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Novel Drug Delivery Approaches for Biopharmaceuticals: Advances in mRNA, Protein, Peptide and Gene Delivery Systems

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Abstract

Biopharmaceuticals, including messenger RNA (mRNA), therapeutic proteins, peptides and gene-based medicines, constitute a rapidly expanding class of advanced therapeutics with considerable potential for treating complex and previously difficult-to-manage diseases. However, their clinical application is frequently limited by enzymatic degradation, inadequate membrane permeability, short systemic half-life, immunogenicity and substantial intracellular delivery barriers. Particular emphasis is placed on lipid nanoparticles (LNPs), polymeric nanoparticles, liposomes, dendrimers, hydrogels, viral and non-viral vectors, and emerging biomimetic and exosome-based delivery systems. The review further considers advances in oral peptide delivery through permeation enhancers, targeted delivery approaches, stimuli-responsive carriers and strategies for improving cellular uptake and intracellular release. LNP technology is highlighted for its pivotal role in enabling the clinical translation of mRNA therapeutics and vaccines. In addition, the emerging application of artificial intelligence and computational approaches in carrier optimisation and formulation design is discussed. Comparative evaluation of these platforms considers delivery efficiency, stability, safety, scalability, therapeutic performance and translational potential. Six comparative tables and four schematic figures summarise key mechanisms, advantages, limitations and developmental status. Finally, major challenges involving toxicity, immunogenicity, regulatory requirements, manufacturing complexity and long-term stability are examined, alongside future opportunities for developing safer, more precise and clinically scalable biopharmaceutical delivery systems.

Keywords: Biopharmaceuticals; Lipid Nanoparticles; mRNA Delivery; Gene Delivery; Nanocarriers

1. Introduction

Biopharmaceuticals constitute a diverse and rapidly expanding class of therapeutic agents that includes recombinant proteins, peptides, monoclonal antibodies, nucleic acids such as messenger RNA (mRNA), small interfering RNA (siRNA) and plasmid DNA, as well as cell- and gene-based products. Over the past two decades, these advanced therapeutics have significantly transformed the management of cancer, metabolic disorders, infectious diseases, autoimmune conditions and inherited genetic disorders by enabling highly specific biological interventions. In contrast to conventional small-molecule drugs, biopharmaceuticals are generally large and structurally complex macromolecules, ranging from kilodaltons to megadaltons, whose therapeutic activity depends on maintaining precise molecular conformation, sequence integrity and biological functionality. Therapeutic proteins and monoclonal antibodies require preservation of higher-order structures to maintain target recognition and pharmacological activity, whereas peptides may undergo rapid enzymatic degradation and possess limited membrane permeability. Nucleic acid therapeutics face additional challenges, including nuclease-mediated degradation, poor cellular uptake, endosomal entrapment and inefficient intracellular release. Cell- and gene-based products introduce further delivery requirements related to cellular viability, genetic stability, tissue targeting and controlled expression. Consequently, the classification of biopharmaceuticals is closely linked to their physicochemical characteristics, biological mechanisms and delivery requirements. Understanding these distinctions is essential for designing appropriate delivery systems capable of protecting therapeutic cargo, improving bioavailability, facilitating cellular and tissue uptake, and enabling controlled or targeted release. Table 1 summarises the principal biopharmaceutical classes, representative examples and their characteristic delivery challenges.

1.1 Therapeutic Potential and the Need for Novel Delivery Systems

The therapeutic promise of mRNA, proteins, peptides and genes is substantial: mRNA can direct transient expression of essentially any protein, including antigens, replacement enzymes or gene-editing machinery, without the risk of genomic integration associated with DNA-based approaches [2,4]; therapeutic proteins and peptides offer exquisite target specificity with generally favourable safety profiles; and gene therapies offer the prospect of a single, durable correction of an underlying genetic defect. Realising this potential in the clinic, however, requires delivery technologies capable of protecting a fragile macromolecular cargo from degradation, ferrying it across biological barriers, and releasing it in a functional form at the intended intracellular or extracellular site of action. Conventional formulation approaches — simple aqueous solutions or suspensions administered parenterally — are frequently

inadequate to meet these demands, motivating the extensive development of novel nanoparticulate and macromolecular delivery platforms reviewed here.

This review aims to provide formulation scientists, graduate students and translational researchers with a consolidated, literature-grounded overview of delivery technologies for mRNA, protein, peptide and gene therapeutics published broadly between 2011 and 2022, spanning underlying biological barriers, platform-specific engineering solutions, targeting and stimuli-responsive strategies, administration routes, characterisation methods, safety and regulatory considerations, and emerging technologies including biomimetic carriers and AI-assisted formulation design.

Table 1. Classification of biopharmaceutical therapeutics.

Biopharmaceutical class	Representative examples	Typical molecular weight	Principal delivery challenge
mRNA therapeutics	COVID-19 vaccines (BNT162b2, mRNA-1273), replacement-protein mRNA	~300–1500 kDa (transcript-dependent)	Extracellular nuclease degradation; endosomal escape; innate immune activation
Therapeutic proteins	Insulin, monoclonal antibodies, enzymes, growth factors	5–150 kDa	Proteolytic degradation; poor epithelial permeability; immunogenicity
Therapeutic peptides	Semaglutide, octreotide, calcitonin, GLP-1 analogues	0.5–5 kDa	Enzymatic hydrolysis; very short half-life; low oral bioavailability
Gene therapies (DNA/RNA)	AAV-based gene therapies, plasmid DNA, siRNA, CRISPR components	Variable (kb-scale nucleic acid)	Nuclease degradation; poor cellular uptake; risk of insertional mutagenesis (viral)

2. Literature Review

2.1 Physicochemical Characteristics and Instability

The physicochemical characteristics that confer biological activity on proteins, peptides and nucleic acids simultaneously contribute to their inherent instability and formulation difficulties. Most biopharmaceuticals possess complex molecular architectures that are highly sensitive to environmental and processing conditions. Proteins and peptides may undergo hydrolysis, deamidation, oxidation, disulphide-bond disruption and aggregation, resulting in conformational changes and loss of biological activity. Their three-dimensional structures can be irreversibly altered by temperature fluctuations, extreme pH, mechanical shear, freeze–thaw cycles and interfacial stress encountered during manufacturing, sterilisation,

transportation and storage. Aggregation is particularly important because it can reduce potency while potentially increasing immunogenicity. Nucleic acid therapeutics exhibit a distinct but equally significant instability profile. mRNA, for example, contains a phosphodiester backbone that is susceptible to degradation by ubiquitous ribonucleases present in biological fluids and cells. Its long, single-stranded structure can also undergo hydrolytic cleavage and structural changes associated with secondary folding, which may influence translation efficiency and biological performance. Consequently, effective delivery systems must provide physical and chemical protection while preserving the native structure or functional integrity of the therapeutic cargo. Encapsulation within lipid or polymeric carriers, incorporation into stabilising formulations and optimisation of storage conditions are therefore central considerations in biopharmaceutical formulation development [2,4].

2.2 Gastrointestinal Degradation and Poor Membrane Permeability

Oral administration represents one of the most attractive but technically challenging routes for biopharmaceutical delivery because the gastrointestinal tract presents multiple sequential barriers. Gastric acidity and pepsin can rapidly denature and degrade protein and peptide therapeutics, while pancreatic enzymes, including trypsin and chymotrypsin, and brush-border peptidases contribute to extensive enzymatic degradation in the small intestine [10,12,13]. The intestinal mucus layer provides an additional diffusional and steric barrier, while the epithelial cell membrane strongly restricts the passage of large, hydrophilic and frequently charged macromolecules. Tight junctions between epithelial cells further limit paracellular transport, whereas inefficient transcellular uptake restricts movement across the lipid-rich plasma membrane. Even when enzymatic degradation is partially prevented, the low intrinsic permeability of proteins and peptides generally results in extremely limited systemic exposure following oral administration. For many peptide and protein candidates, absolute oral bioavailability remains only a few per cent or substantially lower [16]. Advanced approaches therefore seek to overcome these barriers through enzyme inhibitors, permeation enhancers, mucoadhesive systems, absorption-modulating excipients, nanoparticles and lipid-based carriers. Such strategies aim to protect the therapeutic molecule during gastrointestinal transit while simultaneously enhancing epithelial interaction, cellular uptake and transport into systemic circulation.

2.3 Short Biological Half-Life, Intracellular Barriers and Immunogenicity

Following successful absorption or parenteral administration, many biopharmaceuticals exhibit relatively short biological half-lives because of renal filtration, enzymatic or proteolytic degradation, receptor-mediated clearance and uptake by the reticuloendothelial system [17,18]. Smaller proteins and peptides may undergo rapid renal elimination, whereas particulate delivery systems can be recognised and cleared by macrophages and other components of the mononuclear phagocyte system. These limitations can necessitate frequent dosing and may reduce therapeutic adherence. For nucleic acid therapeutics and proteins that act intracellularly, systemic delivery represents only the initial stage of the delivery process. The therapeutic payload must subsequently reach the appropriate tissue and cell, cross or interact with the plasma membrane, undergo efficient endosomal escape and reach its intracellular site of action before degradation occurs. DNA-based therapeutics may face the additional requirement of nuclear entry for transcription. Immunogenicity constitutes another major challenge. Viral vectors may stimulate innate and adaptive immune responses, while therapeutic proteins can induce anti-drug antibodies that alter pharmacokinetics or neutralise pharmacological activity. Nucleic acids, particularly unmodified RNA, may activate innate immune pathways through pattern-recognition receptors, potentially reducing translation and causing inflammatory responses [6,20]. Accordingly, contemporary delivery-system development increasingly focuses on shielding therapeutic cargo, improving tissue specificity, prolonging circulation, promoting endosomal escape and reducing unwanted immune activation. Table 2 summarises the principal physicochemical, gastrointestinal, pharmacokinetic, intracellular and immunological barriers that influence the delivery and clinical performance of biopharmaceutical therapeutics.

Table 2. Physicochemical and biological barriers affecting biopharmaceutical delivery.

Barrier category	Mechanism	Primarily affects
Enzymatic/chemical instability	Nuclease, protease and peptidase degradation; oxidation, deamidation	mRNA, peptides, proteins
Gastrointestinal degradation	Acid hydrolysis, pepsin/pancreatic protease activity	Orally administered proteins and peptides
Poor membrane permeability	Tight junctions, mucus layer, low passive diffusion of hydrophilic macromolecules	All biopharmaceutical classes, especially oral route

Short biological half-life	Renal clearance, proteolysis, reticuloendothelial uptake	Unmodified proteins, peptides, naked nucleic acids
Intracellular delivery barriers	Endosomal entrapment, cytosolic nuclease activity, nuclear envelope	mRNA, siRNA, plasmid DNA, gene-editing proteins
Immunogenicity/toxicity	Innate immune sensing of nucleic acids; anti-drug antibodies; anti-capsid immunity	mRNA, therapeutic proteins, viral vectors

3. Novel Drug Delivery Platforms for Biopharmaceuticals

A diverse toolbox of nanoparticulate and macromolecular carriers has been developed to address the barriers outlined above. Lipid-based nanoparticles, comprising an ionizable or cationic lipid, a helper phospholipid, cholesterol and a PEGylated lipid, are the most clinically advanced platform, underpinning both the approved small-interfering-RNA product patisiran and the mRNA COVID-19 vaccines [1,7]. Polymeric nanoparticles, formed from biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA) or cationic polymers such as polyethyleneimine (PEI) and poly(β-amino esters), offer tunable degradation kinetics and surface functionalisation for both protein/peptide encapsulation and nucleic acid complexation [20]. Liposomes, concentric phospholipid bilayer vesicles, remain widely used for both protein and nucleic acid delivery, while micelles and nanoemulsions offer simpler, often surfactant-based, solubilisation of lipophilic or amphiphilic peptide cargoes.

Hydrogels and implantable systems provide sustained, often months-long, release of therapeutic proteins and peptides from a single administration, circumventing the need for frequent injections [17]. Dendrimers — highly branched, monodisperse macromolecules such as poly(amidoamine) (PAMAM) — present a large number of surface functional groups for nucleic acid complexation or targeting-ligand conjugation and have been explored as both gene and peptide carriers [20]. Beyond these established platforms, targeted and stimuli-responsive delivery systems, discussed in detail in Section 8, incorporate ligands or environmentally responsive chemistries to concentrate therapeutic payload at the intended site of action while minimising off-target exposure.

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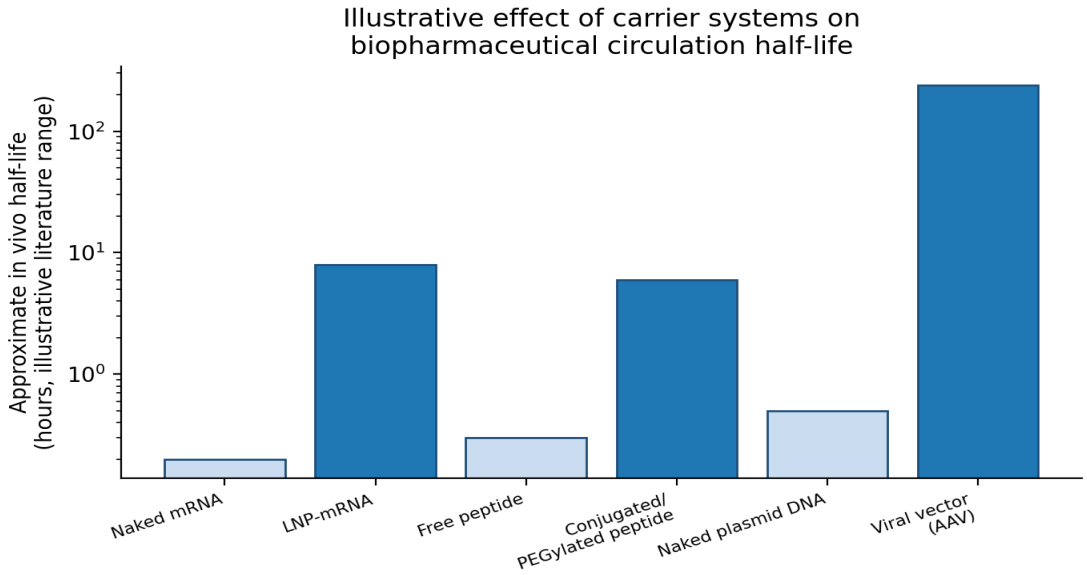


Figure 1. Illustrative effect of carrier/conjugation systems on the in vivo circulation half-life of representative biopharmaceutical modalities (naked vs LNP-delivered mRNA; free vs conjugated/PEGylated peptide; naked plasmid DNA vs AAV vector). Values are illustrative, order-of-magnitude composites drawn from general trends in the reviewed literature.

Table 3. Comparison of novel nanoparticulate and macromolecular delivery platforms.

Platform	Typical size range	Cargo compatibility	Key strength
Lipid nanoparticles (LNP)	60–150 nm	mRNA, siRNA, plasmid DNA	Efficient endosomal escape; clinically validated
Polymeric nanoparticles (PLGA, PEI, PBAE)	50–300 nm	Proteins, peptides, nucleic acids	Tunable degradation; surface functionalisation
Liposomes	50–500 nm	Proteins, nucleic acids, small peptides	Dual hydrophilic/lipophilic loading; established GMP manufacture
Micelles / nanoemulsions	10–100 nm	Amphiphilic/lipophilic peptides	Simple composition; high solubilisation capacity
Hydrogels / implants	Bulk/mm-scale device	Proteins, peptides	Sustained release over weeks to months
Dendrimers	2–15 nm	Nucleic acids, small peptides	Precise, monodisperse architecture; high surface valency

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4. mRNA Delivery Systems

4.1 Structure, Therapeutic Potential and Stability Challenges

Therapeutic messenger RNA (mRNA) is a sophisticated nucleic acid platform comprising a 5′ cap, 5′ untranslated region (UTR), open reading frame (ORF), 3′ untranslated region and 3′ poly(A) tail, with each structural element contributing to RNA stability, translational efficiency and intracellular persistence [2,4]. The 5′ cap facilitates recognition by the translation initiation machinery and protects the transcript from exonucleolytic degradation, whereas optimised UTRs regulate ribosome recruitment, transcript stability and protein production. The poly(A) tail contributes to mRNA stability and translation by interacting with poly(A)-binding proteins. Therapeutic mRNA can be further engineered through codon optimisation, sequence modification and incorporation of chemically modified nucleosides such as N1-methylpseudouridine. These modifications can reduce recognition by innate immune sensors, decrease inflammatory responses and enhance protein expression [3,4]. Despite these advances, mRNA remains inherently unstable because its phosphodiester backbone is highly susceptible to hydrolysis and degradation by ubiquitous extracellular and intracellular ribonucleases. Naked mRNA therefore exhibits extremely limited persistence in biological fluids and is rapidly degraded following administration. Furthermore, its large molecular size, negative charge and hydrophilic character restrict passive cellular uptake. Consequently, protective delivery vehicles are essential for most therapeutic applications, providing nuclease protection, facilitating cellular entry, promoting endosomal escape and enabling efficient cytoplasmic release of functional mRNA.

4.2 Lipid Nanoparticle-Based mRNA Delivery

Lipid nanoparticles are the dominant clinically validated mRNA delivery platform, comprising four principal lipid components: an ionizable lipid (e.g., SM-102, ALC-0315, or the earlier DLin-MC3-DMA), a helper phospholipid such as DSPC, cholesterol, and a PEGylated lipid, typically combined at a molar ratio in the region of 50:10:38.5:1.5 [1,7]. The ionizable lipid is protonated at the acidic pH used during particle formulation, enabling electrostatic complexation with anionic mRNA, but becomes charge-neutral at physiological pH, minimising toxicity during systemic circulation; upon endosomal acidification following cellular uptake, re-protonation of the ionizable lipid promotes membrane destabilisation and endosomal escape of the mRNA cargo into the cytoplasm [1,7]. Cholesterol modulates bilayer rigidity and fusogenicity, while the PEG-lipid controls particle size and confers steric stabilisation, reducing opsonisation and prolonging circulation [1]. Structure–activity work has further shown that branching and chain length within the ionizable lipid tail substantially influence particle stability, fusogenicity and organ-selective delivery [9], and specialised LNP formulations have even been engineered for in utero mRNA delivery to the fetal liver for protein-replacement applications [10].

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4.3 Polymer- and Peptide-Based Carriers, Targeting and Applications

Beyond lipid nanoparticles, cationic and ionizable polymers, polymer–lipid hybrids, and cell-penetrating-peptide-based carriers have been explored as alternative or complementary mRNA delivery vehicles, particularly where tissue-specific targeting or reduced lipid-associated toxicity is desired [5]. Active targeting of LNPs has been achieved through surface conjugation of antibody fragments, peptides or small-molecule ligands, and through 'selective organ targeting' (SORT) approaches in which the inclusion of a supplementary charged lipid biases biodistribution toward the lung, spleen or liver. Clinical and preclinical applications of mRNA therapeutics now span prophylactic and therapeutic vaccination, protein-replacement therapy, and in vivo gene-editing component delivery [6,8], with the clinical success of the BNT162b2 and mRNA-1273 COVID-19 vaccines providing definitive proof of concept for the platform [6].

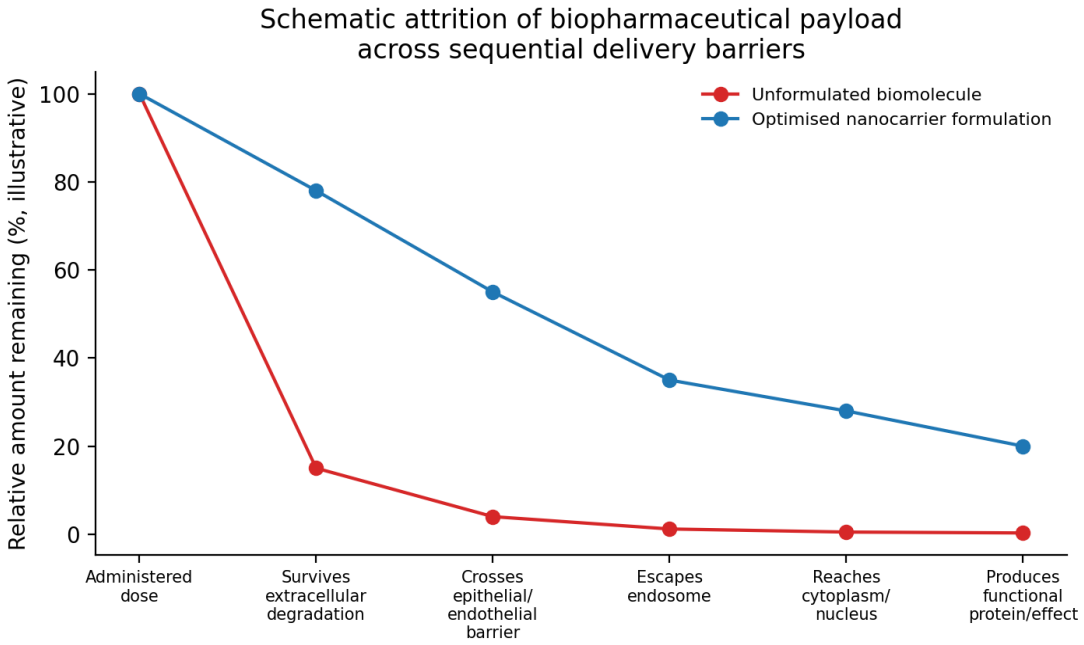


Figure 2. Schematic representation of the sequential attrition of an mRNA (or other biopharmaceutical) payload as it traverses extracellular, epithelial/endothelial, endosomal and intracellular barriers, comparing an unformulated biomolecule with an optimised nanocarrier formulation. Values are illustrative composites of the general trends described in the reviewed literature.

Table 4. Composition and function of a prototypical mRNA lipid nanoparticle (LNP).

LNP component	Representative example(s)	Primary function
Ionizable lipid	SM-102, ALC-0315, DLin-MC3-DMA	mRNA complexation at low pH; endosomal membrane destabilisation
Helper (neutral) phospholipid	DSPC, DOPE	Bilayer structural support; promotes fusogenicity
Cholesterol	Cholesterol	Modulates membrane rigidity and fluidity; particle stability
PEGylated lipid	PEG2000-DMG	Controls particle size; steric stabilisation; reduces opsonisation

5. Protein Delivery Systems

5.1 Therapeutic Proteins, Stability Challenges and Delivery Strategies

Therapeutic proteins, including insulin, monoclonal antibodies, cytokines, growth factors, vaccines and enzyme-replacement therapies, represent an important category of biopharmaceuticals because of their high specificity and ability to replace, supplement or modulate endogenous biological functions. Despite their substantial therapeutic potential, most protein therapeutics are administered by parenteral routes because oral administration exposes them to extensive gastrointestinal degradation and poor epithelial permeability [19]. Proteins are susceptible to proteolytic cleavage, aggregation, oxidation, deamidation and conformational changes during formulation, storage and transport, while their large molecular size and hydrophilic character restrict passive diffusion across biological membranes. Consequently, repeated injections may be necessary for chronic diseases, potentially reducing patient adherence and increasing treatment burden. Several formulation strategies have therefore been developed to improve protein stability, pharmacokinetics and delivery efficiency. Chemical modification approaches, including PEGylation, glycosylation and Fc-fusion, can increase molecular size, reduce renal clearance, improve resistance to proteolysis and prolong systemic exposure. However, excessive modification may influence receptor binding, biological activity or tissue distribution [15]. Encapsulation within nanoparticles, liposomes and polymeric carriers provides physical protection against enzymatic degradation while potentially enabling controlled release and improved tissue distribution. Additional

approaches involve permeation enhancers, enzyme inhibitors and mucoadhesive polymers to improve epithelial interaction and absorption. Together, these strategies seek to preserve protein structure, increase residence time and enhance therapeutic exposure while maintaining biological activity.

5.2 Nanoparticle, Liposomal and Oral Delivery Approaches

Nanoparticle-based systems have emerged as promising platforms for overcoming several barriers associated with protein delivery. Polymeric nanoparticles, particularly those prepared using biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA), can encapsulate proteins within a protective matrix and provide controlled release over extended periods. Liposomes offer another versatile approach because their aqueous internal compartments can accommodate hydrophilic protein molecules, while their lipid bilayers can facilitate interactions with biological membranes. Lipid–polymer hybrid nanoparticles combine selected advantages of both systems, including structural stability, tunable surface properties and controlled cargo release [16]. These carriers have been investigated for the delivery of insulin, growth factors, vaccine antigens and other protein therapeutics, with improvements in protection, cellular uptake and pharmacological activity reported in preclinical studies. Oral protein delivery remains particularly challenging because carriers must survive gastrointestinal conditions while enabling effective transport across the intestinal epithelium. Recent approaches have investigated anionic nanoparticles capable of transiently and reversibly modulating epithelial tight junctions in a size- and charge-dependent manner, thereby increasing paracellular transport and improving the intestinal absorption of encapsulated proteins such as insulin in experimental models [15]. Complementary systems combine mucoadhesive polymers with absorption enhancers and protease inhibitors. Mucoadhesion may prolong contact between the formulation and intestinal mucosa, whereas permeation enhancers temporarily increase epithelial permeability and protease inhibitors reduce enzymatic degradation [16]. Although these approaches demonstrate considerable potential, concerns regarding epithelial safety, reproducibility, dose limitations and variable gastrointestinal conditions remain important barriers to clinical translation.

5.3 Targeted and Sustained-Release Protein Delivery

Targeted protein delivery aims to increase therapeutic concentrations at diseased tissues while reducing systemic exposure and off-target effects. Nanocarriers can be functionalised with antibodies, antibody fragments, peptides, aptamers or other targeting ligands that recognise receptors overexpressed on particular cell populations. Such approaches are particularly

relevant to oncology, where targeted delivery of cytokines, antibodies and other protein therapeutics may enhance antitumour activity while limiting toxicity to healthy tissues. Active targeting can also facilitate receptor-mediated cellular uptake, potentially improving intracellular delivery when the therapeutic mechanism requires access to specific cellular compartments. In parallel, sustained-release systems have been developed to address the short biological half-lives of many protein therapeutics. Injectable depots, biodegradable polymeric microspheres, hydrogels and in-situ forming implants can provide gradual release over days, weeks or, in selected applications, months [17]. These systems may reduce dosing frequency, minimise fluctuations in plasma concentration and improve patient convenience. However, maintaining protein stability during encapsulation and release remains challenging because exposure to organic solvents, interfaces, shear stress or acidic microenvironments can cause aggregation or loss of biological activity. Consequently, successful sustained-release formulations require careful optimisation of polymer composition, particle characteristics, porosity, protein–excipient interactions and release kinetics. Overall, advances in nanoparticulate, targeted, mucoadhesive and depot-based technologies have progressively expanded the possibilities for protein delivery beyond conventional injections. Nevertheless, achieving reproducible absorption, long-term stability, predictable pharmacokinetics and acceptable safety remains essential for translating these technologies into clinically reliable non-invasive and sustained protein-delivery platforms [18].

6. Peptide Delivery Systems

Therapeutic peptides occupy a physicochemical niche between small molecules and proteins, and share many of the same delivery obstacles — enzymatic hydrolysis, short half-life and poor membrane permeability — while offering some advantages for chemical modification and synthesis at scale. Oral peptide delivery has achieved its most significant clinical successes to date through gastrointestinal permeation enhancer technology: the FDA approval of oral semaglutide (Rybelsus®), formulated with the permeation enhancer sodium salcaprozate (SNAC), and of the octreotide capsule (Mycapssa®), employing Chiasma's Transient Permeation Enhancer technology based on sodium caprylate (C8), represent landmark achievements for the field [9].

Medium-chain fatty acid-based permeation enhancers such as sodium caprate (C10) and sodium caprylate (C8), used respectively in the GIPET and TPE technologies, act principally by transiently increasing transcellular and paracellular permeability, through mechanisms that include membrane fluidisation and reversible opening of tight junctions [9,14]. A comprehensive analysis of intestinal permeation enhancers has emphasised that co-

localisation of a sufficiently high local concentration of enhancer and peptide at the epithelial surface — achieved through solid dosage forms or lipoidal dispersions rather than simple solutions — is critical to consistent absorption enhancement [13], while long-term safety data continue to be gathered to confirm that repeated, chronic use of permeation enhancers does not compromise intestinal barrier integrity [12]. Beyond oral delivery, peptide stability and tissue penetration have also been improved through cyclisation, D-amino acid substitution, PEGylation, lipidation (as used in long-acting GLP-1 receptor agonists) and conjugation to cell-penetrating peptides for intracellular cargo delivery [21].

7. Gene Delivery Systems

7.1 Fundamentals and Viral Gene Delivery

Gene therapy aims to treat disease by introducing, correcting or silencing genetic material within target cells, using either viral or non-viral delivery vectors. Viral vectors — retroviruses, lentiviruses, adenoviruses and adeno-associated viruses (AAV) — currently underpin approximately 70% of clinical gene therapy trials owing to their naturally evolved, highly efficient mechanisms for cell entry and nuclear delivery [20]. AAV vectors in particular offer low pathogenicity and long-term transgene expression in post-mitotic tissue, while lentiviruses enable stable genomic integration suited to ex vivo cell therapy manufacture. These advantages are counterbalanced by a modest DNA packaging capacity, complex and costly current Good Manufacturing Practice (cGMP) production, pre-existing anti-capsid immunity in a substantial fraction of the population, and, for integrating vectors, a residual risk of insertional mutagenesis [20].

7.2 Non-Viral Gene Delivery: Lipid and Polymeric Carriers

Non-viral gene delivery vectors — principally lipid nanoparticles, cationic lipids, and cationic or ionizable polymers such as polyethyleneimine (PEI), poly(β-amino esters) and PAMAM dendrimers — offer a safer, more easily manufactured and more immunologically inert alternative to viral vectors, at the cost of generally lower transfection efficiency [20]. Lipid nanoparticles, already established for mRNA and siRNA delivery, are increasingly applied to plasmid DNA and CRISPR/Cas9 ribonucleoprotein or mRNA/single-guide RNA delivery, including CNS-targeted applications following intracerebral administration [1]. Cationic-lipid-based gene vectors were among the earliest non-viral platforms investigated but are limited in vivo by serum-mediated inactivation, aggregation and moderate cytotoxicity [19], motivating continued development of ionizable and biodegradable alternatives.

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7.3 DNA and RNA Delivery Considerations

Plasmid DNA delivery must additionally overcome nuclear envelope translocation to achieve transcription, a barrier absent for cytoplasmically active mRNA and siRNA, which partly explains the more rapid clinical translation of RNA-based non-viral therapeutics relative to non-viral DNA gene therapy. Cell-penetrating-peptide-functionalised nanoparticles have been used to improve both cellular uptake and, for pulmonary gene therapy applications, penetration through the protective airway mucus layer that otherwise excludes conventional nanoparticles [21]. Table 5 compares the principal features of viral and non-viral gene delivery approaches.

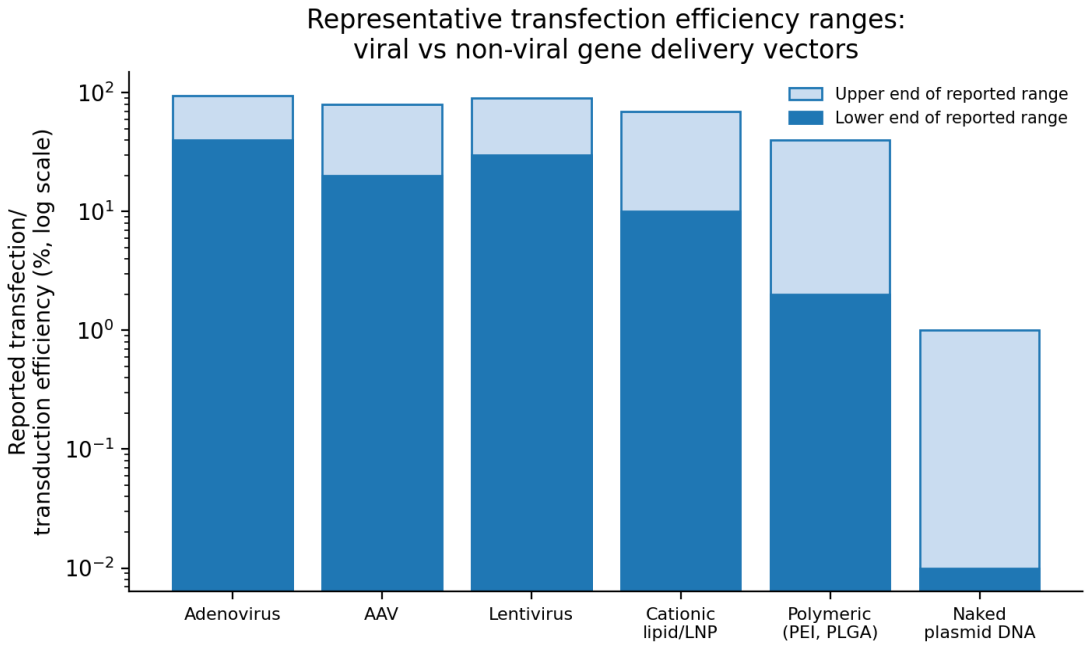


Figure 3. Representative reported ranges of transfection or transduction efficiency for major viral and non-viral gene delivery vectors. Ranges are illustrative and drawn from the general trends reported across the reviewed literature; actual efficiency is highly cell-type, target-tissue and construct-dependent.

Table 5. Comparison of viral and non-viral gene delivery vectors.

Feature	Viral vectors (AAV, lentivirus, adenovirus)	Non-viral vectors (LNP, polymer, dendrimer)

Transfection/transduction efficiency	Generally high	Moderate to low (formulation-dependent)
Packaging capacity	Limited (especially AAV, ~4.7 kb)	Larger and more flexible
Immunogenicity risk	Higher; pre-existing anti-capsid immunity possible	Generally lower, but innate immune activation possible
Insertional mutagenesis risk	Present for integrating vectors (retro-/lentivirus)	Minimal (typically episomal or transient expression)
Manufacturing complexity/cost	High; complex cGMP viral production	Lower; more scalable chemical synthesis
Repeat dosing feasibility	Limited by anti-vector immunity	Generally more feasible

8. Targeted and Stimuli-Responsive Delivery

Passive targeting exploits the enhanced permeability and retention (EPR) effect in tumours and inflamed tissue, whereby nanoparticles of an appropriate size (typically 20–200 nm) preferentially accumulate through leaky vasculature and impaired lymphatic drainage, without requiring a specific targeting ligand. Active targeting instead functionalises the carrier surface with ligands — antibodies, antibody fragments, peptides, aptamers or small molecules — that bind receptors overexpressed on target cells, increasing cellular uptake and specificity beyond what passive accumulation alone can achieve [27]. Receptor-mediated delivery approaches, including transferrin-, folate- and integrin-receptor targeting, have been widely explored to enhance both tumour-specific and, for some peptide/protein cargoes, transcytosis-mediated crossing of restrictive barriers such as the blood–brain barrier [26].

Stimuli-responsive delivery systems are engineered to release their payload selectively in response to a pathological or externally applied trigger. pH-responsive systems exploit the mildly acidic tumour microenvironment or the pronounced pH gradient across the endolysosomal pathway, using acid-labile linkages (e.g., hydrazone bonds) or ionisable polymers that undergo a conformational or solubility change at reduced pH to trigger cargo release or endosomal membrane disruption [28,29]. Enzyme-responsive systems incorporate substrate sequences for proteases or glycosidases that are overexpressed in diseased tissue (e.g., matrix metalloproteinases in tumours), enabling selective cleavage and activation of the carrier or its targeting function only at the intended site [25]. Combinations of dual- or multi-stimuli responsiveness (pH plus enzyme, or pH plus redox potential) are increasingly used to

improve targeting specificity and to enable spatiotemporally controlled release of nucleic acid, protein or peptide payloads.

9. Routes of Administration for Biopharmaceutical Delivery

Route of administration is dictated by the target tissue, required onset and duration of action, and the delivery platform's ability to overcome route-specific barriers. Oral delivery remains the most desirable route for patient convenience and adherence but requires either chemical/formulation protection against gastrointestinal degradation or a permeation-enhancer strategy, as exemplified by oral semaglutide and octreotide [9]. Parenteral delivery (intravenous, subcutaneous, intramuscular) bypasses gastrointestinal and, for intravenous administration, most absorption barriers entirely, and remains the default route for mRNA vaccines, monoclonal antibodies and most current gene therapies.

Pulmonary delivery offers rapid systemic absorption for some macromolecules via the large, thin alveolar epithelium and has been explored for both protein (e.g., inhaled insulin) and gene therapy applications, including lung-targeted, cell-penetrating-peptide-modified nanoparticles designed to penetrate the protective mucus layer of the airway [21]. Nasal delivery exploits the highly vascularised nasal mucosa for rapid absorption and, in some cases, direct nose-to-brain transport bypassing the blood–brain barrier, useful for peptide and small-protein CNS delivery. Transdermal delivery, generally limited to small, lipophilic molecules under passive diffusion, has been extended to larger biomolecules through microneedle arrays that physically breach the stratum corneum. Ocular delivery, buccal/sublingual delivery, and local or site-specific administration (intra-articular, intratumoural, intrathecal) each offer route-specific advantages for particular indications, generally trading reduced systemic exposure and side-effect burden for more invasive or specialised administration procedures.

Table 6. Routes of administration for biopharmaceutical delivery.

Route	Key advantage	Key limitation	Representative biopharmaceutical application

Oral	Patient convenience; high adherence	Enzymatic/acidic degradation; low permeability	Oral semaglutide, oral octreotide
Parenteral (IV/SC/IM)	High bioavailability; bypasses GI tract	Invasive; requires trained administration	mRNA vaccines, monoclonal antibodies, insulin
Pulmonary	Large absorptive surface; rapid onset	Formulation/device complexity; variable deposition	Inhaled insulin, lung-targeted gene therapy
Nasal	Rapid absorption; potential nose-to-brain transport	Limited dose volume; mucociliary clearance	Peptide hormones, CNS-targeted peptides
Transdermal (incl. microneedle)	Non-invasive (minimally); avoids first-pass metabolism	Limited to small/engineered macromolecules	Peptide/vaccine microneedle patches
Ocular / buccal / sublingual / local	Site-specific action; reduced systemic exposure	Restricted to accessible or local indications	Intravitreal proteins, intratumoural gene therapy

10. Characterisation and Evaluation of Delivery Systems

Rigorous physicochemical characterisation is fundamental to the development, optimisation and regulatory qualification of biopharmaceutical delivery systems because the physical attributes of a formulation directly influence its stability, biological performance, manufacturability and safety. Particle size and polydispersity index (PDI), commonly determined using dynamic light scattering (DLS), are important critical quality attributes for nanoparticulate systems. These parameters influence colloidal stability, biodistribution, circulation behaviour, cellular uptake and intracellular trafficking. For lipid nanoparticles (LNPs), particle dimensions also affect encapsulation efficiency and delivery performance, with clinically translated mRNA-LNP systems commonly exhibiting nanoscale dimensions and relatively narrow size distributions [1]. Surface charge, generally evaluated through zeta-potential measurements, provides information regarding colloidal stability and interactions between carriers and biological membranes. Morphological characteristics are further examined using transmission electron microscopy (TEM), cryogenic electron microscopy (cryo-EM) or atomic force microscopy (AFM), with cryo-EM offering detailed information

regarding the internal organisation and morphology of mRNA-loaded LNPs. Encapsulation efficiency and biomolecule loading are additional critical quality attributes and may be quantified using fluorescence-based dye-exclusion assays, such as RiboGreen for nucleic acids, while electrophoretic, immunochemical or chromatographic approaches can be employed for protein and DNA cargo. High encapsulation efficiency is particularly important because it determines the amount of protected and potentially bioavailable therapeutic cargo. The structural and chemical integrity of the encapsulated biomolecule must also be established throughout manufacturing and storage. Circular dichroism spectroscopy can assess protein secondary structure, capillary gel electrophoresis can evaluate mRNA integrity, and mass spectrometry can identify molecular modifications or degradation products. Stability studies should examine both immediate post-manufacturing quality and changes occurring throughout the intended shelf life under defined temperature and cold-chain conditions. In vitro release studies using dialysis, diffusion cells or biorelevant media provide information on release kinetics and can support the development of in vitro–in vivo relationships. Finally, cell-based transfection, expression or potency assays provide essential functional evidence by determining whether the delivered biomolecule retains its intended biological activity. Thus, an integrated combination of physicochemical, structural, stability, release and functional characterisation is necessary to establish consistent product quality and support successful clinical translation of advanced biopharmaceutical delivery systems.

11. Recent Advances and Emerging Technologies

Next-generation lipid nanoparticles incorporate biodegradable ester or disulfide linkages within the ionizable lipid tail to accelerate systemic clearance and reduce accumulation-related toxicity, along with novel helper lipids and PEG-lipid exchange rates engineered for tissue-selective ('SORT') biodistribution. Biomimetic and cell-derived delivery systems, including red blood cell, platelet, leukocyte and cancer-cell membrane-coated nanoparticles, exploit natural cell-surface machinery to confer prolonged circulation and immune evasion (via CD47 'don't-eat-me' signalling) and, in some cases, intrinsic disease-site homing [26,27].

Exosome-based delivery has emerged as a particularly active area: naturally secreted extracellular vesicles derived from mesenchymal stem cells or other donor cells can be loaded with small-molecule, protein, or nucleic acid cargo and exploit endogenous intercellular communication machinery for efficient, low-immunogenicity delivery, as demonstrated for anticancer drug-loaded exosome mimetics and taxol-loaded mesenchymal-stem-cell-derived exosomes targeting metastatic breast cancer [22,23]. Related bioengineering efforts have produced synthetic 'exosome-mimetic' nanovesicles and artificial plasma-membrane-derived

vesicles that replicate key functional features of natural exosomes at greater manufacturing scale and lower cost [24,27], and comprehensive reviews of extracellular vesicle biology continue to catalogue their expanding therapeutic applications in cancer and beyond [25].

Self-assembling nanocarriers, in which peptide, lipid or nucleic acid components spontaneously organise into defined nanostructures under physiological conditions, offer simplified, often solvent-free manufacturing routes. AI-assisted carrier and formulation design is rapidly gaining traction: machine learning models, including random forest regressors, support vector machines and deep neural networks, have been applied to predict LNP potency, transfection efficiency and formulation stability directly from lipid structure and formulation composition, substantially accelerating design–build–test cycles relative to conventional combinatorial screening [30,31,32]. Personalised biopharmaceutical delivery, tailoring formulation composition or dosing to individual patient genetic, immunological or physiological profiles, represents a natural extension of these computational and manufacturing advances, particularly for gene and cell-based therapies where patient-specific manufacturing is already routine.

12. Safety, Regulatory and Translational Considerations

Toxicity and biocompatibility assessment must account for both the biopharmaceutical cargo and the delivery vehicle itself: ionizable lipids, cationic polymers and viral capsid proteins can each independently contribute to dose-limiting toxicity, complement activation or hepatotoxicity, particularly at the repeat doses often required for chronic disease. Immunogenicity remains a central translational concern — unmodified mRNA and certain lipid components can activate innate pattern-recognition pathways (e.g., Toll-like receptors), therapeutic proteins can elicit neutralising anti-drug antibodies, and viral vectors face both innate and pre-existing adaptive immune barriers that can neutralise the vector or trigger serious adverse events at high systemic doses [6,20].

Off-target effects are a particular concern for gene-editing and RNA-interference therapeutics, where imperfect sequence specificity can silence or edit unintended genomic loci, and for nanoparticle carriers whose biodistribution may deliver payload to non-target organs (commonly the liver, spleen and lungs for systemically administered LNPs). Long-term safety data, particularly for integrating gene therapies and for chronic biologic administration, continue to accumulate through extended clinical follow-up and post-marketing surveillance.

Manufacturing and scale-up present distinct challenges for each platform: LNP manufacture requires precise microfluidic or T-junction mixing to achieve reproducible particle size and

encapsulation efficiency at commercial scale; viral vector production requires complex cell-culture-based upstream processes with batch-to-batch potency variability; and exosome-based products face unresolved questions of donor-cell sourcing, purification and potency standardisation. Quality control and reproducibility, encompassing particle size, encapsulation efficiency, potency and impurity profiling, are correspondingly central to regulatory review. Regulatory agencies have developed platform-specific but still evolving frameworks for these products, and clinical translation of genuinely novel delivery systems continues to require careful, iterative dialogue between developers and regulators to establish appropriate analytical and clinical evidentiary standards.

13. Challenges and Future Perspectives

Despite substantial advances in biopharmaceutical delivery, biological barriers continue to restrict delivery efficiency, tissue specificity and therapeutic outcomes. Even highly optimised lipid nanoparticles (LNPs) and viral-vector platforms frequently deliver only a limited proportion of the administered dose to the desired intracellular compartment. Achieving selective delivery beyond organs such as the liver, spleen and lungs remains particularly challenging because of differences in vascular permeability, tissue architecture, cellular receptor expression and carrier clearance. Extrahepatic targeting is therefore an important unmet requirement for the next generation of delivery technologies. In parallel, formulation stability remains a major consideration, particularly for nucleic-acid therapeutics and LNP-based systems. Many mRNA formulations require controlled temperature storage and reliable cold-chain infrastructure, which can increase distribution costs and restrict access in resource-limited settings. Consequently, research is increasingly directed towards lyophilised formulations, improved cryoprotective systems and thermostable delivery platforms capable of maintaining cargo integrity under less restrictive storage conditions.

Manufacturing complexity, production cost and scalability represent additional obstacles to widespread clinical adoption. Viral vectors, exosome-based systems and sophisticated multifunctional nanoparticles often require tightly controlled manufacturing processes and specialised analytical methods. Batch-to-batch consistency can be difficult to maintain because relatively small changes in particle composition, size, surface properties or cargo loading may influence biological performance. Furthermore, the absence of universally standardised methods for physicochemical characterisation, potency evaluation, stability assessment and quality control complicates comparisons between studies and can delay regulatory development. Clinical translation is also influenced by the need for platform-specific safety evaluation, including assessment of immunogenicity, toxicity, biodistribution

and potential long-term effects. Regulatory frameworks must consequently evolve alongside increasingly complex delivery technologies while supporting appropriate global harmonisation.

Future development is likely to depend on the integration of complementary technologies rather than reliance on a single delivery platform. Artificial intelligence and machine-learning approaches may accelerate carrier discovery by predicting formulation–property relationships and identifying optimal combinations of lipids, polymers, targeting ligands and therapeutic cargos. Biomimetic engineering, exosome-inspired systems and modular delivery chemistries may further improve tissue specificity and intracellular trafficking. Combining targeting, controlled release and stimuli-responsive mechanisms could enable more precise delivery while reducing systemic exposure. In addition, continuous manufacturing, process analytical technologies and digitally monitored production systems may improve scalability, reproducibility and quality assurance. Collectively, these advances provide a credible pathway towards safer, more efficient, precisely targeted and globally accessible next-generation biopharmaceutical delivery systems.

14. Conclusion

The development of lipid nanoparticles (LNPs) represents one of the most significant achievements, demonstrating that rationally engineered nanocarriers can protect fragile nucleic acids, facilitate cellular uptake and promote intracellular release at clinically relevant levels. The successful translation of LNP-based mRNA vaccines has provided important validation for this platform and established a foundation for extending mRNA technology towards vaccines, protein replacement, cancer immunotherapy and other therapeutic applications. Similarly, advances in permeation enhancers, protease protection and specialised oral formulations have demonstrated the feasibility of improving the gastrointestinal delivery of selected peptide therapeutics. These developments indicate that non-invasive administration of biopharmaceuticals, although technically challenging, is increasingly achievable through integrated formulation and delivery strategies.

Viral and non-viral gene-delivery systems have likewise continued to develop along complementary pathways. Viral vectors offer high transduction efficiency and established mechanisms for intracellular gene delivery, whereas non-viral systems provide greater flexibility in cargo selection, manufacturing and potentially repeat administration. Polymeric nanoparticles, liposomes, dendrimers, hydrogels, lipid–polymer hybrids and biomimetic carriers have further expanded the technological landscape by addressing specific barriers

including enzymatic degradation, poor membrane permeability, endosomal entrapment and inadequate tissue targeting. Exosome-based systems and other biomimetic approaches are particularly promising because their biological characteristics may facilitate cellular communication and improve delivery specificity. At the same time, artificial intelligence and computational modelling are beginning to support more systematic optimisation of carrier composition, particle characteristics, targeting ligands and formulation conditions.

Future progress will depend on overcoming persistent challenges involving extrahepatic targeting, immunogenicity, toxicity, stability, manufacturing scalability, cost and regulatory standardisation. Greater integration of mechanistic understanding, computational design, biomimetic engineering and advanced manufacturing could enable delivery platforms that are safer, more precise and more reproducible. Ultimately, successful translation will require delivery systems to be designed according to the specific physicochemical properties, biological mechanism and clinical requirements of each biopharmaceutical class. Continued multidisciplinary collaboration among pharmaceutical scientists, engineers, biologists, clinicians and regulatory authorities will therefore be essential for converting emerging delivery technologies into broadly accessible and clinically effective therapeutic platforms.

References

1. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 2021;6:1078–1094.
2. Uchida S, Perche F, Pichon C, Cabral H. Nanomedicine-based approaches for mRNA delivery. Mol Pharm. 2020;17(10):3654–3684.
3. Meng C, Chen Z, Li G, Welte T, Shen H. Nanoplatforms for mRNA therapeutics. Adv Ther. 2021;4(1):2000099.
4. Weng Y, Li C, Yang T, Hu B, Zhang M, Guo S, Xiao H, Liang XJ, Huang Y. The challenge and prospect of mRNA therapeutics landscape. Biotechnol Adv. 2020;40:107534.
5. Kim J, Eygeris Y, Gupta M, Sahay G. Self-assembled mRNA vaccines. Adv Drug Deliv Rev. 2021;170:83–112.
6. Chaudhary N, Weissman D, Whitehead KA. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. Nat Rev Drug Discov. 2021;20(11):817–838.

7. Buschmann MD, et al. Ionization and structural properties of mRNA lipid nanoparticles influence expression in intramuscular and intravascular administration. Commun Biol. 2021;4(1):956.
8. Gebre MS, Brito LA, Tostanoski LH, Edwards DK, Carfi A, Barouch DH. Novel approaches for vaccine development. Cell. 2021;184(6):1589–1603.
9. Kim JC, Park EJ, Na DH. Gastrointestinal permeation enhancers for the development of oral peptide pharmaceuticals. Pharmaceuticals (Basel). 2022;15(12):1585.
10. Hashiba K, et al. Branching of the hydrophobic tail of an ionizable lipid influences the stability, fusogenicity and functional delivery of mRNA lipid nanoparticles. [As cited in: Recent advances in the lipid nanoparticle-mediated delivery of mRNA vaccines. Vaccines. 2023]. 2022.
11. Riley RS, et al. Ionizable lipid nanoparticles for in utero mRNA delivery. Sci Adv. 2021;7(3):eaba1028.
12. Aguirre TAS, Teijeiro-Osorio D, Rosa M, Coulter IS, Alonso MJ, Brayden DJ. Current status of selected oral peptide technologies in advanced preclinical development and in clinical trials. Adv Drug Deliv Rev. 2016;106:223–241.
13. Brayden DJ, Maher S. Intestinal permeation enhancers for oral peptide delivery. Adv Drug Deliv Rev. 2016;106(Pt B):277–319.
14. Maher S, Mrsny RJ, Brayden DJ. Overcoming poor permeability: translating permeation enhancers for oral peptide delivery. Drug Discov Today Technol. 2012;9(2):e71–e174.
15. Banerjee A, Qi J, Gogoi R, Wong J, Mitragotri S. Anionic nanoparticles enable the oral delivery of proteins by enhancing intestinal permeability. Nat Biomed Eng. 2020;4:1075–1086.
16. Liu C, Kou Y, Zhang X, Cheng H, Chen X, Mao S. Strategies and industrial perspectives to improve oral absorption of biological macromolecules. Expert Opin Drug Deliv. 2018;15:223–233.
17. Agarwal P, Rupenthal ID. Injectable implants for the sustained release of protein and peptide drugs. Drug Discov Today. 2013;18:337–349.
18. Bruno BJ, Miller GD, Lim CS. Basics and recent advances in peptide and protein drug delivery. Ther Deliv. 2013;4:1443–1467.
19. Sachdeva S, Lobo S, Goswami T. What is the future of noninvasive routes for protein- and peptide-based drugs? Ther Deliv. 2016;7:355–357.

20. Author(s). Emerging non-viral vectors for gene delivery: lipid nanoparticles, PEI, PAMAM dendrimers and related polymeric carriers. J Nanobiotechnology. 2022 (approx.).
21. Osman G, Rodriguez J, Chan SY, Chisholm J, Duncan G, Kim N, Tatler AL, Shakesheff KM, Hanes J, Suk JS. PEGylated enhanced cell penetrating peptide nanoparticles for lung gene therapy. J Control Release. 2018;285:35–45.
22. Kalimuthu S, Gangadaran P, Rajendran RL, Zhu L, Oh JM, Lee HW, Gopal A, Baek SH, Jeong SY, Lee SW, Lee J, Ahn BC. A new approach for loading anticancer drugs into mesenchymal stem cell-derived exosome mimetics for cancer therapy. Front Pharmacol. 2018;9:1116.
23. Melzer C, Rehn V, Yang Y, Bähre H, von der Ohe J, Hass R. Taxol-loaded MSC-derived exosomes provide a therapeutic vehicle to target metastatic breast cancer and other carcinoma cells. Cancers (Basel). 2019;11:798.
24. Kim H, Kim EH, Kwak G, Chi SG, Kim SH, Yang Y. Exosomes: cell-derived nanoplatforms for the delivery of cancer therapeutics. Int J Mol Sci. 2020;22(1):14.
25. Choi JU, Park IK, Lee YK, Hwang SR. The biological function and therapeutic potential of exosomes in cancer: exosomes as efficient nanocommunicators for cancer therapy. Int J Mol Sci. 2020;21(19):7363.
26. Villata S, Canta M, Cauda V. EVs and bioengineering: from cellular products to engineered nanomachines. Int J Mol Sci. 2020;21(17):6048.
27. Martinelli C, Gabriele F, Dini E, Carriero F, Bresciani G, Slivinschi B, Dei Giudici M, Zanoletti L, Manai F, Paolillo M, Schinelli S, Azzalin A, Comincini S. Development of artificial plasma membrane-derived nanovesicles suitable for drug encapsulation. Cells. 2020;9(7):1626.
28. Manchun S, Dass CR, Sriamornsak P. Targeted therapy for cancer using pH-responsive nanocarrier systems. Life Sci. 2012;90:381–387.
29. Patil R, Portilla-Arias J, Ding H, Konda B, Rekechenetskiy A, Inoue S, Black KL, Holler E, Ljubimova JY. Cellular delivery of doxorubicin via pH-controlled hydrazone linkage using a multifunctional nanovehicle based on poly(β-L-malic acid). Int J Mol Sci. 2012;13(9):11681–11693.
30. Đuriš J, Kurćubić I, Ibrić S. Review of machine learning algorithms' application in pharmaceutical technology. Arh Farm. 2021;71(4):302–317.

31. Allen C. Machine learning directed drug formulation development. Adv Drug Deliv Rev. 2021;175:113806.

32. Jiang J, Ma X, Ouyang D, Williams RO III. Emerging artificial intelligence (AI) technologies used in the development of solid dosage forms. Pharmaceutics. 2022;14(11):2257.