ≈ 4.7 kb), which restricts delivery of large genes or complex regulatory elements, and challenges in large-scale manufacturing, such as high production costs, scalability issues, and lot-to-lot variability.[4,240,241] These limitations have driven increasing interest in non-viral vectors, including physical methods (eg, electroporation, microinjection, and ultrasound) and chemical carriers such as NPs.[4]

Nanotechnology has become an integral component of gene therapy, offering versatile platforms for non-viral gene delivery. However, clinical translation remains limited due to ongoing concerns regarding safety, efficacy, and specificity. To overcome these barriers, future research must focus on a deeper mechanistic understanding of NP–cell interactions, including cellular attachment, endocytosis, intracellular trafficking, and gene expression pathways. Elucidating these processes will allow for the rational design of nanocarriers that can efficiently navigate extracellular and intracellular barriers.[242] A key challenge in non-viral delivery is non-specific biodistribution, which can lead to systemic toxicity. Therefore, developing specific targeting strategies, either through ligand-functionalized nanocarriers or localized delivery techniques, is essential. NPs functionalized with peptides, antibodies, or aptamers have shown promise in enhancing cellular specificity and reducing off-target effects, particularly for hard-to-reach tissues such as the brain.[243] Equally important is the design of multifunctional and stimuli-responsive nanostructures, capable of releasing their cargo upon physiological cues such as pH, redox potential, or enzyme activity. These systems can enhance gene release in target cells while minimizing premature degradation.[244,245]

Besides NPs, alternative delivery platforms such as conjugation-based systems have also emerged as promising approaches for nucleic acid therapeutics. In particular, small-molecule conjugates including N-acetylgalactosamine (GalNAc), cholesterol, peptides, antibodies, and aptamer-mediated systems have demonstrated significant potential for improving cellular uptake, tissue specificity, pharmacokinetics, and therapeutic efficacy.[246,247] Among these, GalNAc conjugation has achieved notable clinical success in liver-targeted siRNA delivery through receptor-mediated uptake via the asialoglycoprotein receptor, leading to the development of several approved RNA therapeutics.[248,249] These conjugation strategies are structurally and mechanistically distinct from nanoparticle-based carriers; however, the integration of conjugation technologies with NP systems may further enhance targeting efficiency, stability, and controlled delivery, paving the way for the next generation of multifunctional nucleic acid delivery platforms.

Table 3 Representative FDA-Approved Nucleic Acid Therapeutics and Gene Therapy Products, Including mRNA Vaccines, siRNA Therapeutics, Antisense Oligonucleotides, Viral Vector-Mediated Gene Therapies, and CRISPR-Based Genome Editing Platforms, Together with Their Corresponding Delivery Systems and Therapeutic Applications

Product	Therapeutic Modality	Delivery System	Indication	Regulatory Status	Ref
BNT162b2	mRNA vaccine	LNPs	COVID-19 prevention	FDA/EMA approved	[232]
mRNA-1273	mRNA vaccine	LNPs	COVID-19 prevention	FDA/EMA approved	[232]
Onpattro	siRNA therapeutic	LNPs	Hereditary transthyretin amyloidosis	FDA approved	[232]
Givlaari	GalNAc-siRNA conjugate	GalNAc conjugation	Acute hepatic porphyria	FDA approved	[233]
Leqvio	GalNAc-siRNA conjugate	GalNAc conjugation	Hypercholesterolemia	FDA approved	[233]
Oxlumo	GalNAc-siRNA conjugate	GalNAc conjugation	Primary hyperoxaluria type 1	FDA approved	[234]
Spinraza	Antisense oligonucleotide (ASO)	Intrathecal administration	Spinal muscular atrophy	FDA approved	[235]
Luxturna	AAV gene therapy	Adeno-associated viral vector	Inherited retinal dystrophy	FDA approved	[236]
Zolgensma	AAV gene therapy	Adeno-associated viral vector	Spinal muscular atrophy	FDA approved	[237]
Casgevy	CRISPR-based gene editing	Ex vivo edited hematopoietic stem cells	Sickle cell disease and β-thalassemia	FDA approved	[238]

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Furthermore, the use of advanced in vitro models, including tissue-engineered constructs and organoids, as well as computational simulations and AI-based predictive modeling, can help in understanding NP biodistribution, kinetics, and optimization of design parameters. Such tools will be invaluable for accelerating the preclinical evaluation of gene delivery platforms.[250–252] Ultimately, the goal is to engineer nanocarriers that are non-toxic, do not provoke immune responses, degrade naturally with high loading capacity for genetic material, extend circulation time in vivo, and effectively penetrate through barriers including the BBB. Achieving this will not only enable clinical breakthroughs in treating genetic disorders and cancers but will also solidify the convergence of nanotechnology and gene therapy as a cornerstone of next-generation medicine. This evolving field has already attracted significant interest from academia, industry, and biotechnology entrepreneurs, paving the way for impactful innovations and transformative patient outcomes.[253,254]

Conclusion

Non-viral gene delivery systems offer a safer and more versatile alternative to viral vectors, with advantages including reduced immunogenicity, greater cargo capacity, and scalable synthesis. Despite progress in vector design, challenges such as low transfection efficiency, poor endosomal escape, and limited in vivo targeting persist. Future efforts should focus on engineering stimuli-responsive, biodegradable carriers with enhanced targeting and intracellular delivery capabilities. Integration with gene-editing tools (eg, CRISPR/Cas), development of theranostic platforms, and incorporation of disease-specific ligands hold promise for improving therapeutic outcomes. Advancing in vivo validation, standardizing evaluation methods, and addressing translational barriers will be essential to realize the full clinical potential of non-viral gene delivery.

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During the preparation of this work, the authors used AI (quillbot.com) in the writing process to improve the readability and language of the manuscript. After using this AI, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Disclosure

The authors report no conflicts of interest in this work.

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