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Recent progress on nanosystems for nucleic acid delivery

Shanka Walia[a] and Mohit J. Mehta *[b,c,d]

Nucleic acid (NA) based therapeutics have witnessed tremendous progress and breakthroughs in treating pathological conditions, including viral infections, neurological disorders, genetic diseases, and metabolic disorders. NAs such as plasmid DNA (pDNA), short interfering RNA (siRNA), microRNA (miRNA), and antisense oligonucleotides (ASOs) can be modified to revolutionize personalized medicine. Despite the great potential of NA-based therapeutics, their clinical transformation is significantly hampered by instability, degradation, and inefficient delivery to the targeted site in the *in vivo* system. Lipid-based delivery systems hold great potential to overcome these shortcomings to enhance the delivery and bioavailability, improve stability, and increase the therapeutic effect of the NAs by delivering them to the active site. This review emphasized various nucleic acid-based therapeutics and their enhanced and improved delivery using different nanocarriers. Ultimately, the importance of lipid-based nanocarriers for delivering NAs is discussed and provides perspective in this field.

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1. Introduction

Nucleic acids (NAs) are immensely recognized for their therapeutic potential during the COVID-19 pandemic. NAs, including DNA, antisense oligonucleotides (ASO), aptamers, short interfering RNA (siRNA), microRNA (miRNA), and messenger RNA (mRNA), provide a highly versatile platform at the underlying level of transcription and translation for the treatment of diseases as compared with traditional drugs.[1,2] Nucleic acids' high specificity and pharmacological effect offered unprecedented opportunities to combat viral infections and complicated diseases, including cancer, diabetes, cardiovascular diseases, genetic disorders, and acquired conditions.[3] The majority of the NAs bind selectively to their target molecules *via* complementary Watson–Crick base pairing, making target molecule detection simple.[4] NAs are good candidates for the personalized medicinal approach because genes and their mutations can be easily questioned. Hence, NAs can target patient-specific genes with minimal off-target effects.[5] In contrast, pharmaceutical drug molecules require extensive screening and medicinal chemical optimization.

However, the intracellular uptake of NAs is quite challenging because of their anionic nature and poor stability, which makes the intracellular uptake of NAs difficult. Due to their highly hydrophilic and polyvalent anionic properties, NAs are degraded by extracellular enzymes and, thus, poorly absorbed by cells. When injected intravenously, they tend to accumulate in the liver and kidneys.[6] All these factors reduced the efficiency of the delivered cargo to elicit the desired response.[7] Furthermore, due to their high molecular weight, negative zeta potential, and hydrophilicity, NAs cannot penetrate the cellular membrane. However, clinical application is the primary goal of any therapeutic molecule; hence, nanovehicles should ensure adequate delivery of drugs to the targeted cell or organ without causing any harmful effect on the healthy cells. The therapeutic payload is delivered to the targeted cells *via* two main approaches, namely, passive targeting and active targeting. The foundation of passive targeting is the enhanced permeability and retention (EPR) effect. For example, tumor vasculature is often leaky compared with healthy blood vessels. This helps nanoparticles to penetrate the cancer cells more efficiently.[8] In contrast to this, active targeting facilitates surface modifications of nanovehicles with specific ligands, resulting in enhanced binding to the receptors of targeted cells. Presently, a structure capable of improving the bioefficacy and controlled release of NAs at the desired site is of the utmost importance. Nucleic acids possess versatile physicochemical properties and tunability. These features allow them to be easily functionalized with various bio-

[a]*Swami Sahajanand College of Computer Science, Plot No. 639, ISCON Mega City, Gujarat, 364002, India*
[b]*Nanomedicine Lab, Department of Biological Sciences and Bioengineering, Inha University, 100 Inha-ro, Michuhol-gu, Incheon, 22212, Republic of Korea*
[c]*Adult Stem Cell Group, Faculty of Medicine and Health Technology, Tampere University, Tampere, 33520, Finland*
[d]*Tays Research Services, Wellbeing Services County of Pirkanmaa, Tampere University Hospital, Tampere, 33520, Finland. E-mail: mohitjmehta@gmail.com, mohit.mehta@tuni.fi*

molecules and nanoparticles (NPs) for enhanced therapeutic effect with selective binding to the targeted molecules. Different nanoparticle-based platforms such as metal NPs, polymers, dendrimers, proteins, lipids, and other biomolecules are widely exploited in conjugation with NAs.[9,10] For instance, liposomes and micelles, which have now received FDA approval, were part of the first generation of nanoparticle-based therapy.[11] Lipid-based nanocarriers were introduced as promising tools to retain the structural integrity and therapeutic potential of NAs. Lipid-based nanocarriers offer a versatile platform for NA encapsulation, which resulted in the clinical translation of several NA therapeutics. This review aims to provide an in-depth discussion on various nanosystems for nucleic acid delivery and therapeutics. They offer a powerful approach to address the limitations of traditional treatments, and hence can potentially overcome these challenges.

2. Nucleic acid-based therapeutics

In the last decade, nucleic acid-based therapies have emerged as promising approaches for treating multiple disorders by regulating molecular pathways.[12] NAs target disease-causing genes in a specific manner, enabling precise and personalized treatment for life-threatening diseases.[13] NA-based therapeutics knock down, upregulate, or alter targeted gene expression. Currently, several NA-based therapeutics are used for the treatment of severe ailments (Table 1).

2.1 Plasmids

Plasmids are circular, double-stranded, high molecular weight (<1000 to >200 000 bp) DNA constructs. They are often used as vectors to introduce a foreign gene into a target cell for various purposes such as gene therapy, vaccine production, and protein expression.[14,15] The plasmid backbone contains the gene for antibiotic resistance controlled by a prokaryotic promoter (a prokaryotic origin of replication for plasmid replication),[14] and an expression cassette with a promoter to initiate the transcription of a transgene that encodes a therapeutic protein.[15] Plasmid DNA molecules typically contain several regulatory signals, such as promoter and enhancer sequences, responsible for regulating gene expression.[14] Promoters (cytomegalovirus (CMV) and Rous sarcoma virus or human alpha-actin, human beta-actin promoter) commence gene transcription by providing a recognition site for the RNA polymerase. Enhancers play a vital role in the large-scale production of a gene with specificity. They are localized either downstream or upstream from the promoter site. Transcription efficiency can be elevated by selecting the promoter and enhancer of interest; for example incorporating SV40 enhancer in the expression plasmid enhanced muscle-specific gene expression.[26] Plasmids also play an essential role in generating vaccines for genetic immunization. Plasmid-derived vaccines are easy to produce if a genetic sequence of the concerned variant is identified, and are also known to control the infection.[27] Pardridge *et al.* (2020) developed a plasmid DNA approach for the treatment of Niemann–Pick C1 (NPC1), a lysosomal cholesterol storage disorder affecting the brain.[28] Plasmid DNA was encapsulated in pegylated liposomes encoding the functional human *NPC1* gene. Quantitative PCR has shown the successful delivery of pDNA and NPC1 mRNA expression in the brain, liver, and spleen. Malardo and coworkers (2012) demonstrated that injecting a low dose of pDNA (pcDNA3) in the Wistar rat endotoxemia model has increased plasma vasopressin and regulates blood pressure.[29] The rats treated with 10–20 μg pDNA showed lower levels of inflammatory cytokines, namely, IL-6 and TNF-α.

2.2 Antisense oligonucleotide (ASO)

Antisense oligonucleotides (ASOs) of therapeutic importance comprise single-stranded nucleic acids and are 18–30 base pairs in length.[30] ASOs form complementary base pairs with their target RNA by classical Watson and Crick base pairing. After binding with the target RNA, ASOs resulted in gene silencing by altering or degrading the expression of the target RNA *via* cleavage or blockage.[16,17] The various mechanisms adopted by ASOs to degrade target RNA include (1) modified splicing by skipping and inclusion of exons, and inhibition of 5′ cap formation, (2) steric blockage of ribosomal functions as they inhibit the binding of target RNA with ribosomes through the translation arrest of the target RNA, and (3) induction of RNAse H that recognizes ASO–RNA hybrids and degrade the target RNA present in the hybrid.[31,32] ASOs are primarily administered *via* transfection and transduction *in vitro* and *in vivo*.[33] For example, Passini *et al.* (2011) used 2′-*O*-2-methoxyethyl-modified ASOs for the treatment of severe spinal muscular atrophy (SMA).[34] SMA is an autosomal recessive neuromuscular disorder related to the deficiency of survival motor neuron (SMN) protein caused by mutations in the *SMN1* gene. ASO injection resulted in increased levels of SMN protein in

Table 1 Applications of different types of nucleic acid in clinical research

S. no.	Nucleic acid	Biomedical application	Ref.
1	Plasmid DNA	Gene cloning, gene therapy, DNA vaccine, protein expression, gene editing	14 and 15
2	Antisense oligonucleotides (ASOs)	Targeting RNA functioning of genetic disorders, gene silencing	16 and 17
3	Aptamers	Cell tracking, bacterial and viral protein sensing	18
4	siRNA and miRNA	Gene therapy, antiviral therapy, drug discovery Biomarkers, cancer treatment	19–21
5	mRNA	Vaccine production, cancer immunotherapy, gene editing	22 and 23
6	CpG DNA	Immunotherapy, vaccine adjuvant, gene therapy, anti-inflammatory	24 and 25

SMA-infected mice. *In* vivo studies suggested improved therapeutic effects on muscle physiology, functioning, and the survival rate of motor neurons. Some examples of FDA-approved ASO-based drugs include eteplirsen, golodirsen, and nusinersen.[4] Several others are under clinical trials for the treatment of neurological disorders, hepatitis B virus infections, solid tumors, and renal diseases.[35]

Although ASOs might have shown enhanced efficacy *in vitro* and *in vivo*, they showed drawbacks related to low cellular uptake and poor stability in body fluids that hindered their potential from bench to bedside.

2.3 Aptamers

Aptamers are short single-stranded synthetic oligonucleotides of 10–100 nucleotides folded into three-dimensional shapes.[36,37] Aptamers possess a remarkable tendency to bind non-covalently to their target with high selectivity and specificity. Aptamers are widely explored to target biomolecules of interest, including proteins, peptides, carbohydrates, antibodies, small molecules, toxins, live cells, and even heavy metals.[18] Thus, aptamers are potential candidates for cell tracking, bacterial and viral protein sensing, medicine, and analytical chemistry. Aptamers are primarily generated *in vitro* from the systematic evolution of ligands by exponential enrichment (SELEX).[38] Aptamers are often compared with antibodies because of their similar function in binding the proteins with specificity. Unfortunately, antibodies are fraught with significant shortcomings, such as limited and unreproducible synthesis, being expensive, and being prone to generating immunogenicity. However, aptamers with versatile properties have gained much attention in the research community since their discovery in 1990.[39,40] Aptamers are a fascinating alternative to conventional antibodies due to their facile and reproducible production, small size, reduced immunogenic effect, physicochemical stability, longer shelf life, and *in vitro* chemical synthesis.[41]

2.4 Small interfering RNA and microRNA

Small interfering RNA (siRNA) and microRNA (miRNAs) are representative modulators of the interference RNA (RNAi) mechanism. RNAi comprises a group of agents that use double-stranded RNA containing homologous sequences complementary to the target gene and perform sequence-specific gene silencing.[19] The primary function of RNAi is to build a robust defense mechanism for protecting the genome from mobile genetic material released from viruses, which, on activation, produce abnormal dsRNA or RNA.[42] For example, stable RNAi nanocomplexes with redox-sensitive glycol chitosan derivatives were synthesized based on rolling circle transcription. This nanocomplex ensured systemic and targeted siRNA delivery with enhanced therapeutic effects *in vivo via* the EPR effect.[43] A nanomedicine platform based on AuNPs covalently functionalized with siRNA duplexes was efficiently used to neutralize oncogene expression in glioblastoma multiforme.[44] *In vivo* studies indicated that the AuNP–siRNA nanocomplex penetrates the blood–brain barrier. AuNP–siRNA nanocomplex effectively knocked down endogenous Bcl2L12 mRNA and protein levels and sensitized glioma cells toward therapy-induced apoptosis by targeting the oncoprotein Bcl2L12. Elbashir and coworkers (2001), demonstrated that 21-nucleotide siRNA duplexes mediated the knockdown of endogenous and heterologous genes in human embryonic kidney (293) and HeLa cells.[45] In 2018, the FDA approved the first siRNA-based drug, patisiran (Onpattro), to treat familial amyloid polyneuropathy.[46] Other siRNA-based medications approved by the FDA for clinical use include givosiran (to treat acute hepatic porphyria), lumasiran (for the treatment of primary hyperoxaluria type 1), and inclisiran (to treat atherosclerotic cardiovascular disease).[47] Vir Biotechnology and Alnylam Pharmaceuticals recently developed ALN-COV (VIR 2703) based on siRNA therapeutics to cure SARS-CoV and SARS-CoV-2 infections.[20]

microRNA (miRNA) is an attractive therapeutic tool, particularly in cancer treatment. miRNA interacts with the 3′ or 5′ untranslated region of the targeted mRNA, resulting in degradation and translational suppression.[21] Li *et al.* (2021) reported intracellular miRNA imaging and gene silencing using a let-7a miRNA-activated DNA nanomachine.[48] Multifunctional miRNA-515 sponge-loaded magnetic nanodroplets combined with ultrasound and magnetism were used to treat hepatocellular carcinoma (HCC).[49] *In vivo*, studies showed the suppression of xenograft HCC because miRNA-515 upregulated the expression of anti-oncogenes, namely CD22, P21, TIMP1, NFKB, and E-cadherin in cancerous cells. However, no miRNA drug is available and approved by the FDA for therapeutic purposes. Most of the miRNA drugs are still under clinical trials; for example, miravirsen (miR-122 inhibitor) for treating hepatitis (Hep) C has completed Phase II clinical trials. Similarly, lademirsen, or RG-012 (miR-21 inhibitor), is in Phase II clinical trials for treating Alport syndrome (NCT02855268). MRG-110 has completed phase I clinical trials, and further studies are underway to treat impaired wounds. MRG-110 (miRNA-92a inhibitor) improved wound healing in preclinical models and can effectively treat impaired wound healing conditions in diabetic patients. In short, several miRNA drugs are under preclinical and clinical trials to treat various disorders.

2.5 Messenger RNA (mRNA)

Messenger RNA (mRNA) is a type of genetic material that contains the information for producing proteins from DNA, the genetic code found in the nucleus of a cell.[50] In recent years, scientists have investigated the use of mRNA as a therapeutic agent, specifically in the medical field. mRNA therapeutics, commonly termed mRNA vaccines or mRNA drugs, are designed from synthetic or modified mRNA molecules to stimulate the production of specific proteins in the body to treat diseases.[51] The Pfizer-BioNTech and Moderna COVID-19 vaccines, which employ mRNA to guide cells to generate the spike protein present on the surface of the SARS-CoV-2 virus, are among the most noteworthy milestones of mRNA therapies.[22] The immune system then recognizes these spike proteins as foreign moieties and stimulates an immune response

against the virus. This method has proved to be efficient in preventing critical illness and death from COVID-19, and regulatory agencies around the globe have permitted its emergency usage.[51,52]

Additionally, mRNA therapies can cure many ailments, including cancer, genetic abnormalities, and viral infections.[23,53] For instance, mRNA is used in cancer immunotherapy to engineer T cells and natural killer (NK) cells with antigen receptors and as a template for immunologically active proteins in various immune and non-immune cells.[23] mRNA therapeutics also have the potential to treat genetic disorders by providing cells with functional copies of genes that are missing or non-functional. Researchers have already demonstrated the ability to use mRNA to correct genetic mutations in animal models of certain diseases, such as inherited retinal diseases. They are working on advancing these therapies to clinical trials.[54] mRNA therapeutics have several advantages over traditional drugs. Because mRNA does not integrate into the genome, it does not have the potential to cause permanent genetic changes.[55] Moreover, mRNA can be easily synthesized, thus allowing the rapid development and production of new therapeutics. Despite these advantages, some challenges still need to be overcome to fully realize the potential of mRNA therapeutics. One of the main challenges is ensuring mRNA's safe and effective delivery to the targeted cells. Researchers are developing various delivery methods, such as NPs and viral vectors, to overcome this challenge.[56] Furthermore, mRNA therapeutics are relatively new, and more research is needed to fully understand their safety and efficacy.[55] Overall, mRNA therapeutics represent a promising new approach to treating and preventing various diseases and conditions. With continued research and development, mRNA therapeutics have the potential to transform the way we think about medicine and improve the lives of millions of people worldwide.

2.6 Cytosine-phosphate-guanine (CpG) DNA

Cytosine-phosphate-guanine (CpG) is a synthetic oligonucleotide studied as a therapeutic agent for several undruggable conditions. CpG activates the immune system by binding to Toll-like receptor 9 (TLR9), expressed in immune cells such as dendritic and B cells.[57] Consequently, these cells are activated, and an immunological response is initiated. Preclinical investigations demonstrated that CpG has antitumor properties and enhances the immune response to cancer cells, increasing tumor cell death.[24,58] It has been reported that CpG has anti-inflammatory properties, making it a suitable candidate for treating autoimmune illnesses such as rheumatoid arthritis and multiple sclerosis.[59] CpG has been shown to enhance the immune response to viral infections, including influenza and herpes simplex virus, HIV,[60,61] hepatitis B and C,[62,63] and bacterial infections such as *Streptococcus pneumoniae*.[25,64,65] In addition, CpG has been studied as a potential treatment for allergies. In preclinical studies, CpG has been found to reduce the severity of allergic reactions and is currently being investigated as a treatment for allergies such as asthma and allergic rhinitis.[66–68] Overall, CpG is a promising therapeutic agent with many potential applications. However, additional research is required to completely comprehend its mechanism of action and determine its human safety and efficacy.

3. Classification of spherical particles/nanoparticles for potential complexation with nucleic acids

The development of NPs provides diverse platforms for delivering drugs with enhanced effects. However, it is reported that NP morphology (*viz*., shape and size) can directly affect their response in biological systems.[69] Amidst them, spherical NPs such as metallic NPs, polymeric NPs, protein, nucleic acids, and lipid-based complexes having a shape in common are the most promising drug delivery agents and therapeutic probes. It has been reported that under *in vivo* conditions, the NPs behave differently from biomolecules in therapeutic applications, particularly in vaccine delivery.[70] (1) The larger surface area to volume ratio and permeability of these nanocomplexes allow efficient and targeted delivery of the cargo (DNA/RNA *etc*.) and reduce unwanted side effects.[71] (2) Nanocarriers can be tailored to slowly release loaded their cargo, providing controlled and sustained release within the targeted tissue or organ. (3) In biological systems, small molecules face challenges such as degradation by enzymes and other biological processes. Nanostructures can encapsulate the therapeutic molecules, protecting them from degradation and ensuring their effective and targeted delivery. This is especially important for fragile molecules like DNA and RNA used in nucleic acid vaccines.[72] (4) Naked DNA or RNA can sometimes trigger unwanted immune responses. Nanocarriers can shield the nucleic acids, overcoming such reactions and promoting a more focused immune response toward the target antigen.[73,74] Overall, under *in vivo* conditions, nanostructures provide distinct advantages over small molecules. Their ability to deliver drugs directly to target sites, offer controlled release, and protect sensitive molecules makes them a powerful tool for improved treatment efficacy and reduced side effects.

Table 2 shows the merits and demerits of these drug delivery vehicles. One can choose the ideal carrier to deliver particular drugs/genes based on these advantages and disadvantages. A perfect carrier transports the drug to its target site and releases it at the site of action. Several conditions must be addressed, including specific and selective interactions, sufficient drug delivery, and sustained drug release at the targeted area. For example, nanoparticles can be made from various materials to encapsulate hydrophobic and hydrophilic drugs with increased stability. Similarly, polymeric NPs can be used to encapsulate hydrophobic and hydrophilic drugs and can be designed to release drugs in a controlled manner over time. Lipid-based drug delivery agents can encapsulate hydrophobic drugs, protecting them from degradation and helping them reach the targeted area in the body.

Table 2 Merits and demerits of advanced delivery systems

S. no	Delivery agent	Advantages	Disadvantages	Ref.
1	Inorganic NPs	✓ Ease of synthesis and surface functionalization ✓ Enhanced intracellular uptake ✓ Able to entrap both hydrophilic and hydrophobic drugs ✓ Enabled active and passive targeting ✓ Photoluminescence properties ✓ Stability (w.r.t. wide range of pH and temperatures)	• Intracellular toxicity • Rapid elimination by the reticuloendothelial system • Lack of clinical trials • Poor biodegradability • May trigger the immune system	75
	Nobel metal (AuNPs, AgNPs) NPs	✓ Strong biocompatibility ✓ Tunable optical properties ✓ High surface-to-volume ratio ✓ High binding affinity	• Expensive for large-scale applications • Potential toxicity concern • Stability issues due to aggregation at elevated temperatures	76
	Quantum dots (QDs)	✓ High quantum yield ✓ Resistance to photobleaching as compared with traditional organic dyes ✓ Size-dependent emission wavelength from visible to NIR region	• High cytotoxicity • Exponential decrease in fluorescence and blinking of different QDs • Instability and elevated hydrodynamic diameter on interaction with serum proteins	77
	Silicon NPs (SiNPs)	✓ Good biocompatibility and biodegradable ✓ Tunable optical properties ✓ Ease of functionalization ✓ Thermal stability	• Low quantum yield • Potential toxicity concerns	78
	Carbon nanomaterials (CMs)	✓ Tunable photoluminescence ✓ Photostability ✓ Ease of fabrication ✓ Economical ✓ Eco-friendly, and biocompatible	• Toxicity concerns due to ROS generation, inflammation, DNA damage • Limited understanding of the long-term effect on human health • Limited control over size and structure	79
	Iron oxide NPs	✓ Biocompatibility ✓ Magnetic properties ✓ High surface-to-volume ratio ✓ Tunable properties	• Toxicity concerns • Stability issues due to aggregation • Long-term effects • Costly production	80
2	Polymeric NPs	✓ Biocompatible and biodegradable ✓ Able to entrap both hydrophilic and hydrophobic drugs ✓ Ease of surface functionalization ✓ Controlled and sustained release ✓ Protect the drug from metabolic degradation ✓ Prolonged residence time	• Burst effect • Limited drug loading • Deep knowledge of polymer–receptor molecular interactions required • Limited knowledge about long-term side effects	81–83
3	Dendrimers	✓ Increasing solubility of highly lipophilic drugs ✓ Tunable physicochemical properties ✓ Ease of surface functionalization for targeted drug delivery ✓ Covalently associating drugs	• Not a suitable candidate carrier for hydrophilic drugs • Cellular toxicity • Elimination and metabolism depend on the generation and materials • High cost for their synthesis	84
4	Nanofibers	✓ Large surface area ✓ Biocompatibility ✓ Encapsulation and targeted delivery ✓ Tissue engineering	• Toxicity • Degradation • Expensive for large-scale production	85 and 86
5	Proteins/peptides	✓ Various anticancer effects ✓ High cell permeability ✓ Low systemic toxicity ✓ Improved target selectivity ✓ Bypass biological barriers	• Size influenced pharmacokinetics • Short half-life	83
6	SNAs	✓ High specificity and potency ✓ High drug loading efficiency ✓ Biocompatibility and prolonged circulation time ✓ Dense packing and delivery of therapeutics ✓ Resistant to nuclear degradation ✓ Effective gene regulation	• Low yield and high cost of synthesis • Low stability w.r.t. temperature and UV exposure • Limited targeting ability • Limited knowledge about their behavior in *in vivo* systems	87 and 88
7	Liposomes	✓ Biocompatible and biodegradable ✓ Able to entrap both hydrophilic and hydrophobic drugs ✓ Controlled release ✓ Protect the drug from metabolic degradation. Prolonged circulation time ✓ Low systemic toxicity	• Poor stability • Short shelf life and instability in circulation • A special storage system is needed • Batch-to-batch variation in the size of liposomes	83

Table 2 *(Contd.)*

S. no	Delivery agent	Advantages	Disadvantages	Ref.
8	Lipid-based nanocomplexes (LNPs and SLNs)	✓ Production on a large-scale industry level ✓ Easy sterilization ✓ Able to entrap both hydrophilic and hydrophobic drugs ✓ Able to target specific cells or tissues ✓ Modulated and controlled release ✓ Low toxicity due to their biocompatible and biodegradable components and the absence of organic solvents in their process ✓ Protecting drugs from environmental conditions ✓ Low cost compared with liposomes	• Limited drug loading for SLNs • Instability in the bloodstream • SLNs tend to gelation • Polymorphic transition for SLNs • Particle size growth and drug expulsion during storage	84

3.1 Inorganic NPs

Inorganic NPs are vital in biomedical applications, *viz.*, bioimaging, therapy, diagnosis, and drug delivery.[89,90] Inorganic NPs are mainly metal oxides, semiconductors, and noble metals with sizes ranging from 1–100 nm. These NPs possessed intrinsic physical, optical, magnetic, and electrical properties due to the characteristics of the core material. Additionally, these properties can be controlled by tailoring the size, shape, structure, and composition to achieve enhanced sensing and therapeutic effects.[91] NPs gain positive responses as nanocarriers because of their tunable properties, improved efficacy, and decreased side effects by boosting their targeting ability.[92,93] The loading can be done *via* four methods: physical adsorption, electrostatic interaction, encapsulation inside the NP core, or covalent binding. For example, gold NPs (AuNPs) are well known for their therapeutic and delivery capabilities due to their stability, inertness, high binding affinity to thiols, amines, and disulfides, and ease of functionalization with ligands such as drugs, proteins, and nucleic acids. Excellent fluorescence due to the surface plasmon resonance effect resulted in absorption in the visible and NIR regions. Therefore, it is essential in diagnosis and sensing applications.[94] Also, depending upon the shape and size of the AuNPs, the free electrons on the surface of AuNPs oscillate continually, granting them photothermal properties.[95,96] Thiolate NAs can be easily conjugated to AuNPs *via* covalent and electrostatic interactions between sulfur and gold.[9] Gracezyk *et al.* (2021) developed an AuNP carrier to deliver siRNA by functionalizing AuNPs with a thiol-modified tectoRNAs trimer (structural RNA). They applied this conjugate to regulate the CopGFP expression in MDA-MB-231 GFP/RFP cells (Fig. 1a).[97] The cellular uptake of AuNP: to tectoRNA conjugate was determined by TEM studies, showing

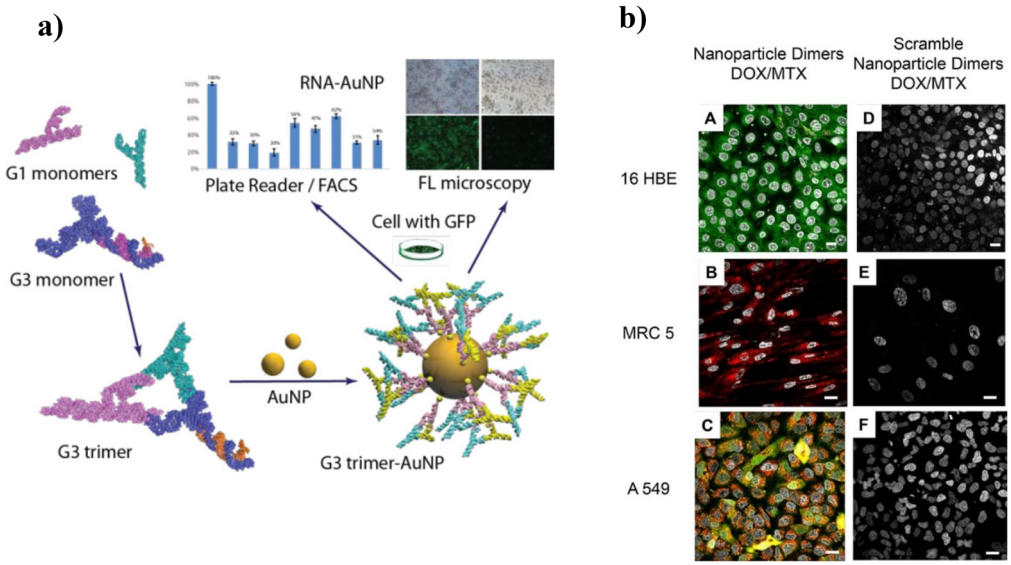

Fig. 1 (a) Impact of AuNP:structural RNA complex on MDA-MB-231 GFP/RFP cells. *In vitro* studies show the regulation of CopGFP expression in the targeted cells. Adapted with permission from ref. 97. Copyright 2021 ACS Chemical Society. (b) Confocal microscopy images of cells incubated with (A–C) nanoparticle dimers and (D–F) "non-targeting" scrambled nanoparticle dimers. Sixteen HBE cells express only keratin 8, and MRC 5 cells express only vimentin. A549 expresses both keratin 8 and vimentin. A fluorescence signal corresponding to the presence of keratin 8 mRNA (A), vimentin mRNA (B), and both vimentin and keratin 8 mRNA (C) are observed. When incubated with nanoparticle dimers designed with "non-targeting" scramble sequences, all three cell lines display no response. Adapted with permission from ref. 98. Copyright 2018 ACS Chemical Society.

their presence in the cytoplasm both inside the cytosol and membrane structure. Also, the prepared AuNP:tecto RNA conjugates effectively regulated gene expression, as demonstrated by GFP expression studies using fluorescence techniques. Kyriazi *et al.* (2018) synthesized a DNA–AuNP dimer-based multifunctional platform for mRNA sensing and targeted drug delivery.[98] The synthesized DNA–AuNP dimer specifically recognizes keratin 8 and vimentin mRNAs in keratin 8-expressing 16HBE (epithelial cells), vimentin-expressing MRC 5 (mesenchymal cells), and A549, showing the expression of both keratin 8 and vimentin respectively. Furthermore, two anticancer drugs, doxorubicin (Dox), which detects keratin 8 mRNA, and mitoxantrone (MXT), which detects vimentin mRNA, were intercalated into a DNA duplex, resulting in cell death in response to specific mRNA signatures. Contrary to this, the scrambled NPs, without having recognition sites for targeted mRNA, failed to release the loaded drugs (Fig. 1b).

Another class of inorganic NPs includes semiconductor quantum dots (QDs) due to their nanosize showing size-tunable 3D quantum confinement effects, thus giving rise to exquisite optical properties.[99–101] QDs are typically made of semiconducting materials such as group II–VI elements (CdS, CdSe, CdTe, ZnS, ZnSe, ZnTe), group III–V elements (InP or InAs), group I–III–VI_2 elements ($CuInS_2$, $AgInS_2$), group IV–VI elements (PbS, PbSe, PbTe), or group IV elements (C, Si, Ge).[102–105] QDs are important fluorophores as they provide emission in the UV, visible, and near-infrared ranges with an impressive quantum yield compared with traditional organic dyes, with drawbacks including photobleaching and poor signal intensity.[106,107] All these properties of QDs make them suitable probes for optical bioimaging and targeted molecular sensing. For instance, Ma *et al.* (2019) designed a CdTe:Zn^{2+} QD nanobeacon conjugated with black hole quencher (BHQ1) and phosphorothioate co-modified DNA by hydrothermal synthesis for single RNA detection and imaging (Fig. 2a(A)).[108] This nanobeacon was highly sensitive and efficiently detected low-abundance nucleic acids in live cells *via* FRET. QDs functionalized with BHQ1, and single DNA were applied to detect and image single HIV-1 RNAs in live HIV-1 integrated cells (Fig. 2a(B, C)). Similarly, gene silencing in tumor cells of the central nervous system was done using siRNA-loaded polyethylenimine (PEI) functionalized CdSSe/ZnS QD-based nanocarriers.[109] siRNA-loaded PEI-CdSSe/ZnS QDs efficiently target human telomerase reverse transcriptase (TERT). Two glioblastoma cell lines, U87 and U251, after transfection showed a decrease in gene and protein expression levels of TERT with a high level of gene transfection efficiency within 48 h.

The rapid development of silicon nanostructures provides a potential class of sensitive sensors and therapeutic agents for real-time diagnosis and therapeutic applications.[110–112] Silicon NPs (SiNPs) are indirect band gap semiconductors and thus exhibit longer excited-state lifetimes; after entering the cellular system, SiNPs are biodegraded into silicic acid (nontoxic compound) and easily excreted out of the body without showing any sign of toxicity.[78,113] Chaix *et al.* (2019) reported amine-functionalized porous SiNPs for the loading and improved delivery of pDNA[114] (Fig. 2b). *In vitro* studies suggested that the SiNPs showed better biocompatibility and successful transfection of up to 10^7 RLU mg^{-1} proteins in HEK 293 cells.

Carbon nanomaterials (CNMs), also called green NMs, are admirable fluorescent NMs with fascinating characteristics such as tunable photoluminescence, photostability, ease of fabrication, economical production, eco-friendliness, and biocompatibility.[115] In contrast to QDs, the mechanism behind the fluorescence property of CNMs involves π-plasmon and surface defects generated from radiative recombination of the surface-confined electrons and holes.[116] Wang *et al.* (2014) synthesized fluorescent carbon dots (CDs) for simultaneous imaging and efficient siRNA delivery for cancer therapy.[117] PEI-functionalized CDs were applied for the adsorption of survivin siRNA. After transfection for two hours with siRNA-loaded CDs/PEI complexes, MGC-803 gastric cancer cells showed blue fluorescence in the cytoplasm, thus suggesting the internalization of loaded siRNA into the cancerous cells (Fig. 2c). Furthermore, the expression of survivin mRNA in MGC-803 cells was downregulated to 96.4 ± 8.7 when exposed to siRNA-loaded CD/PEI complexes. Iron oxide NPs (IONPs), with their remarkable superparamagnetic properties and excellent surface-to-volume ratio, have attracted worldwide interest.[80] Magnetic NPs are widely used for magnetic particle imaging, magnetic resonance imaging, hyperthermia, cell tracking, targeted genes, and drug delivery.[118,119] Iron oxide NPs are comparatively less toxic and cytocompatible, thus allowing clinical translation. IONPs are usually synthesized from iron oxides such as maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4), metal alloys (*e.g.*, FeCo and FePt), as well as the doping of magnetically susceptible elements (*e.g.*, $MnFe_2O_4$ and $CoFe_2O_4$).[120,121] Efficient gene (pDNA) delivery to mesenchymal stem cells was accomplished using positively charged, PEI-coated IONPs.[122] The uniform and narrow-sized (15 nm) IONPs exhibit good magnetic properties. *In vitro* studies suggested that IONPs under the external magnetic field efficiently delivered 99% of the loaded pDNA within 30 min, followed by nuclear importing of the carried genes, resulting in enhanced gene expression of the treated cells. The caveolin-mediated pathway facilitated the internalization of pDNA-loaded IONPs into the cytoplasm. Zhang *et al.* (2010) designed PEI-coated IONPs and applied them to deliver interfering RNA (siRNA) GFP plasmid *via* an external magnetic force in 3D cell culture (NIH 3T3 cells).[123] The NPs facilitated 64% and 77% transfection efficiency for siRNA and GFP plasmid, respectively. These transfection complexes significantly reduced the GFP-expressed cells' growth, with 80–82% silencing efficiency. Furthermore, these complexes delivered four toxic shRNA to the 3D cell culture, enhancing cell death (41–51%) (Fig. 2d).

Other commonly used inorganic NPs include silica NPs, which are hydrophilic and porous, have access to surface functionalization due to silane groups, and are widely used for drug delivery, biomolecule conjugation, and many more applications.

However, for *in vivo* applications, the toxicity of these inorganic NPs is an inevitable issue among researchers.

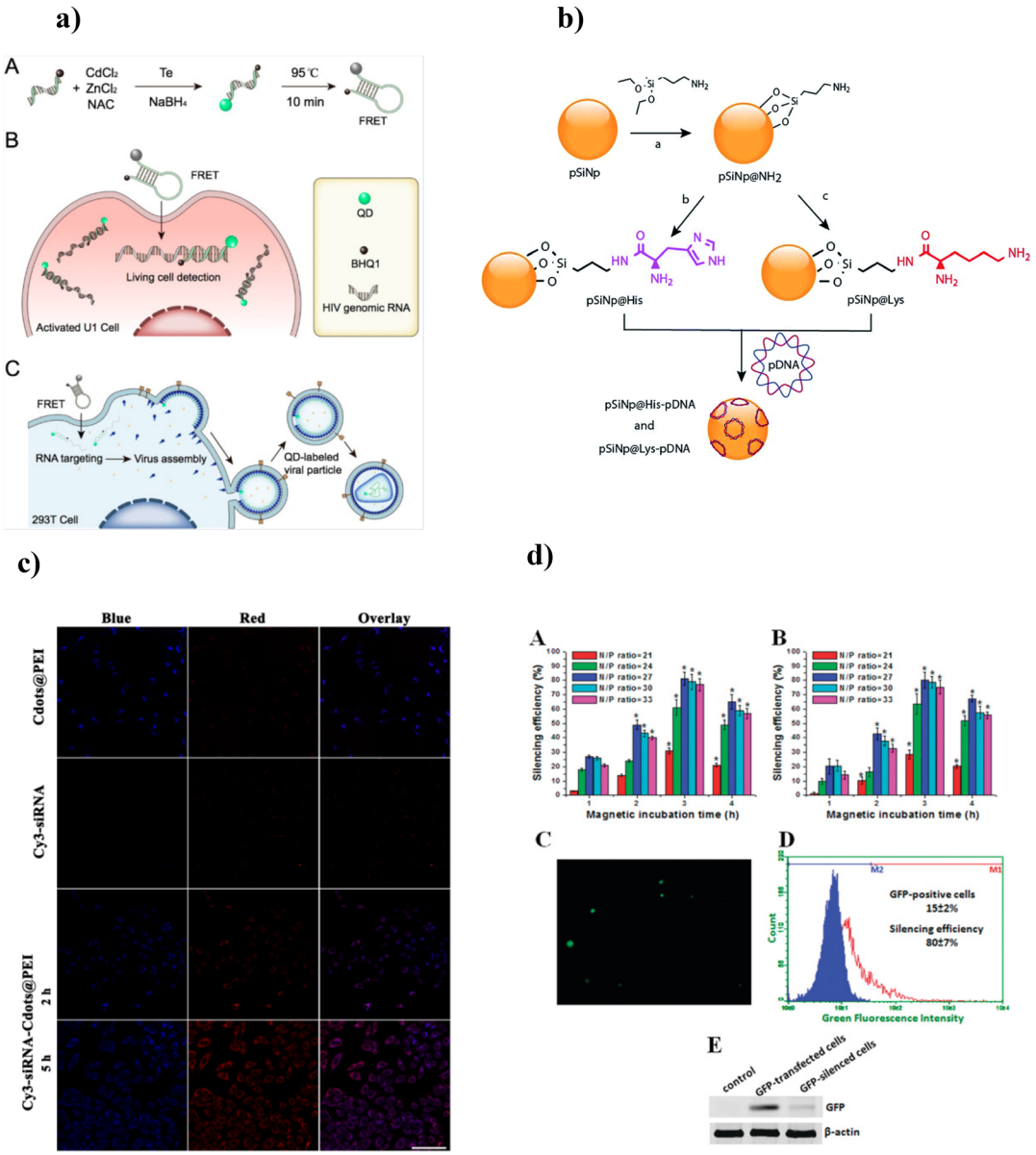

Fig. 2 (a) Schematic illustration of QD-NBs for HIV-1 genomic RNA detection. (A) Schematic diagram of the QD-NBs' preparation; schematic illustration of QD-NBs for (B) HIV-1 genomic RNA detection in living cells, and (C) fluorescence labeling of single virus particles. Adapted with permission from ref. 108. Copyright 2019 American Chemical Society. (b) Reaction scheme for the chemical functionalization of SiNPs with histidine and lysine and the complexation of pDNA. Adapted with permission from ref. 114. Copyright 2019 Royal Society of Chemistry. (c) Confocal laser scanning microscopic images of MGC-803 cells incubated with Cdots@PEI or Cy3-siRNA for 2 h, or Cy3-siRNA-Cdots@PEI complexes for 2 and 5 h. Adapted with permission from ref. 117. Copyright 2014 Springer Nature. (d) GFP silencing by PEI-coated SPMNs/GFP shRNA in GFP-transfected 3D cell cultures. Adapted with permission from ref. 123. Copyright 2010 American Chemical Society.

Additionally, the cellular uptake, circulation, and clearance of NPs depend upon the physicochemical properties of the NPs. After entering the bloodstream, NPs come across the biological barrier composed of lipids, proteins, and other components. The interaction of NPs with biomolecules resulted in the formation of biomolecule coronas around the NPs before reaching the target site, thus influencing the biological fate of the NPs.[100] To circumvent these issues, researchers developed biocompatible surface modification approaches to inorganic NPs to avoid or decrease the nonspecific interactions of these NPs with biomolecules and increase prolonged accumulation at the site of interest.

3.2 Polymeric NPs

Polymeric nanoparticles have shown great potential in recent years due to their small size, ranging from 1–1000 nm. Polymeric nanoparticles are mostly spherical with a solid structure. The main advantages of polymeric NPs (PNPs)

include (1) the high molecular weight and polyvalent nature of these molecules, which enable the encapsulation and delivery of bulky and long-chain NAs,[124] (2) biocompatibility and biodegradability: PNPs easily break down inside the body into water and carbohydrates and hence can be quickly eliminated from the body,[125] (3) facilitation of successful loading and controlled drug delivery,[126,127] (4) retention of the bioactivity of drugs or biomolecules by evading the immune system, thus enhancing their bioavailability and therapeutic potential, and (5) the provision of a platform for ligand functionalization, resulting in the targeted and stealthy delivery of drugs.[96] Polymeric nanoparticles can be synthesized by natural and synthetic polymers.[128] Natural polymers, namely chitosan, hyaluronic acid, starch, alginate, cellulose, and lignin, and synthetic polymers, *viz*., polylactide-*co*-glycolide (PLGA), polylactides (PLA), polyethylenimine (PEI), polyethylene glycol (PEG), polycaprolactones, and polyacrylates, have been widely explored for the synthesis of PNPs.[125,126,128] The most common polymeric NPs include nanocapsules (polymeric capsules surrounding a cavity) and nanospheres (solid matrix). For instance, a nanovehicle based on cationic cyclodextrin-polyethyleneimine 2k conjugate delivered mRNA encoding HIV glycoprotein120 for treating HIV-1.[129] The delivery system enhanced the intranasal delivery of mRNA by crossing the nasal epithelial barrier through intracellular pathways, thus resulting in a solid anti-HIV immune response. Biodegradable chitosan-alginate 3D porous injectable gel was designed for *in vivo* mRNA vaccine delivery.[130] Increased levels of IFN-α secretion, luciferase reporter protein expression, and T-cell proliferation were observed from mRNA lipoplex-loaded gel scaffolds compared with the systemic injection of naked mRNA and mRNA:lipoplex.

A nanovehicle based on PLGA-encapsulated antisense microRNA-21 (miRNA-21), known to be overexpressed in glioblastoma cells, was applied for the improved therapeutic effect of temozolomide (TMZ) on glioblastoma cells (Fig. 3a).[131] Enhanced drug delivery and sustained gene silencing of miRNA 21 were observed in glioblastoma cells, namely U87 MG, LN229, and T98G cells. This nanovehicle also showed a significant decrease in cell viability ($p < 0.001$) with a 1.6-fold increase in cell arrest at the G2/M phase in the TMZ-treated cells (Fig. 3b–d). Furthermore, the intracellular co-delivery of the nanocomplex and TMZ in glioblastoma cells resulted in a 67% and 15% enhancement in the expression of miRNA-21 targeted phosphatase and tension homologue (*PTEN*) genes and apoptosis-associated caspase-3 respectively (Fig. 3e). The PLGA–SNA complex was designed to accommodate the chemotherapeutic drug coumarin spatially and nucleic acid to independently improve the controlled loading and tunable release of encapsulated moieties.[132] *In vitro* studies performed on RAW blue cells to confirm the immunotherapeutic response of PLGA–SNAs depicted dose- and time-dependent activation of TLR9. PLGA–SNAs were cytocompatible at concentrations ranging from 10×10^{-9} M to 2×10^{-6} M. Also, the cellular uptake efficiency of PLGA–SNAs was tenfold higher than their linear counterpart and at a shorter time of 0.5 h. However,

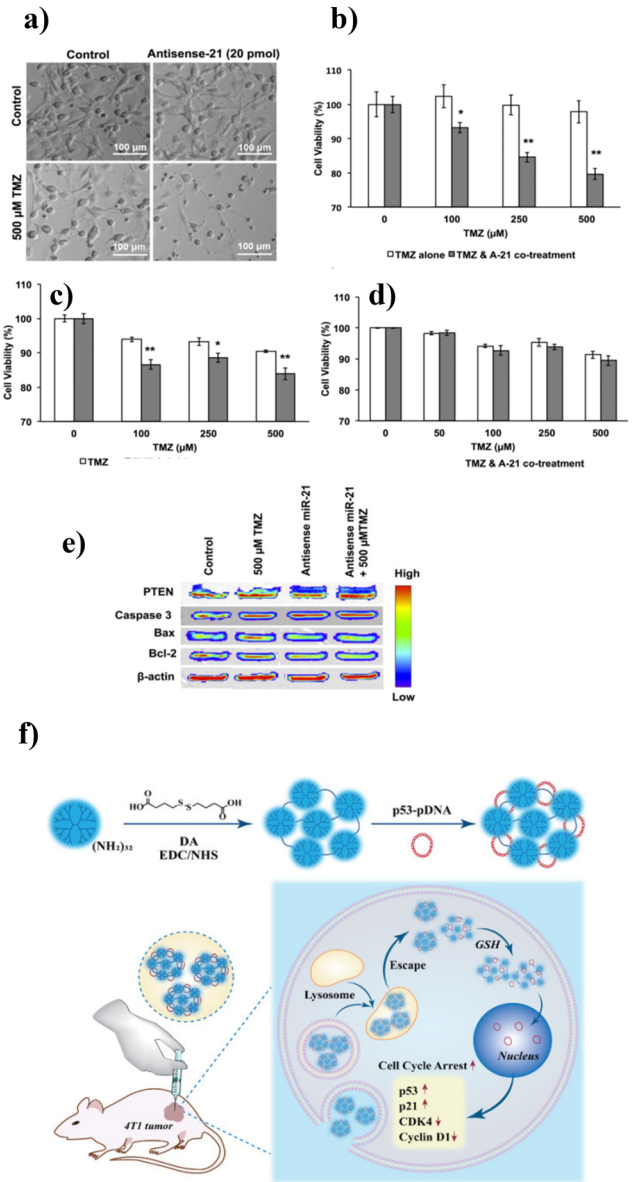

Fig. 3 (a) Phase contrast microscopic images of control and antisense miR-21 transfected cells treated with 500 μM TMZ. *In vitro* cell viability MTT assays on control and antisense miR-21 transfected (b) U87 MG cells, (c) LN 229 cells, and (d) T98G cells treated with different concentrations of TMZ. (e) Cellular pathway analysis of antisense miR-21 and TMZ co-treatment on U87 MG cells. Adapted with permission from ref. 131. Copyright 2015 American Chemical Society. (f) Schematic representation of PAMAM dendrimer/pDNA-p53 nanocarrier preparation for cancer gene therapy. Adapted with permission from ref. 139. Copyright 2021, American Chemical Society.

PNPs also suffer from drawbacks, including toxicity and a high risk of particle aggregation. The FDA approves very few PNP-based drugs for clinical applications.[133]

Dendrimers or "dense stars" are globular, hyperbranched 3D structures, and their physicochemical properties can be controlled for the desired application. Dendrimers can be synthesized by divergent or convergent techniques.[134,135] The

remarkable properties of dendrimers make them suitable vehicles for the delivery of nucleic acids. For instance, the polycation nature of dendrimers provides multiple sites for the electrostatic binding of the negatively charged phosphate backbone of NAs, resulting in solid DNA complexation. Also, the formation of the dendrimer–NA complex protects against nuclease degradation of the attached NAs. It is speculated that the presence of tertiary amines in the structure of dendrimers enhanced the cellular uptake and delivery of NAs through endosome escape *via* the sponge effect.[136] Other merits include the attachment of biomolecules or ligands to the functional groups available on the exterior surface. In contrast, drugs and small cargo can be encapsulated in the interior of the dendrimer.[133] Various dendrimers are studied for the delivery of NAs, such as poly L-lysine, triazine, polyglycerol, poly(propyleneimine), and poly(amidoamine) (PAMAM) based dendrimers. At the same time, PAMAM is the most investigated nanocarrier because of facile synthesis and functionalization.[137] Palombarini and coworkers (2021) demonstrated the targeted delivery of miRNA to myeloid leukemia cells (which are otherwise challenging to transfect) by incorporating nucleic acid with ferritin-poly(amidoamine) (PAMAM) dendrimer NPs.[138] The cellular internalization of this nanocomplex drives morphological changes and enhances the expression of the retinoic acid receptor, alpha (RARα), an early hallmark of granulocytic differentiation. Mekuria *et al.* (2021) developed a nanocarrier by covalently binding PAMAM dendrimer with 4,4′-dithiodibutryic acid (DA) to successfully deliver p53-pDNA. The nanocarrier reflected a 2.3- and 2.1-fold increased gene transfection in 4T1 and mouse breast cancer cells.[139] Furthermore, *in vitro* and *in vivo* studies showed an upregulated expression of mRNA and p53 and p21 protein and downregulation of cyclin-D1 and CDK-4 protein, thus facilitating cell cycle arrest in the G1 phase (Fig. 3f).

Dendrimers are ideal for delivering NAs with enhanced stability and improved cellular internalization compared with other therapeutics. However, regarding the safety concerns, the toxicity of these dendrimers is still unaddressed. The strong cationic amine groups on the surface of dendrimers resulted in solid binding with the negatively charged cell membrane, which led to destabilization, disruption of cell components, and eventually lysis.[140]

Nanofibers are a promising class of biomaterials for nucleic acid delivery, offering several advantages including a large surface area, biocompatibility, encapsulation and targeted delivery (gene, growth factors, protein, and peptide delivery), and scaffolding for tissue engineering.[85] Nanofibers can be synthesized by different methods, namely, phase separation, electrospinning, and physical and chemical fabrication. Various natural, semisynthetic, and synthetic polymers are widely used for their synthesis. Furono *et al.* (2022) reported plasmid DNA delivery using horseradish peroxidase cross-linked gelatin nanofibers.[141] Nanofibers immobilized with Lipofectamine/pDNA resulted in the transfection of pDNA delivery in HEK293 cells. Furthermore, genome-editing molecules including Cas9 protein and guide RNA (gRNA) were expressed in nanofiber-treated HEK293 cells, resulting in gene knock-in and knock-out. Polycaprolactone nanofiber-encapsulated siRNA showed controlled release for up to 28 days with successful transfection of the treated HEK 293 cells.[142] The cells showed enhanced cellular uptake and *GAPDH* gene silencing of 61–81%. Nanofibrous scaffolds made up of collagen type 1 were used for the controlled and long-term delivery (at least 5 months) of siRNA/silica NPs.[143] *In vivo* studies revealed that the nanofiber-based scaffolds showed more effective gene silencing ($p < 0.05$) as compared with traditional bolus delivery. An *in vivo* biodistribution study revealed that siRNA stayed confined up to ~290 μm from the implants. As compared with negative scrambled siRNA therapy, a reduction in fibrous capsules of ~45.8% was observed after 4 weeks.

Along with the advantages, nanofibers do have some drawbacks that researchers are working to overcome.[86] (1) Nanofibers may have inherent toxicity or cause inflammatory responses in the body. (2) Depending on the material, nanofibers may degrade too quickly or too slowly, affecting the release profile of the drugs they carry. (3) Manufacturing nanofibers for large-scale clinical use can be complex and expensive.

3.3 Proteins/peptides

Proteins, a class of natural biomolecules, stand out as an attractive biocompatible substitute for synthetic polymers in nanomedicine due to their biodegradability, natural abundance, mild synthesis, and fast metabolization.[144,145] Additionally, proteins showed remarkable chemical modification properties due to their surface abundance of functional groups such as carboxyl, amine, and hydroxyl. The amphiphilic nature makes them amenable for small molecules, metallic NPs, and hydrophobic and hydrophilic drugs.[145,146] Protein-based NPs, when administered inside the body, showed weak immunogenicity, were efficiently degraded by the enzymes and were eliminated through hepatic clearance. Abraxane is an FDA-approved drug composed of paclitaxel-bound albumin-based NPs used to treat metastatic breast cancer by inhibiting the mitosis of cancer cells.[147,148] The drug avoids hypersensitivity due to albumin protein, a major drawback of traditional anticancer drugs. Protein NPs can be easily synthesized *via* electrospray, emulsion, and desolvation processes using natural proteins, namely albumin, fibroins, 30Kc19, gelatin, lipoprotein, legumin, zein, gliadin, and ferritin proteins.[144,149]

Peptides are short chains of amino acids linked by a covalent amide bond (peptide bond). They exhibit remarkable sequence and functional diversity, merits employed to design spherical NPs *via* a self-assembly approach.[150] Several peptides are currently used for biomarker imaging, targeted drug and gene delivery, bioprinting, wound healing, and tissue engineering.[151,152] Different peptides such as nuclear localization, tumor-targeted, and cell penetration peptide sequences are extensively reported for biomedical applications. Jia *et al.* (2020) reported enhanced siRNA delivery and improved gene silencing using hyaluronic acid (HA)-modified transmembrane peptide octa-arginine (R8)-based R8-bipolar (HA-bibola/siRNA)

and R8-monobola (HA-bola/siRNA) amphiphilic nanocomplexes.[153] Cell viability studies reflected better cytocompatibility of HA-bibola/siRNA compared with HA-bola/siRNA and control samples (PEI/siRNA) in 4T1 cells. Additionally, HA-bibola/siRNA showed enhanced cell uptake efficiency and down-regulation of Bcl-2 protein expression due to the presence of cell-penetrating peptides on the surface of the nanocomplex. *In vivo*, studies reflected higher antitumor efficacy, improved targeted ability, and increased Bcl-2 gene suppression in 4T1 tumor-bearing Balb/c mice.

3.4 Spherical nucleic acids (SNAs)

The primary function of nucleic acids includes storing and transduction of genetic information. Nucleic acid-based (DNA, RNA, CRISPR/Cas9 gene editing system) spherical NPs hold great significance in the biosensing, therapy, and silencing of various diseases ranging from viral infections to neurological disorders, cardiovascular diseases, and cancer by taking advantage of cellular pathways.[154–156] In the present era, therapeutic technologies related to nucleic acids are at the forefront of fighting the COVID-19 pandemic around the globe.[157,158] They consist of highly compact self-assembled oligonucleotide layers oriented in 3D geometries based on typical Watson–Crick base-pairing.[119,120] Selective and precise base-pairing of DNA/RNA NPs differentiates them from traditional biomolecules, which might be otherwise difficult to design with such ease.[159] Various chemical modifications (nucleotide sequence, sugar, or phosphate backbone modification), biocompatibility, and programmable therapeutic approaches are other fascinating properties of these nanostructures.[160] Most nucleic acid-based NPs interact *via* complementary base pairing with their target molecules, thus resulting in specific and rapid action.[161] Spherical nucleic acids (SNAs) comprise (1) the outer shell of densely packed nucleic acid radially encasing the contents, and (2) an inner core of NPs. They were initially made of DNA shells and AuNP cores.[161,162] Since then, various inorganic nanoparticles such as Ag,[163] QDs, magnetic NPs,[164] silica,[165] organic nanocomposites such as polymers, proteins, and liposomes,[166,167] and hybrid structures of inorganic–organic materials were used to explore different biomedical applications. The three-dimensional architecture of SNAs provides them with unique physicochemical properties and makes them superior to their linear counterparts.[87] The spherical shape allows the dense packing of the oligonucleotide into a limited space.[168] This compact structure of SNAs leads to an enhanced electric charge, increasing the stability of the SNAs and providing resistance to nuclease degradation and prolonged cell accumulation *via* scavenger receptor engagement and endocytosis.[169] SNAs can easily evade the immune system's attack due to strong electric charges because of the dense packing. Also, the interaction of SNAs with receptors on the cell surface resulted in easy penetration through the cell, tissue membranes, and even the blood–brain barrier.[170] Melamed *et al.* (2018) suggested that the spherical architecture of SNAs functionalized with polyethylenimine has improved siRNA-mediated GFP gene silencing 10-fold.[171] Wang *et al.* (2019) reported an SNA-based vaccine for treating a mouse tumor.[172] SNA co-delivered CpG oligonucleotide (adjuvant) and peptide (antigen) to generate an antitumor immune response. The results suggested that the vaccine could increase the survival rate to 31 days, also delaying tumor growth by 15 days. SNAs efficiently promote gene regulation for the treatment of skin diseases.[173] Randeria *et al.* (2015) showed the application of siRNA-encapsulated SNAs in impaired wound healing by downregulating ganglioside GM3 synthase (GM3S) in diabetic mice.[174] The expression of GM3S was reduced by >80% at the wound site *via* the siRNA pathway, and the wound was observed to heal within 12 days in the SNA-treated mice.[174]

Although nucleic acid-based drugs are in the infant stages of clinical trials, they have revolutionized therapeutics' fate in recent years. Despite the remarkable merits of SNA-based therapeutics, challenges still hinder their further clinical and translational applications.

4. Classification of lipid nanocomplexes for efficient delivery of NAs

The clinical application of NPs depends on their successful delivery to the disease site. The effectiveness of synthesized NPs in treating diseases depends upon their therapeutic response, which can be achieved by delivering a required dose to the targeted site. During their journey to a target site, NPs must evade multiple biological barriers at different sites, including (1) phagocytosis by liver cells while NPs are circulating in the blood.[175] (2) Even after reaching the target site, NP entry into the disease site is restricted by the endothelial cell walls. (3) The immune system also recognizes and destroys foreign components (NPs) and vectors (spherical DNA/RNA containing genetic information).[176] (4) The internalization of NPs in the cell membrane or nucleus enhanced the effect of the former. The overall effect of these barriers restricted the penetration of most NPs into the targeted cell or organ and thus reduced their therapeutic efficiency. Recently, remarkable progress has been made in designing and developing delivery systems that enhance the therapeutic trajectory of drugs, inorganic NPs, and DNA/RNA-based genetic drugs.

Lipid-based delivery cargos, namely micelles, liposomes, and lipid nanoparticles (LNPs), are promising delivery agents as they can protect nanomaterials from degradation and enhance circulation by avoiding early clearance by the immune system.[177,178] Lipids are widely used nonviral delivery agents and are fascinating because of their easy synthesis, characterization, greater payload, homogeneity, biodegradability, and marginal toxicity profile. A broad range of lipid-based NPs is utilized to deliver NAs, including cationic lipids, ionizable lipids, zwitterionic lipids, LNPs, liposomes, and solid lipid NPs (SLNs) (Table 3).

Table 3 A summary of lipid-based NA delivery systems and their biomedical applications

S. no	Type of lipid vehicle	Composition of vehicle	Cargo	Method of synthesis	Treatment	Ref.
1	Cationic	DOTAP/cholesterol	mRNA, pDNA, and oligonucleotide	Thin-film evaporation	Ovarian cancer	179
2	Cationic	9322-O16B/Chol/DOPE	mRNA	Chemical	B-cell lymphoma	180
3	Cationic	DOPE-steaylated octaarginine (STR-R8), DOTMA-YSK05, cholesterol-GALA peptide	pDNA	Chemical	Efficient and selective delivery of pDNA to the lungs	181
4	Ionizable cationic	DLin-MC3-DMA/DLin-KC2-DMA/DODAP/DSPC/PEG-DSPE, cholesterol	siRNA	Microfluidic	pDNA transfection	182
5	Ionizable cationic	C-12-200 (IL)/DOPE, cholesterol/PEG-lipid conjugate	pDNA	Microfluidic	Cardiovascular diseases	183
6	Ionizable cationic	DLin-MC3-DMA, DSPC, cholesterol, DMG-PEG2K	mRNA	Chemical	Hepatic reticuloendothelial diseases	184
7	Ionizable	DSPC, CHO, DMG-PEG_{2000}	siRNA	Chemical	Hyperlipidemia	185
8	Zwitterionic	Phosphatidylcholine, DPPC, cholesterol	pDNA	Chemical	pDNA transfection	186
9	Liposome	DOTAP/DOPE/DSPE-PEG	6-Carboxyfluorescein-labeled 14-mer oligonucleotide	Ethanol dilution	*In vivo* labeling of human microbiota	187
10	Cationic liposome	DPPC/DOTAP/cholesterol	GFP-mRNA	Lipid film hydration	Neurodegenerative diseases	188
11	Protamine liposome	DOTAP/cholesterol	mRNA	Chemical	Colorectal cancer gene therapy	189
12	Lipid nanoparticles	C-14-4 IL/DOPE/cholesterol/PEG	mRNA	Microfluidic	Engineering of CAR T cells to kill cancer cells	190
13	Lipid nanoparticles	DLin-MC3-DMA/DAP/phospholipid/cholesterol/PEG	Spherical DNA/RNA	Ethanol dilution	Organ-specific delivery of nucleic acid	191
14	Solid lipid NPs	DOTAP/lecithin/cholesterol/lipopolysaccharide	TNF-α siRNA	Ultrasonication	Rheumatoid arthritis	192
15	Solid lipid NPs	Stearic acid, soya lecithin	pDNA, Dox	Solvent displacement	Lung cancer therapy	193
16	Cationic solid lipid NPs	Peptide-cationic lipid CDO14	siRNA, upconversion NPs	Thin-film dispersion	Bioimaging and gene therapy	194

Lipid-based delivery agents, *viz*., micelles, liposomes, and lipid NPs, have been extensively studied to encapsulate and deliver NAs, primarily due to their ease of synthesis and interaction with NAs (Fig. 4a).[195] The essential components of FDA-approved LNPs for NA delivery developed by pharmaceutical companies include cationic or ionizable lipids, cholesterol, a helper lipid, and a PEG-lipid (Fig. 4b and c), for example DLin-MC3-DMA, PEG: PEG-2000-C-DMG (Alnylam), SM-102, PEG-2000-DMG (Moderna), and ALC-0315(Pfizer/BioNTech) ALC-0159 (Pfizer/BioNTech/Acuitas), along with DSPC and cholesterol (Fig. 4d).

Moreover, lipid-based nanovehicles can be surface functionalized to achieve the targeted delivery and release of therapeutics in a specific tissue or cell.[196,197] However, it is important to consider the chemical properties of the lipids used in NA delivery for efficient drug development. One of the critical parameters of lipids is their surface charge. Differently charged lipids can have varying degrees of compatibility with nucleic acids. In general, positively charged lipids tend to have a higher degree of compatibility with negatively charged nucleic acids, such as DNA and RNA, as electrostatic interactions between the positive charges on the lipids and the negative charges on the nucleic acids can help to stabilize the interactions between the two. Conversely, negatively charged lipids may have reduced compatibility with NAs, as the negative charges on the lipids may repel the negative charges on the NAs. Also, it is well known that the charge of NPs alters their tissue selectivity after intravenous administration. Taking advantage of this approach, different tissue-targeted LNPs have been designed by incorporating cationic or anionic lipids into the general composition of LNPs to tune their charge.[198] For example, using zwitterionic lipids, which have both a positive and negative charge, has been shown to enhance the stability of NPs and improve *in vivo* efficacy.[199] Additionally, using lipids with a high melting point can help improve the NPs' stability and enhance their ability to penetrate cell membranes.[200] Another critical factor to consider is the size of the NPs formed by the lipids. Smaller NPs have been shown to penetrate cell membranes more effectively and deliver NAs to target cells.[201] However, it is also important to note that the size of the NPs should not be so small that they are rapidly cleared from the body by the immune system. Besides considering the lipids' structural and chemical properties, the NPs' composition is also an essential consideration for NA delivery.[202] For example, using PEG as a surface coating on lipids has been shown to improve the stability of the particles and reduce their clearance from the body.[203] Additionally, using other polymers, such as PEI or PLA, can also help improve the stability and efficacy of the lipid-based delivery agents. Overall, the design of lipids for NA delivery is a complex process that requires a thorough understanding of the structural, chemical, and composition-based factors that influence their efficacy. Through the use of structure–activity relationships and careful consideration of the properties of the lipids, it is possible to design nanoparticles that can effectively deliver NAs to target cells and improve the efficacy of gene therapy.

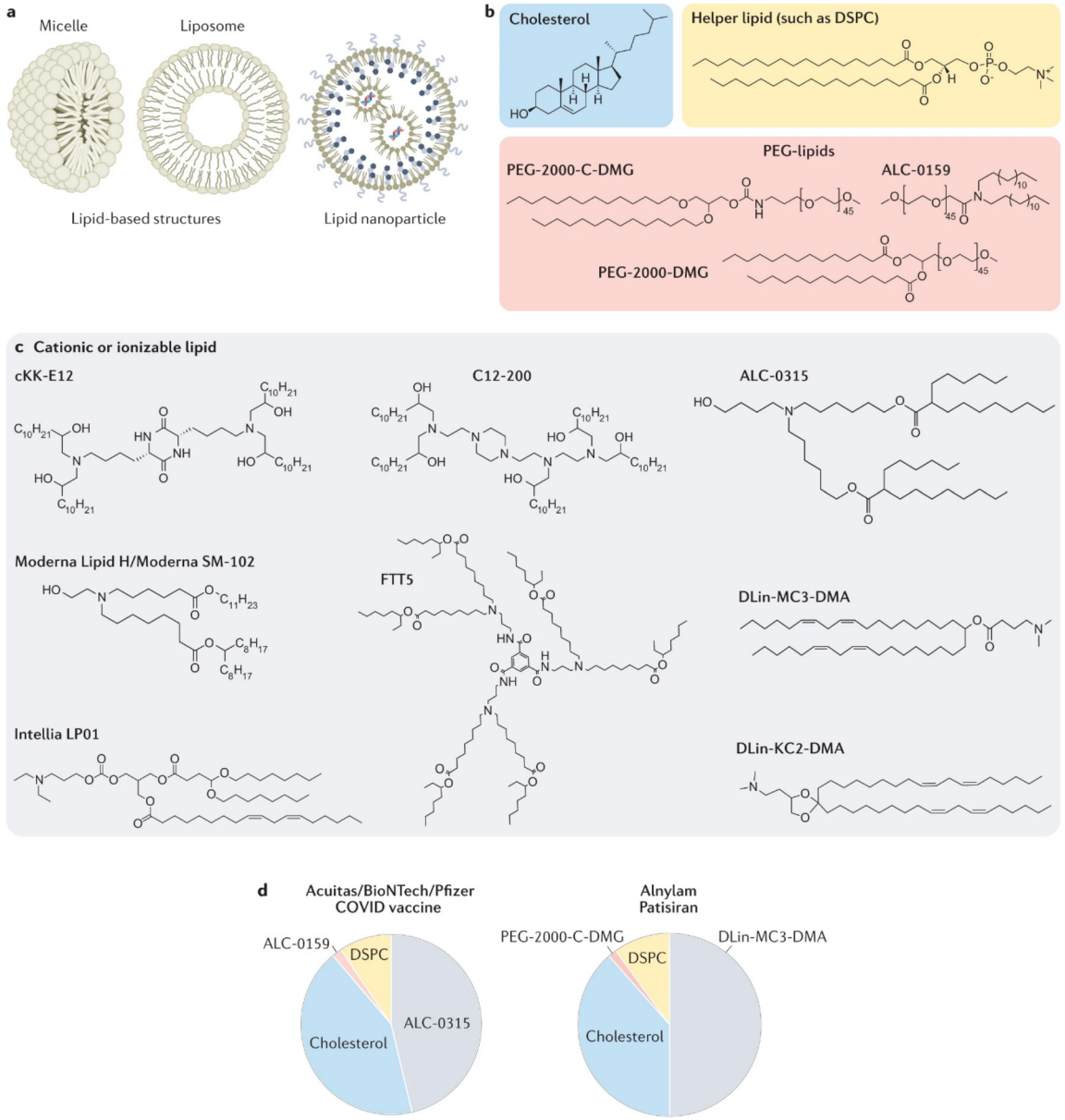

Fig. 4 FDA-approved lipid-based structures contain some variation of the four essential components: cholesterol, a helper lipid, a PEG-lipid, and a cationic or ionizable lipid. (a) Lipid-based structures can include micelles, which consist of a lipid monolayer, or liposomes, which consist of a bilayer. Lipid nanoparticles comprise multiple lipid layers and lipid and nucleic acid microdomains. (b) and (c) In addition to the RNA payload, LNPs often consist of cholesterol, a helper lipid, a PEG-lipid (all shown in part b), and a cationic or ionizable lipid (part c). (d) The molar ratios of the four components constitute the FDA-approved Acuitas/BioNTech/Pfizer COVID-19 vaccine and patisiran, which delivers siRNA to the liver. Adapted with permission from ref. 195. Copyright 2022, Springer Nature.

4.1 Cationic lipids

Cationic lipids (CLs) are well characterized by their positively charged hydrophilic head (such as quaternary ammonium, amines), linker (such as ester, ether, disulfide), and negatively charged hydrophobic tail (cholesterol, tocopherol). CLs are well-explored for the delivery of negatively charged nucleic acids. Cationic lipid, namely *N*-[1-(2,3-dioleyloxy) propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA) bearing a quaternary ammonium head group and unsaturated phosphatidylethanolamine (DOPE), was first employed for nucleic acid delivery for gene therapy by Felgner's group in 1987.[204] The lipid formed is still commercially available with Lepofectin and is extensively used for the *in vitro* delivery of nucleic acids.[205] CLs formed complexes with negatively charged phosphate groups in the polynucleotides' DNA. The complexes are formed through positively charged head groups *via* electrostatic interactions, whereas hydrophobic tails exist as bilayers around DNA.[206] This lipid envelope surrounding DNA provides resistance against omnipresent nucleases and overcomes immuno-

genicity and mutagenicity. Additionally, it can easily combine with other anionic sulfate groups of cell membrane proteoglycans, thus ensuring cell uptake. 1,2-Dioleoyl-3-(trimethylammonium) propane (DOTAP) and polyethylene-glycol-2000-1,2-distearyl-3-sn-phosphatidylethanolamine (PEG-DSPE) based cationic lipid was used to coat hydrophobic IONPs *via* self-assembly synthesis.[207] The lipid-coated IONPs showed an average size of 46 nm and improved delivery in three cell lines: HeLa, PC-3, and Neuro-2a. Furthermore, *in vivo*, studies revealed that the growth status of the tumor cells in Balb/c mice was efficiently monitored through MR images, and the magnetic property of IONPs was retained up to 15 days after administration. Sun *et al.* (2022) demonstrated the efficacy of DOTAP/cholesterol-based CL NPs for successfully delivering mRNA, pDNA, and oligonucleotide.[208] The nanocomplex was stable for up to 60 days at 4 °C storage conditions without affecting transfection efficacy. The effective DOTAP/cholesterol CL NP:mRNA ratio for *in vitro* studies was 62.5 μM lipid to 1 μg mRNA. It was speculated that a lower lipid concentration carried a lower surface charge, resulting in decreased cell interaction and endosome escape. In contrast, a higher concentration might lead to cytotoxicity and inhibition of mRNA dissociation from the nanocomplex.

Based on the chemical structure of a positively charged head group, lipids can be classified into six classes, namely quaternary ammoniums, amines (primary to quaternary), amino acids or peptides, guanidiniums, heterocyclic headgroups, and some uncommon headgroups.[177,209] Headgroups containing charge and dimensions impact cell interaction, endosome escape, and access to the target cell.[210]

Linkers typically comprise non-biodegradable (*e.g.*, ethers and carbamates) and widely used biodegradable (*e.g.*, esters, amides, and thiols) functional moieties. Linkers form a junction between the headgroup and tails.[211] The linker affects the stability, biodegradability, cytotoxicity, and transfection efficiency of LNPs.

The negatively charged, lipophilic aliphatic chains of sterols form the last part of cationic lipids. The hydrophobic tails elicit NP formation and potency by maintaining fluidity, hydrophobicity, and fusion with the cell membrane.[209]

Nevertheless, despite the advantages CLs offer for the delivery of therapeutic cargo, researchers are motivated to continue their development and optimization in preclinical research. Their drawbacks include reduced *in vivo* efficacy, low circulation time, rapid elimination by RES, off-target accumulation in the negatively charged cellular system, and unacceptable toxicity related to inflammatory responses, which has challenged their clinical acceptance.[212–214]

4.2 Ionizable lipids

Ionizable lipids (ILs) or ionizable cationic lipids are small amphiphilic vesicles containing an ionizable protonatable tertiary-amino head group, a spacer/linker, and a hydrophobic moiety.[215] ILs showed the unique feature of electrostatic charge dependency on the lipid pK_a and pH of their environmental surroundings and, thus, become positively charged at acidic pH, ensuring the binding of therapeutic cargos while maintaining a neutral charge at physiological pH, reducing toxicity effects.[216,217] ILs with a suitable pK_a can modify their electrostatic charge to ensure prolonged circulation and cytosolic release of therapeutics, elevated transfection efficiency, access to targeted sites, and reduced toxicity. The transfection efficiency of ILs depends mainly on the pK_a, with an optimal pK_a value of 6.2–6.5.[176,218]

Ionizable lipids can be categorized into various subclasses based on their structure.

(1) Unsaturated ILs: the degree of tail unsaturation significantly impacts the fluidity and delivery efficiency of ILs. The high unsaturation of lipid tails leads to nonbilayer lipid formation, enhancing membrane disruption and cargo release. Some common unsaturated ILs include DLin-MC3-DMA (MC3), A18-iso5-2DC18, OF02, *etc*.

(2) Polymeric ILs: the structural basis of polymeric ILs is the substitution of free amines on cationic polymers with alkyl tails, and their hydrophobic aggregation results in improved particle formation. Common examples include (1,2 bis(triclosan-10,12-diynoyl)-snglycero-3-phosphocholine) (DC8,9PC), 7C1, G0-C14 *etc*.

(3) Biodegradable ILs: the main objective of using different materials for biomedical applications includes improved biocompatibility and biodegradability with reduced toxicity and inappropriate accumulation inside the body.[202] Adding biocleavable ester bonds (simple structure, chemical stability) to ILs provides biocompatibility *via* undergoing enzymatic hydrolysis *in vivo*. Maier *et al.* (2013) synthesized a library of biodegradable ILs by incorporating ester bonds at different lipid positions.[202]

Recently, anti-SARS-CoV-2 vaccines have also used ILs for their formation.[219] The vaccines mainly comprise mRNA motifs and LNPs composed of pH-sensitive ILs. Kim *et al.* (2021) reported on the target delivery of RNA using engineered ILs into specific liver cells, namely hepatocytes and liver sinusoidal endothelial cells.[220] The delivery of ILs to the targeted site was ensured by controlling the size and PEG:lipid ratio (Fig. 5a). Moreover, active targeting was achieved by adding mannose to the ILs. *In vivo*, gene silencing studies showed the selectivity of the engineered ILs towards the targeted liver cells (Fig. 5b).

ILs are widely explored by modifying their domains, including hydrophobic tails, hydrophilic heads, or linkers. Walsh *et al.* (2013) reported a new class of IL with a lysine head group linked to long-chain dialkylamine *via* an amide linker for siRNA delivery.[221] The resulting pH-dependent ILs contain a carboxylate group and two ionizable amines. This ionizable lipid exhibits electrostatic charge-dependent membrane disruption advantages, successful *in vitro* siRNA transfection, and enhanced siRNA-mediated knockdown in transfected HeLa cells. Nucleic acid delivery was also accomplished using different aminoglycoside-derived ILs.[222,223]

Lipid-based delivery systems are widely used for improved chemotherapy for cancer treatment. Broma *et al.* (2019) reported ILs as a Trojan horse in delivering AuNPs with a size

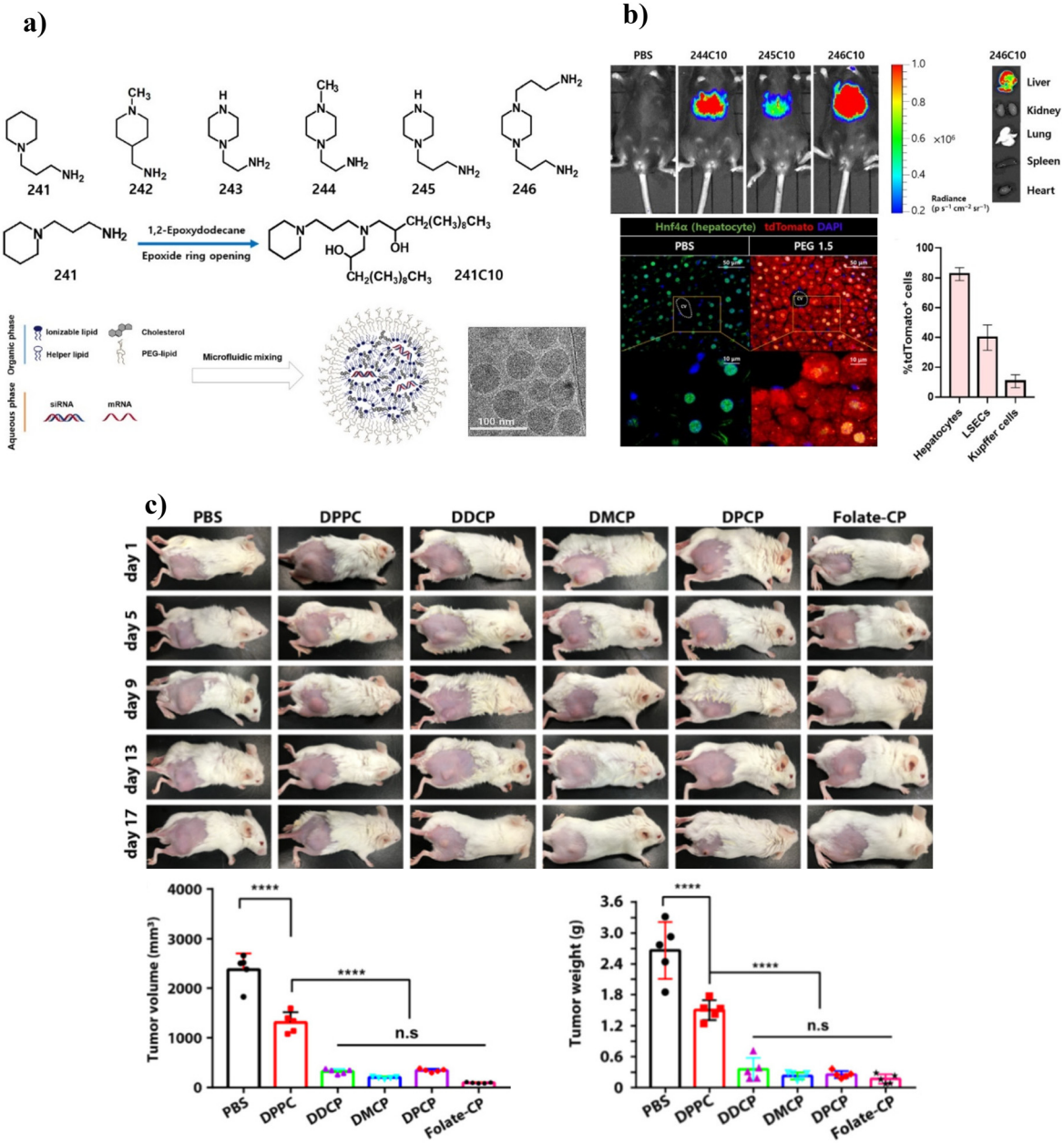

Fig. 5 (a) Preparation of LNPs derived from ionizable lipids. (b) *In vivo* evaluation of ILs showed potent luciferase expression. *Ex vivo* organ images showed that LNPs were mostly taken up into the liver. Liver histology image and transfection efficiency showed significant tdTomato fluorescence in the hepatocyte. cv, central vein. Adapted with permission from ref. 220. Copyright 2021, American Association for the Advancement of Science. (b) Synthesis and *in vivo* application of CP liposomes. (c) Tumor therapy efficacy of CP liposomes (DDCP-c, DMCP-c, DPCP-c, and folate-CP) with DPPC as the control. Adapted with permission from ref. 228. Copyright 2021, American Chemical Society.

range of 5 nm for enhanced outcomes in the radiation therapy of triple-negative MDA-MB-231 cells.[224] The ILs with the composition DLin-MC3-DMA/DSPC/cholesterol/PEG were used to coat AuNPs. The complex of IL–AuNPs showed a ~73-fold increase in the uptake of small-sized AuNPs in cancerous cells.

4.3 Zwitterionic lipids

The zwitterionic lipids (ZILs) contain an equal number of covalently bonded anionic and cationic moieties.[225] ZILs have gained the utmost attention in biomedical applications, including drug delivery and increased uptake of loaded molecules by reducing the adsorption of proteins on nanocarriers in serum.[226] Obata *et al.* (2010) designed endosomal-pH-responsive liposomes functionalized with glutamic acid-based zwitterionic lipids for enhanced drug delivery applications.[227] The liposomes showed a positive zeta potential at lower pH and became negatively charged at basic pH due to the carboxyl group moiety in the glutamic acid. Furthermore, synthesized pH-responsive lipids reflected high fusogenic potential with the anionic membrane of cancer cells. Thus, it ensures the

improved release of encapsulated Dox in HeLa cells and high antitumor activity *in vivo* against a xenograft breast cancer tumor. Cancer treatment with targeted and enhanced drug delivery was ensured using a novel biorthogonal zwitterionic lipid (choline phosphate (CP)) based liposome functionalized with folic acid using a click reaction.[228] Furthermore, compared with phosphatidylcholine (PC) based liposomes, supramolecular ionic pair interactions of zwitterionic lipid exhibit adhesive characteristics with the cell membrane of cancer cells, enhanced biocompatibility in normal cells, significantly enhanced cytotoxicity and inhibition of tumor growth (Fig. 5c).

4.4 Liposomes

Liposomes are small spherical-shaped artificial vesicles synthesized from cholesterol and nontoxic phospholipids. Liposomes are widely explored as delivery agents due to their size and ability to load both hydrophilic and hydrophobic molecules.[229] Doxil was the first liposomal formulation approved in 1995 by the FDA of the USA for the treatment of refractory acquired immune deficiency syndrome (AIDS)-related to Kaposi's sarcoma.[230] The remarkable journey of liposomes as delivery agents includes ligand-targeted delivery, nucleic acid/gene delivery, and delivery of active drugs, polymers, anesthetics, and antimicrobial agents.[231] Spherical liposomes are readily taken up by irregular and distorted tumor cells *via* an enhanced permeability and retention effect, resulting in elevated drug distribution at the tumor site.[232] Curcumin and metformin-loaded DSPE-PEG2000-hyaluronic acid liposomes were designed to target hepatocellular tumors and drug resistance.[233] *In vitro* and *in vivo* studies revealed that this formulation exhibits more potent antiproliferation and antimetastasis. The inhibition of drug resistance and tumor growth was attributed to the down-regulation of multidrug resistance-related P-glycoprotein and the inducing epithelial–mesenchymal transformation of tumor cells. Michel *et al.* (2017) developed cationic liposomes to load mRNA and improve cell transfection to treat alpha-1-antitrypsin deficiency.[234] Liposomes showed a prolonged transfection effect with negligible cytotoxicity in A549 cells. Liposomes had a long-acting transfection effect on cells, resulting in increased expression of a functional alpha-1-antitrypsin protein. Dhaliwal *et al.* (2020) developed a cationic liposome-based nanovehicle for intranasal delivery and potent mRNA transfection to a murine model's brain.[188] The incubation of mRNA-loaded cationic liposomes with J774.1 macrophage cells showed stable GFP expression in the cytosol up to 24 h (Fig. 6a and b). Furthermore, intranasal administration of mRNA-loaded cationic liposomes in mice compared with control

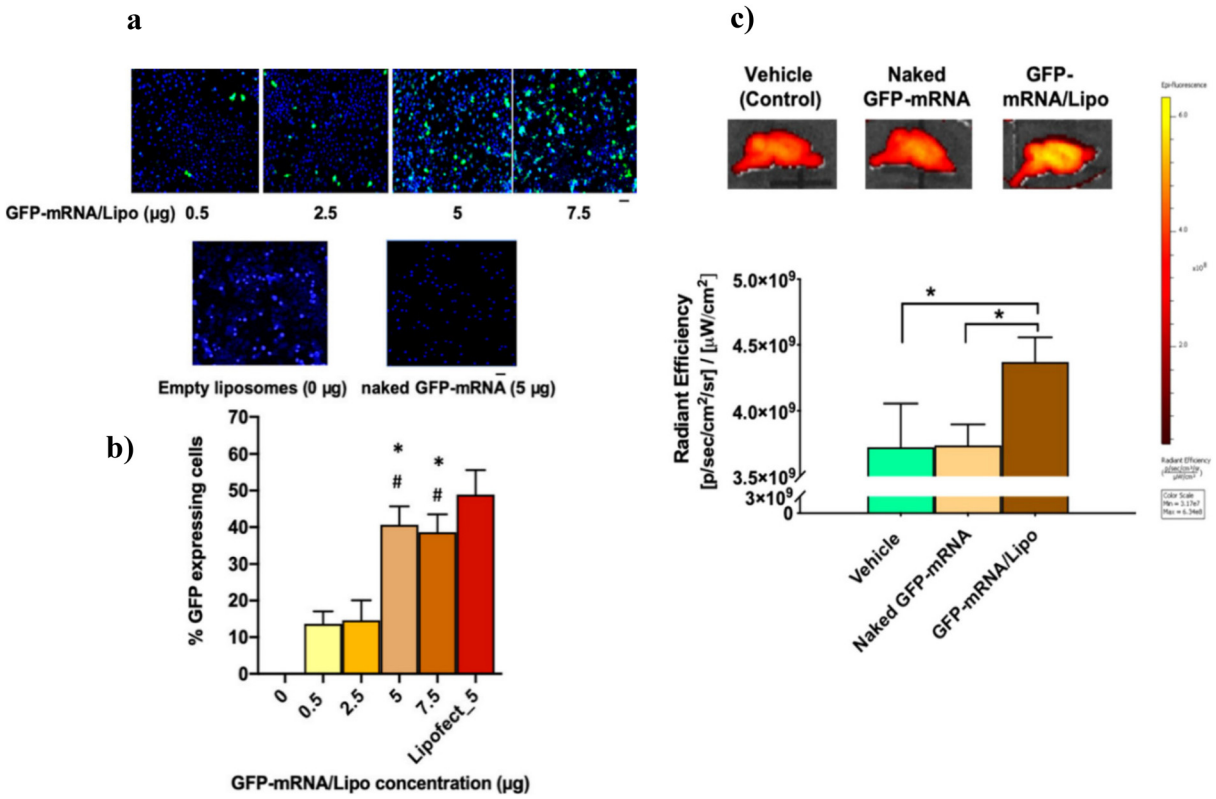

Fig. 6 (a) GFP-mRNA transfection studies in J774A.1 macrophages using cationic liposomes at different concentrations. (b) Quantification of % GFP-expressing cells. Lipofect_5 represents Lipofectamine (with 5 μg GFP-mRNA)-treated cells as a positive control, and the 0 μg GFP-mRNA/Lipo group represents empty liposome-treated cells as a negative control (showed no GFP signal). (c) GFP-mRNA expression in mouse brain, 24 h post intranasal administration of GFP-mRNA-loaded cationic liposomes. Adapted with permission from ref. 188. Copyright 2020, American Chemical Society.

(GFP-mRNA) and vehicle (liposome)-treated groups showed significantly higher GFP expression by ~15% ($p < 0.05$) up to 24 h (Fig. 6c).

Lei *et al.* (2020) demonstrated colorectal cancer gene therapy using a protamine/liposome-based delivery carrier loaded with IL-15 mRNA (CLPP-mIL-5 complex).[189] Cytokine IL-15 can be a cancer gene therapy due to its immune stimulation characteristics. *In vitro* studies showed the successful delivery of mRNA in C26 cells. The accumulation of mRNA in cancer cells induced cytotoxicity and lymphocyte stimulation. Following systemic delivery in three C26 murine colon cancer-bearing mice, CLPP-mIL-5 complex inhibits cancer rates up to 70%, 55%, and 69% in abdominal cavity metastasis tumor, subcutaneous, and pulmonary metastasis models, respectively.

4.5 Lipid nanoparticles (LNPs)

Lipid NPs are the highly advanced clinically viable vector for NA delivery with Onpattro (patisiran) approval for amyloidosis treatment and their application in COVID-19 vaccine around the globe.[177,235] Lipid NPs are composed of four different lipids, namely (1) ionizable cationic lipid, (2) three neutral helper lipids, namely phospholipid, (3) cholesterol, and (4) PEGylated lipid.[236] In general, ionizable cationic lipids form an electrostatic complex with anionic nucleic acid, enabling intracellular uptake and endosomal escape. The stability and fluidity of the complex are maintained by cholesterol. Furthermore, phospholipids enhance the structural integrity of the complex. PEGylated lipid is an important module that maintains stability, enables cellular uptake, and protects the lipid–NA complex from being captured by the protein corona.[237,238] Billingsley *et al.* (2022) developed a sequential library of biocompatible lipid NPs *via* microfluidic mixing to successfully deliver mRNA to T cells (Fig. 7a and b).[190] Their study revealed that lipid NPs composed of C14-4 ionizable lipid improved the delivery of mRNA by a 3-fold increase in primary human T cells. Additionally, lipid NPs with minimal cytotoxicity induced chimeric antigen receptor (CAR) expression and effectively killed cancer cells (Fig. 7c). Ly *et al.* (2022) developed lipid NPs using FDA-approved ionizable lipids, namely, MC3, ALC-0315, and SM-102, and applied them for the loading and delivery of self-amplifying mRNA (saRNA), protein expression, and cytokine activation *in vitro* (triggering different levels of IL-6 response).[236] Furthermore, it was found that PEG facilitates the encapsulation and stability of saRNA, which is otherwise complicated to preserve as compared with mRNA. The type of ionizable lipid highly influenced the protein expression. Protein expression was highest in ALC-0315, followed by SM-102, whereas MC3 failed to show potent protein expression. The study provides an insight into lipid NPs required for specific applications, including the delivery of heavy RNA molecules (saRNA, Cas9), therapies related to protein replacement, and vaccine production.

4.6 Solid lipid nanoparticles

Solid lipid NPs (SLNs) have been extensively studied over the past decade because they provide the combinatorial effect of various carrier systems, including polymeric nanoparticles, emulsions, and liposomes.[239] SLNs have changed the dimensions of gene and drug delivery because of their advantages, including biocompatibility and safety profile, avoidance of organic solvents, ease of large-scale synthesis, water-based technology, physicochemical stability, ability to encapsulate proteins and nucleic acids along with hydrophilic, hydrophobic drugs and bioactive compounds, and controlled and sustained release of loaded components.[240–242] SLNs are spherical nanoparticles with diameters of 50–1000 nm.[239,242] SLNs remain solid at room temperature because of the core of SLNs. The core of SLNs is composed of solid lipids such as glycerides, steroids, fatty acids, or waxes, whereas LNPs comprise solid and liquid crystalline lipids.[201,243] SLNs are superior to lipid nanoparticles in protecting nucleic acids

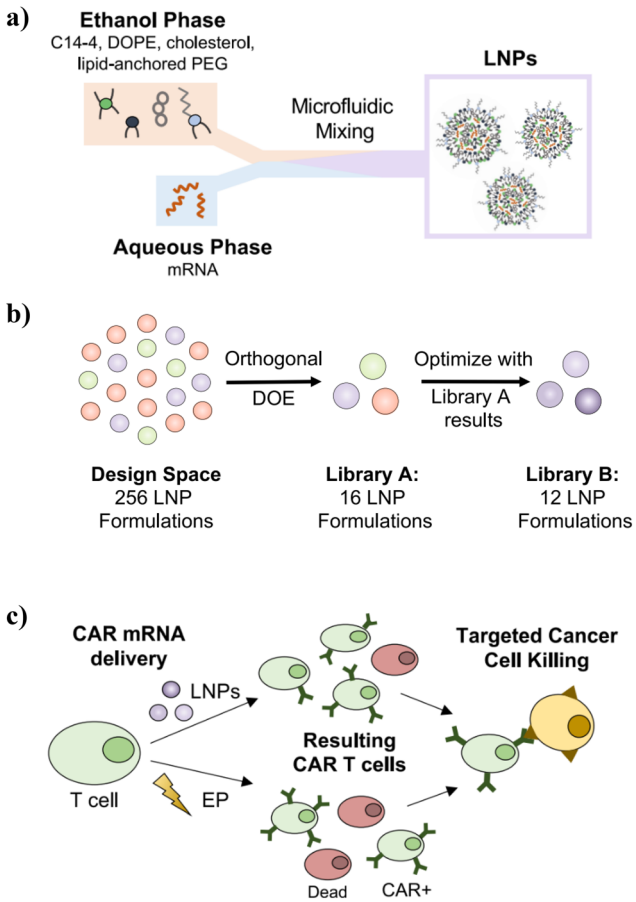

Fig. 7 (a) Schematic of LNP synthesis, including the components used to make LNPs *via* microfluidic mixing and the expected resulting structure. (b) Visualization of the design process used to generate libraries A and B with library A resulting from orthogonal DOE screening of a wide range of excipient molar ratios, and library B examining more formulations within a narrowed range of excipient ratios based on the results from the library A screen. (c) Schematic of the CAR T cell production utilizing either LNPs or EP for mRNA delivery to T cells. The treated T cell populations may differ in viability and CAR potency depending on the transfection method. Still, both can generate functional CAR T cells to induce targeted cancer cell killing. Adapted with permission from ref. 190. Copyright 2022, American Chemical Society.

from degradation and leakage during storage. SLNs are synthesized using physiological, biodegradable, and biocompatible lipids in a solid state at room temperature, emulsifiers, or a combination of pharmaceutical agents and solvents. Different methods have been extensively investigated for the synthesis of SLNs, such as emulsion-solvent evaporation,[244] microemulsion,[245] high-shear homogenization,[246] ultrasonication,[247] solvent injection methods,[248] nanoprecipitation (solvent displacement technique) and so on.[193] SLNs possess a solid lipid core loaded with the active ingredient and stabilized with an outer surface of surfactant. Nonviral delivery of plasmid DNA was efficiently done using SLP-based cargos.[249] The key ingredients of the SLNs prepared *via* the solvent-emulsification method include cholesteryl oleate glycerol trioleate, and DOPE cholesterol3β-[*N*-(dimethylaminoethane)carbamoyl (DC)-cholesterol]. Furthermore, plasmid DNA was incorporated into SLNPs. *In vitro* studies suggested improved transfection efficiency of plasmid DNA in dendritic cells. Song *et al.* (2017) synthesized cationic solid lipid NPs (CSLNs) for the delivery of siRNA and β-$NaYF_4$:Yb,Er upconversion nanoparticles (UCNPs) and applied these for bioimaging and gene therapy in A549 cells.[194] CSLPs were synthesized *via* the thin-film dispersion method using a peptide-based cationic lipid, CDO14, and further conjugated to UCNPs and siRNA by electrostatic interaction. Gene silencing, bioimaging, and cytocompatibility studies demonstrated that encapsulated siRNA and UCNPs could efficiently be delivered and internalized into the cells and displayed superior cellular imaging and gene silencing with CSLPs (Fig. 8a & b). Cytotoxicity studies performed on A549 cells with an incubation of 48 h showed that CLSPs were cytocompatible, with cell viability higher than 90% (Fig. 8c).

4.7 Commercial history of lipid-based drug delivery agents

The commercial history of lipidic formulations for drug and gene delivery can be traced back to the late 20th century when the first liposomal drug, Doxil (doxorubicin hydrochloride liposome injection), was approved by the US FDA in 1995.[250] Since then, lipid formulations have become a popular and effective means of delivering a wide range of therapeutic agents, including cancer drugs, anti-inflammatory agents, and nucleic acids for gene therapy. Liposomes, LNPs, and spherical structures can encapsulate drugs and protect them from rapid clearance and degradation, enabling targeted delivery to cells and tissues. The market for lipid-based drug delivery systems has snowballed in recent years, with several new drugs and gene therapies using lipidic formulations in development and commercialization. One of the main advantages of lipidic formulations is their ability to enhance the efficacy of drugs and reduce their toxic side effects. All these advantages have led to the approval of several lipid-based drugs for the treatment of cancer, including Doxil,[250] Camptosar (lipid NPs),[200] and DaunoXome (daunorubicin citrate liposome injection).[251] In recent years, the use of lipidic formulations for gene delivery has also become increasingly popular, leading to the develop-

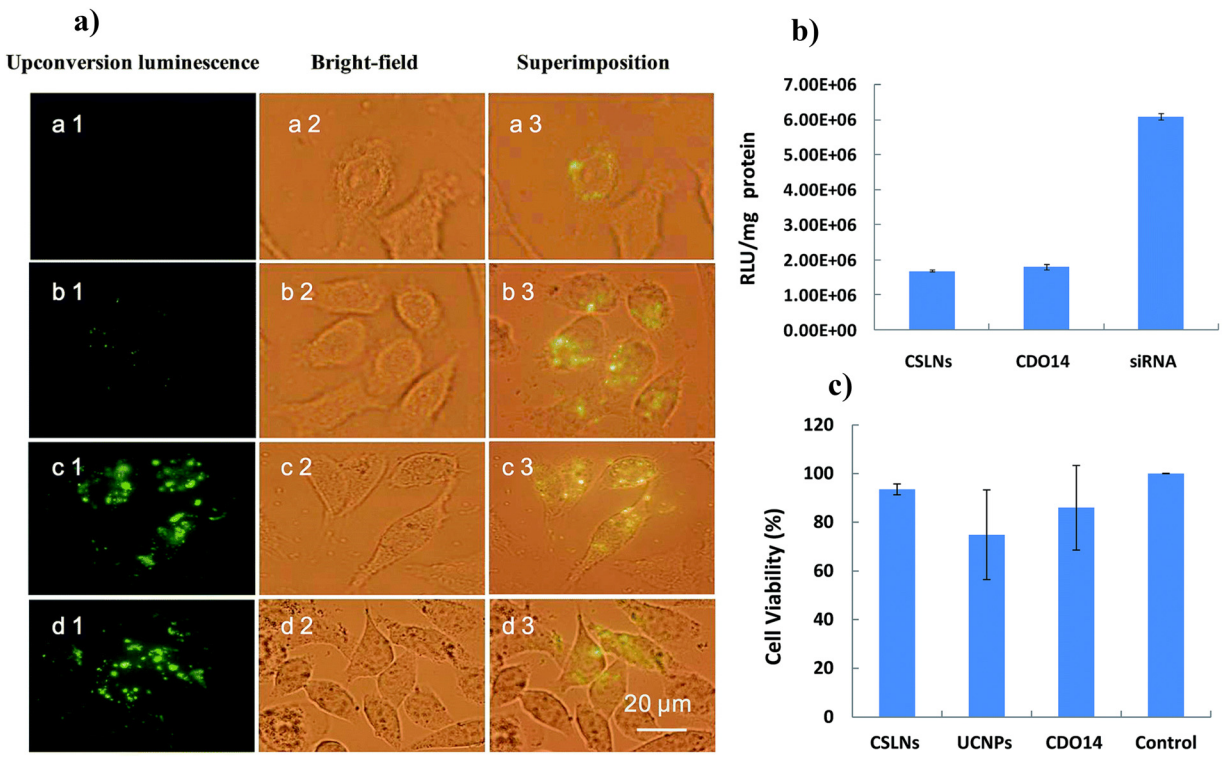

Fig. 8 (a) Uptake of CSLNs into A549 cells. The fluorescence microscope images after treatment with CSLNs (excited by 980 nm light) at concentrations of (a) 5 μg μL^{-1}, (b) 10 μg μL^{-1}, (c) 15 μg μL^{-1} and (d) 20 μg μL^{-1}. (b) Gene silencing mediated by CSLNs. (c) Cytotoxicity of CSLNs/siRNA and UCNPs/siRNA in A549 cells by MTT assay. Adapted with permission from ref. 194. Copyright 2017, The Royal Society of Chemistry.

Table 4 Overview of the FDA-approved lipid-based therapeutics

S. no.	Commercial name	Developer	Date of approval	Lipid	Drug	Treatment	Ref.
1	Diprivan	Crucell	Feb 23, 1989	Liposome	Propofol	Induction and maintenance of anesthesia	251
2	Doxil®	Janssen	Nov 17, 1995	Liposome	Dox	Ovarian and breast cancer	250
3	Daunoxome®	Galen	Apr 8, 1996	Liposome	Daunorubicin	HIV-associated Kaposi's sarcoma	251
4	AmBisome	Gilead Sciences	Nov 8, 1997	Liposome	Amphotericin B	Invasive fungal infections	251
5	Cytosar-U®	Pfizer	Jan 8, 1999	Lipid NPs	Cytarabine	Leukemia and lymphoma	200
6	Visudyne	Bausch and Lomb	Apr 30, 2002	Liposomal	Verteporfin	Macular degeneration, pathologic, or ocular histoplasmosis	251
7	Camptosar®	Pfizer	June 24, 2004	Lipid NPs	Irinotecan	Metastatic colon or rectum cancer	200
8	Vinlon™	Talon Therapeutics, Inc.	Sept 8, 2012	Liposome	Vincristine	Acute lymphoblastic leukemia	200
9	Onivyde®	Merrimack	Oct 22, 2015	Liposome	Irinotecan	Various metastatic cancers, including breast, pancreatic, sarcomas, or brain	251
10	Nocita®	Aratana therapeutics	August 12, 2016	Liposome	Bupivacaine	Anesthetic	253
11	Vyxeos CPX-351®	Jazz Pharmaceuticals	Aug 3, 2017	Liposome	Cytarabine : daunorubicin	Acute myeloid leukemia	251
12	Onpattro® (patisiran)	Alnylam Pharmaceuticals Inc.	Aug 8, 2018	Lipid NPs	siRNA	Hereditary transthyretin amyloidosis	235
13	Givosiran (Givlaari®)	Alnylam Pharmaceuticals Inc.	Nov 20, 2019	Lipid NPs	siRNA	Hepatic porphyria	254
14	Comirnaty®	BioNTech, Pfizer	Aug 23, 2021	Lipid NPs	mRNA	COVID-19	177
15	Spikevax®	Moderna	Jan 31, 2022	Lipid NPs	mRNA	COVID-19	252

ment of new treatments for genetic diseases and other disorders. During the outbreak of SARS-CoV-2, several LNP mRNA vaccines were developed to fight the disease, such as Comirnaty and Spikevax.[177,252] Lipid-based delivery systems are widely accepted in clinical applications and have been approved by the FDA to deliver various therapeutics, including drugs and nucleic acids (Table 4).

5. Nanomaterial delivery vehicles for nucleic acids: promise and challenges

Nucleic acids are widely explored for the treatment of a broad spectrum of diseases including cancer, heart diseases, genetic disorders, and viral and bacterial infections. However, the applicability of nucleic acid in therapeutics is challenging due to enzymatic degradation, and unfavorable physiocochemical properties (hydrophilicity and high molecular weight, negative charge) resulting in reduced cellular uptake, poor *in vivo* stability, rapid excretion or uptake by non-targeted cells.[72,255] Suitable vectors that can overcome inadequate efficacy, susceptibility to enzymatic degradation, low bioavailability, and off-target side effects are required to increase the therapeutic efficacy of nucleic acids. Nanomaterials offer exciting possibilities for the therapeutic delivery of nucleic acids, like DNA or RNA.[223] These nanocarriers hold immense potential for the pharmaceutical landscape as they revolutionized gene therapy, RNA interference, and vaccine development. Compared with traditional methods of delivering nucleic acids into cells, nanomaterials offer a multitude of advantages. (1) Nucleic acids on their own have difficulty penetrating cell membranes and reaching their target sites. Nanomaterials can encapsulate and protect nucleic acids from enzymatic degradation, facilitating their cellular uptake and delivery across biological barriers.[234] (2) Nanocarriers can be engineered with specific molecules on their surface that recognize and bind to particular cell types. This targeted delivery allows researchers to focus the effects of nucleic acids on the desired cells, reducing side effects on healthy tissues.[249] (3) Nanocarriers can be designed to release their nucleic acid cargo in a controlled manner over time. This sustained release can be crucial for some therapies, allowing for longer-lasting effects.[256] (4) Some nanocarriers can be engineered to combine functionalities like delivering therapeutic molecules and imaging capabilities, offering a more comprehensive approach to treatment and monitoring.[257] (5) Nanocarriers can be designed to release their cargo in response to specific stimuli like pH changes or light exposure.[237] This allows for a more controlled and localized release of nucleic acids within the body.

While nanomaterials offer exciting possibilities for nucleic acid delivery, there are also some limitations and potential downsides to consider. (1) Despite targeting strategies, there is a risk that nanocarriers could deliver nucleic acids to unintended cell types. This can lead to unwanted side effects or even toxicity.[74] (2) The body's immune system may recognize nanocarriers as foreign invaders and trigger an inflammatory response. This can limit the effectiveness of the therapy or cause complications.[258] (3) Developing and manufacturing safe and effective nanocarriers for nucleic acid delivery can be complex and expensive. This can hinder the accessibility and affordability of these therapies. (4) Some nanomaterials may not be efficiently cleared from the body after delivering their payload. This can raise concerns about potential long-term effects.[259]

6. Future strategies

Researchers are actively exploring several strategies to further improve nanomaterial-based delivery vehicles for nucleic acids, addressing the current limitations and unlocking their full potential. (1) Attaching targeting ligands to the surface of nanocarriers can significantly improve their specificity. These ligands can bind to receptors present on specific cell types, directing the delivery of nucleic acids to the desired location. (2) Developing nanocarriers that respond to specific environmental cues within the body. For example, carriers could release their cargo in response to changes in pH or temperature, allowing for targeted release at the disease site.[260] (3) Modifying the surface properties of nanocarriers to minimize interactions with the immune system and reduce the risk of inflammatory responses.[261] This can involve using biocompatible coatings or manipulating surface charges. (4) Developing nanocarriers from biodegradable polymers that are naturally broken down and eliminated by the body, reducing long-term accumulation concerns.[259] (4) Optimizing the rate at which nucleic acids are released from nanocarriers. This can help to minimize off-target effects and improve the overall therapeutic efficacy. (5) Developing nanocarriers that can co-deliver nucleic acids with other therapeutic agents, such as drugs or imaging molecules, for combination therapy and improved treatment outcomes.[262] (6) Creating nanocarriers that can be tracked within the body using imaging techniques, allowing researchers to monitor their delivery pathway and optimize targeting strategies. (7) Developing efficient and cost-effective production methods for well-defined and consistent nanocarriers, facilitating their wider clinical application.[263] By addressing these challenges and focusing on these future strategies, researchers can unlock the full potential of nanomaterial-based delivery vehicles for nucleic acids. This has the potential to revolutionize how we treat various diseases, offering more targeted, effective, and safer therapies based on gene therapy, RNAi, and DNA vaccines.

7. Conclusion

Nucleic acid-based therapeutics has experienced the transformation from concept into clinical reality in recent years. The first FDA-approved siRNA-based drug, Onpattro (2018), provides a platform for further developing NA therapeutics to cure rare conditions, cancers, and infectious diseases.[235,264] Since then, significant progress has been made to improve their therapeutic effect, further amplified in the development of the SARS-Cov-2 vaccine worldwide.[157] However, clinical availability usually requires modification of NAs and their carriers to increase nuclease resistance and enhance cellular uptake. SNAs with densely packed 3D structures have overcome some challenges without further modification. SNAs have minimized the nuclease degradation of delivered NAs.[191] Despite this, significant issues still need to be addressed, most notably in effectively providing therapeutic NAs to the targeted site in the required quantity. The stability of SNA structures can be improved by tuning them with other NPs, resulting in tissue/organ-specific delivery. Among all the classes of delivery agents, lipid-based nanocomplexes have shown ideal delivery efficiency at the clinical level.[265] The clinical trials of the different diseases have proved the importance of lipid-based NPs in NA therapeutics. Over the past decade, several NA therapeutics have been in clinical trials, and only a few enjoyed commercial successes. These points highlight the challenges in translating new drugs from animals to humans. With the success story of Onpattro, lipid-based technology is accepted worldwide and is ready to uplift the next wave of NAs therapeutics for vaccination, gene therapy, and protein production. Undoubtedly, the field of NAs is undergoing a massive expansion, and their potential applications in the treatment of chronic disorders, immunotherapy, and personalized medicine will ensure their development to revolutionize the biomedical field in the near future.

Abbreviations

DOTAP	1,2-Dioleoyl-3-(trimethylammonium) propane
ASO	Antisense oligonucleotides
CNMs	Carbon nanomaterials
CLs	Cationic lipids
FDA	Food and drug administration
AuNPs	Gold NPs
GFP	Green fluorescent protein
ILs	Ionizable lipids
IONPs	Iron oxide NPs
LNPs	Lipid NPs
mRNA	Messenger RNA
miRNA	MicroRNA
NPs	Nanoparticles
NAs	Nucleic acids
pDNA	Plasmid DNA
PAMAM	Poly(amidoamine)
PEG	Polyethylene glycol
PEG-DSPE	Polyethylene-glycol-2000-1,2-distearyl-3-sn-phosphatidylethanolamine
PEI	Polyethyleneimine
PLGA	Polylactide-*co*-glycolide
PNPs	Polymeric NPs
QDs	Quantum dots
siRNA	Short interfering RNA
SiNPs	Silicon NPs
SLNPs	Solid lipid NPs
SNAs	Spherical nucleic acids
UCNPs	Upconversion nanoparticles
ZILs	Zwitterionic lipids.

Author contributions

All the authors read and approved the manuscript.

Data availability

No primary research results, software or code has been included and no new data were generated or analysed as part of this review.

Conflicts of interest

The authors declare that they have no competing interests.

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