REVIEW ARTICLE

Advances in Nanoparticles for Effective Delivery of RNA Therapeutics

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Abstract

RNA therapeutics, including messenger RNA (mRNA) and small interfering RNA (siRNA), are genetic materials that mediate the translation of genetic direction from genes to induce or inhibit specific protein production. Although the interest in RNA therapeutics is rising globally, the absence of an effective delivery system is an obstacle to the clinical application of RNA therapeutics. Additionally, immunogenicity, short duration of protein expression, unwanted enzymatic degradation, and insufficient cellular uptake could limit the therapeutic efficacy of RNA therapeutics. In this regard, novel platforms based on nanoparticles are crucial for delivering RNAs to the targeted site to increase efficiency without toxicity. In this review, the most recent status of nanoparticles as RNA delivery vectors, with a focus on polymeric nanoparticles, peptide-derived nanoparticles, inorganic nanoparticles, and hybrid nanoparticles, is discussed. These nanoparticular platforms can be utilized for safe and effective RNA delivery to augment therapeutic effects. Ultimately, RNA therapeutics encapsulated in nanoparticle-based carriers will be used to treat many diseases and save lives.

Keywords RNA delivery · Nanoparticles · Nanomedicine · Gene therapy · Biomaterials

1 Introduction

Nucleic acid acts as a potent therapeutic agent by manipulating gene expression to treat various diseases [1, 2]. Messenger RNA (mRNA) can be used as a protein replacement therapy for cystic fibrosis and lung cancer [3–5]. Recently, mRNA has been utilized to develop the COVID-19 vaccine; it encodes the spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), making it resistant to infection through acquired immunity. As mRNA is not inserted into the genome but translated into the cytoplasm, there is no risk of insertional mutation. Furthermore, mRNA has shown substantial potential in a range of therapeutic applications, including vaccines for various infectious diseases and cancer immunotherapies. To achieve therapeutic effects, mRNA molecules must reach specific target cells

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and produce a sufficient level of proteins. However, naked mRNA molecules are susceptible to enzymatic degradation and are not easily taken up by cells because they are intrinsically unstable and large in size [6]. Thus, to develop effective mRNA therapeutics, it is crucial to improve their stability in the body and effectively deliver them to the target cells. According to recent studies, mRNA-loaded nanoparticle-based delivery systems showed higher protein expression than naked mRNA [7–9].

mRNA delivery nanotechnology can be classified into three categories. The first is ionic nanocomplexes, which are produced by mixing cationic delivery carriers and anionic mRNA. As mRNA has a negative charge, nanosized particles can be achieved by electrostatic force with a cationic

polymer. Its most significant advantage is that it can be produced in a short time simply by a mixing process. The second method of mRNA delivery is the modification of the nanoparticle surface by a cationic polymer or by carrying out an exchange reaction with cationic functional groups, which generates a positive surface charge. In this case, mRNA can be readily absorbed by electrostatic interactions. The last method involves the encapsulation of mRNA by the nanoparticles, which has the advantage of safely delivering mRNA to the target site by preventing cargo degradation via endogenous ribonuclease (RNase) (Table 1).

Numerous mRNA-based therapeutics are currently being developed or will be developed for a variety of therapeutic applications, including infectious diseases

Table 1 Polymeric vehicles based on naturally derived polymers and synthetic polymers for RNA therapeutics to enhance delivery efficiency and treat cancer and other diseases

Type	Delivery vehicle	Therapeutic modality	Target	Ref
Natural polymer	CS deacetylation, HA	mRNA	Promoting in vitro transfection efficiency	[25]
	CS NP	miRNA	Efficient delivery of miRNA to macrophages/can be used to target atherosclerotic lesions	[27]
	CS-Heparin NP	siRNA	Improvement of pH-sensitive tranfection efficiency	[28]
	CS-HAD NP		CD44 targeting for bladder cancer therapy	[30]
	CuVa NP		Gene knockdown of in vitro melanoma cell line B16	[31]
	Milk exosome		Anti-tumor activity against lung tumor xenograft	[34]
	Exosome	mRNA	Tumor-suppressing function in glioma model	[35]
			Attenuation of in vivo models of Parkinson's disease	[36]
			Treatment of genetic disease, familial hypercholesterolemia	[37]
	CS NP	mRNA	Transcript therapy for the treatment of cystic fibrosis	[38]
Synthetic polymer	PEG-PGBA	mRNA	Promoting gene expression levels by modulating the stiffness	[39]
	hPBAE		Inhaled delivery to the lung epithelium	[40]
	ω-Cholesteryl-PEG-Pasp(DET)		Efficient mRNA introduction into the mouse lung	[41]
	PEG-Plys		Efficient protein expression in mouse brain	[42]
	PEG-Pasp(TEP)		Induction of mRNA to the mouse brain	[43]
	Pasp(DET/CHE)		Targeting the brain for Alzheimer's disease, Huntington's disease	[44]
	PAsp(DET)		Genome editing in the brain	[45]
	fluorinated PEI	siRNA	Promotion of siRNA delivery to the lung	[46]
	PACE-PEG	mRNA	Increased inhalation delivery to the lung	[47]
	PEG-PAsp(DET)		Delivery of mRNA to the kidney	[48]
	PEI-γ-PGA		High protein expression in the liver and spleen	[49]
	CARTs		Anti-tumor activity against lymphoma and colon carcinoma tumors	[52]
	PbAE		Antigen-specific immunity to treat ovarian cancer, melanoma, and glioblastoma	[53]
	tyrosine-modified PEI	siRNA	High knockdown efficacies to treat melanoma	[54]
	ECV-modified PEI		Inhibition of tumor growth in prostate carcinoma xenografts	[55]
	PbAE		Considerable knockdown in the glioblastoma cells	[56]
	PbAE		Platform of glioblastoma treatment	[57]
	PGS	mRNA	Vaccine against SARS-CoV-2 delivery system	[59]
	PVES		SARS-CoV-2 RBD mRNA vaccine	[60]
	PEG-PAsp(DET/GlcAm)		ATP-responsiveness for selective mRNA release	[61]

and cancers [10]. Since et al. 2019 article [11], clinical trials for more than 35 new nanoparticle technologies have commenced. Cancer treatment and imaging applications are among the novel nanoparticle technologies being tested in these new trials [12]. Moderna and AstraZeneca have been conducting phase II clinical research in recent years to evaluate the local administration of vascular endothelial growth factor (VEGF) mRNA for heart regeneration following myocardial infarction. Additionally, Moderna has initiated a phase I trial for the intratumoral administration of mRNA targeting OX40-binding partner, a member of the tumor necrosis factor receptor (TNFR) and tumor necrosis factor (TNF) superfamily expressed on activated CD4 and CD8 T cells [13].

Although RNA therapeutics have been thoroughly investigated and have successfully entered the clinic through lipid nanoparticles (LNP) [14], there are still limitations in delivery efficiency and organ- and tissue-specific targeting. In detail, cationic LNP components have potential toxicity since they can interact with enzymes, such as protein kinase C (PKC), to cause cytotoxicity [15, 16]. Cationic lipids, a component of LNP, interact with RNA to facilitate encapsulation but can also be toxic [17]. Additionally, a high concentration of cationic lipids can disrupt the cellular membrane structures, resulting in cell lysis and necrotic death [15, 16].

To overcome these limitations, various nanoparticle carriers, such as polymer-based nanoparticles (natural and synthetic), inorganic-based nanoparticles, hybrid nanoparticles, and biological-based approaches using exosomes and peptide nanoassemblies have been actively studied worldwide [18]. In fact, nanotechnology has been studied for decades for the treatment and diagnosis of diseases [19]. This review focuses on investigating advanced nanoparticles for the effective delivery of mRNA or small interfering (siRNA) studies over the last 3 years (Table 2).

2 Polymeric Vehicles

Research on polymeric drug delivery has progressed significantly since the 1980s [20]. The major advantages of polymeric delivery systems include high tailoring ability, biodegradability, ease of functionalization, and good drug-release profile [21]. For the delivery of nucleic acids, the gene delivery system has some barriers to overcome. Vectors should increase transfection efficiency to penetrate the cell membrane and protect genes from harsh environments. In this regard, polymeric nanoparticles have many advantages as non-viral vectors for gene delivery. The fate of polymeric vectors is easily determined by structural characteristics such as molecular weight, surface charge, hydrophobicity, and chain compliance to promote delivery efficiency [22, 23]. Therefore, polymeric nanoparticles are promising candidates for gene vectors because their properties can be controlled to meet the eventual targets of the delivery system. Depending on the source material, mRNA/siRNA delivery studies can be divided into either naturally derived polymers or synthetic polymers (Fig. 1).

2.1 Naturally Derived Polymers

2.1.1 Efforts to Improve Delivery Efficiency

Naturally derived polymers such as hyaluronic acid (HA) or chitosan (CS) are used in drug delivery as mRNA carriers because they are biocompatible and controllable via various modifications. In particular, CS has the potential to act as an mRNA or gene carrier through complexation or surface adsorption owing to its unique advantages, cationic charge, and facile modification (Fig. 2a) [24]. One study showed

Table 2 Peptide-derived nanoparticles for efficient RNA therapeutics transfection and RNA-based cancer therapy

| Type	Delivery vehicle	Therapeutic modality	Target	Ref
Peptide-derived NP	HK (histidine-lysine) peptide	mRNA	Increased mRNA transfection in vitro	[64]
	PEG-KL4 peptide		Inhalable dry powder for mRNA pulmonary delivery	[1]
	OAA with s–s building block		Enhanced transfection efficiency and tolerability	[65]
	CPP PepFect 14		Induction of specific proteins in ovarian cancer	[67]
	AmPPD	siRNA	Effective therapy in prostate cancer model	[68]
	p5RHH NP		Targeting drivers of tumor progression pancreatic adeno-carcinoma model	[69]
	chimeric peptide NP	miRNA	Plectin-1 targeted delivery in pancreatic cancer	[70]
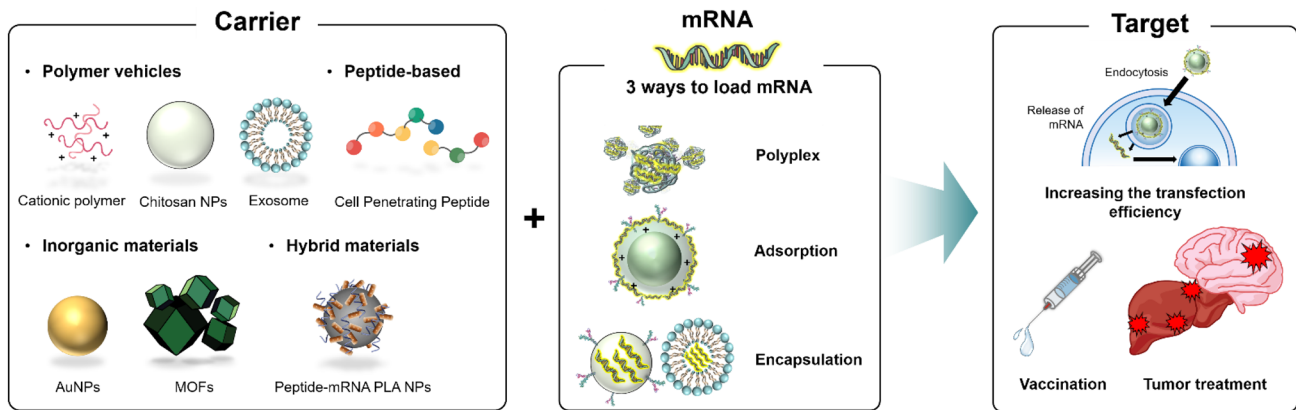
Fig. 1 Schematic diagram of mRNA delivery using nanomaterials

that CS-based nanoparticles for mRNA delivery achieved enhanced transfection efficiencies by modulating some factors. Specifically, polymer length, degrees of deacetylation and HA, charge densities, and nucleic acids were controlled to optimize formulations reaching 60–65% transfection efficiency in vitro [25].

In the case of microRNAs (miRNAs) that have become an ideal class of biomarkers in diverse cancers [26], miRNA mimics are delivered by CS nanoparticles to target macrophages for treating cardiovascular diseases. miRNA/CS complexes can alter the expression of target genes both in vitro and in vivo. Moreover, mice treated with miRNA/CS nanoparticles exhibited reduced reverse cholesterol transport, indicating that this system has the potential to be applied in vivo to target atherosclerotic lesions [27]. The incorporation of heparin into CS nanoparticles is an effective way to increase transfection efficiency. In one study, the oligonucleotide release from heparin-included CS nanoparticles was pH-dependent following polyplex swelling and collapse of the polysaccharide network. The in vitro release studies showed an increased release rate of the oligonucleotide under slightly acidic conditions (pH 4.5); moreover, the efficacy of siRNA silencing vascular endothelial growth factor (VEGF) of ARPE-19 cells was 25% higher with heparin than that of CS nanoparticles without heparin [28] (Table 3).

2.1.2 Cancer Therapy

Recently, the importance of appropriate cancer therapy is increasingly emerging since it has become very important to detect and treat cancers in a healthy way [29]. Owing to the feasibility of polymer functionalization, many studies have reported enhanced targeting effects when polymeric nanoparticles are decorated with different peptides or ligands. For the delivery of siRNA targeting CD44, CS-based nanoparticles were conjugated with HA and presented a tumor-specific nanosystem (siRNA@CS-HAD NPs). In vivo

experiments showed a significant accumulation of nanoparticles at the tumor site, substantially inhibiting the targeted oncogene and bladder cancer [30]. Another natural polymer, curdlan, which is a water-insoluble linear beta-1,3-glucan, was modified with amine groups, and its alkylation derivatives, denoted as CuVa polymers, exhibited an increase in biocompatibility as well as siRNA delivery efficiency. In vitro results showed that CuVa nanoparticles induced considerable knockdown of a disease-related gene, STAT3, in the mouse melanoma cell line B16 [31].

Exosomes are naturally derived from extracellular vesicles produced in cells and are released to the outside for intercellular communication in eukaryotic cells [32]. They are promising candidates as natural carriers because they are easy to handle, non-cytotoxic, and non-immunogenic [33, 34]. Interestingly, bovine milk exosomes, which are stable and resistant to degradation, were utilized as novel carriers for siRNA delivery. siRNA delivered via milk exosomes could silence key oncogenes, ranging from 2- to 10-fold knockdown in multiple cancers in vitro. In addition, intravenous administration of siRNA-loaded exosomes into the lung tumor xenograft in a nude mouse model induced anti-tumor activity [34]. Exosomes with targeting moieties have also been shown to enhance mRNA delivery in brain tumors by penetrating the blood–brain barrier (BBB). Large-scale exosomes were produced using cellular nanoporation (CNP) biochips, which can stimulate cells to release exosomes loaded with mRNA (Fig. 2b). To achieve glioma targeting, two different peptides were inserted into the N-terminus of CD47 and demonstrated tumor-suppressing function in implanted brain gliomas by inhibiting tumor growth and prolonging animal survival [35].

2.1.3 Applications for Non-cancerous Diseases

As mentioned earlier, exosomes are potent cell-derived nanovesicles (50–150 nm) that can mediate intracellular
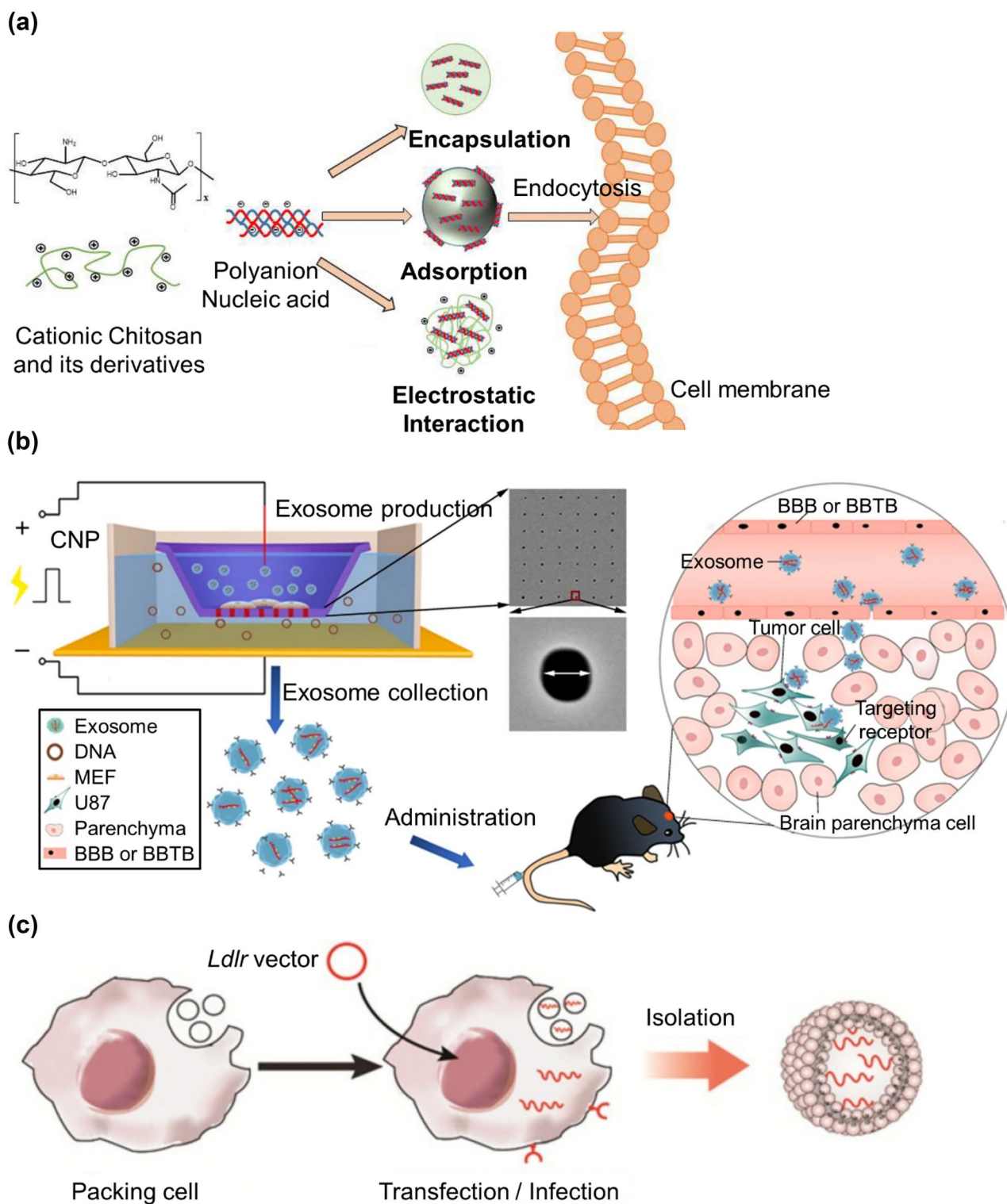
Fig. 2 Schematic illustrations of nanoparticles based on naturally derived polymers. **a** Chitosan structure, fabrication mechanisms of particles based on chitosan–nucleic acid (reproduced with permission from [24] Copyright 2019, Marine drugs). **b** Schematic representation of CNP-generated EVs for targeted nucleic acid delivery (repro-duced with permission from [35] Copyright 2019, Nature Biomedical Engineering). **c** Schematic illustration of *Ldlr* mRNA encapsulation procedure into the exosomes (reproduced with permission from [37] Copyright 2021, Theranostics)

Table 3 Inorganic nanoparticles for efficient mRNA delivery and anti-tumor effects

Type	Delivery vehicle	Therapeutic modality	Target	Ref
Inorganic materials	PAMAM-G5D-grafted AuNP	mRNA	Promotion of transfection activity	[72]
	SeNP		Hepatocellular targeted delivery of mRNA	[74]
	AuNPs-DNA		Inhibition of tumor growth in tumors formed by HeLa cells	[75]
	CS-functionalized SeNP		Potential cancer immunotherapy agent	[76]

communication to achieve therapeutic outcomes. Intriguingly, a series of synthetic biology-inspired control devices, denoted as EXOsomal Transfer Into Cells (EXOtic), were investigated to enable efficient exosomal mRNA delivery without concentrating exosomes. In detail, the combination of exosome production boosters encoded in exosome producer cells and a specific mRNA packaging device could increase the delivery of exosomal mRNA into target cells. Exosome producer cells equipped with EXOtic devices have demonstrated their potential in therapeutic applications. Catalase mRNA delivery by exosome producer cells attenuated neurotoxicity and neuroinflammation in both in vitro and in vivo models of Parkinson's disease [36] (Table 4).

For the treatment of the genetic disease familial hypercholesterolemia (FH), *Ldlr* mRNA was encapsulated into exosomes to restore low-density lipoprotein receptor (LDLR) expression (Fig. 2c). Following tail-vein injection into $Ldlr^{-/-}$ mice, reversals of phenotypes such as steatosis, high-LDL cholesterol, and atherosclerosis were demonstrated in vivo. Short-term delivery systems can cause exosomes to accumulate in the liver, restore receptor expression, and treat disorders in $Ldlr^{-/-}$ mice even without targeting moieties. Thus, the exosome-mediated *Ldlr* mRNA delivery system represents a promising therapeutic approach for the treatment of FH [37]. Another genetic disease, cystic fibrosis (CF), is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that requires transcript therapy. CS nanoparticles were used to incorporate capsaicin, then were surface-loaded with wtCFTR-mRNA to restore CFTR function. With increasing transfection efficiency, this mRNA/CS complex effectively restored CFTR function in the CF cell line CFBE41o [38].

2.2 Synthetic Polymers

2.2.1 Efforts to Improve Delivery Efficiency

Polycation stiffness plays a significant role in determining mRNA delivery performance. Recently, two block copolymers, poly(ethylene glycol)–poly(glycidyl butylamine) (PEG-PGBA) and PEG-poly(L-lysine) (PEG-PLL), were compared to confirm the influence of additional flexibility (Fig. 3a). Twenty-four hours after the administration of GLuc mRNA/micelles into the mouse lung, the luminescence intensity from mRNA/PEG-PGBA was significantly higher than that from mRNA/PEG-PLL. PEG-PGBA, which has a less rigid segment, enhanced gene expression levels in vivo [39]. As another method to facilitate functional protein production, hyperbranched poly(beta-amino esters) (hPBAEs) were synthesized (hDD90-118) to deliver in vitro transcribed (IVT) mRNA to the lung via inhalation. A vibrating mesh nebulizer was used to deliver the IVT-mRNA-encoding firefly luciferase to mice. Repeated administration of inhaled hPBAE-mRNA resulted in consistent protein production in the lung epithelium (Fig. 3b) [40].

Table 4 Hybrid nanoparticles for RNA therapeutics delivery to treat cancer and induce immune responses

Type	Delivery vehicle	Therapeutic modality	Target	Ref
Hybrid system	Zr-based MOF	mRNA	Increase of mRNA transfection activities	[80]
	AMOF	mRNA	Quantitative analysis and in situ imaging of mRNA level	[82]
	DOTAP/protamine		High transfection efficiency	[83]
	Zr-based MOF	siRNA	increased therapeutic effect against in vitro ovarian cancer cells	[86]
	ZIF-8	miRNA	Suppression of tumor growth in breast cancer model	[87]
	CPP-functionalized DOTAP/mPEG-PCL	mRNA	Anti-cancer effects in colon cancer models	[88]
	PLGA/lipid		Strong immune responses for vaccination	[89]
	PLA/CPP		Modulation of DC immune response for mRNA vaccine	[90]

In another study, mRNA was able to reach the lung by tightening the mRNA into polyplex micelles. To accomplish this, mRNA was pre-hybridized with cholesterol (Chol)-tethered RNA oligonucleotides (Chol (+)-OligoRNA), followed by the combination of ω-cholesteryl (ω-Chol) with PEG-polycation block copolymers. Chol (+)-OligoRNA on mRNA strands played a central role in tightening the mRNA packaging in the polyplex micelles because it attracts the ω-Chol moiety of the block copolymers. After the administration of mRNA into the mouse lung, the remaining amount of pre-hybridized mRNA was much higher than that of non-hybridized mRNA, based on qRT-PCR analysis. Therefore, this approach promotes stabilization of the mRNA delivery system even under harsh in vivo conditions [41]. Similarly, the technique of regulating the mRNA steric structure inside the polyplexes was also used in other studies. The bundled structure of mRNA impacted the physicochemical properties, stability, and functionality of polyplex micelles, resulting in efficient protein expression in cells in vitro and mouse brain in vivo (Fig. 3c). The bundled mRNA in block copolymers consisting of PEG-poly(lysine) (PLys) can improve the stability of polyplex micelles by effectively avoiding polyion exchange reactions and RNase attacks [42].
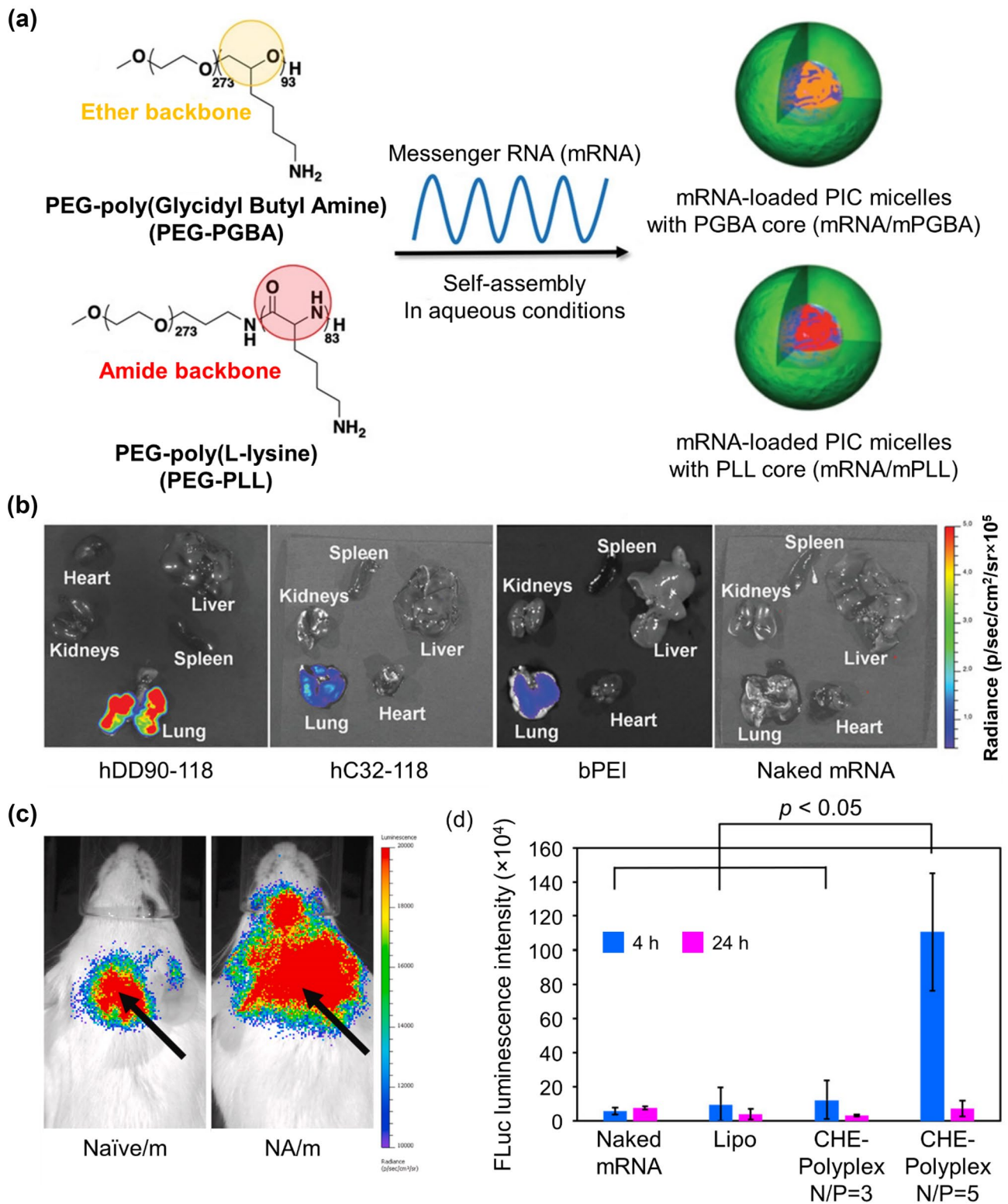
Several studies have suggested promising carriers for mRNA medicine in brain tissues to cure neuron-related diseases. Specifically, amino ethylene-repeating groups were conjugated to the side chains along the backbone of a polyaspartamide polymer terminated with PEG to enhance its ability to bypass the BBB. This PEG-based nanocomplex encapsulated with luciferase (Luc2) mRNA was infused into mouse brains via intracerebroventricular injection and exhibited the best Luc2 mRNA delivery efficiency [43]. In addition to incorporating PEG chains, the hydrophobic effect is a critical factor in controlling the properties of mRNA-loaded polyplexes. Among the series of amphiphilic polyaspartamide derivatives containing various hydrophobic moieties with cationic diethylenetriamine (DET) in the side chain, the polyaspartamide derivative with 11 residues of 2-cyclohexylethyl (CHE) moieties (PAsp(DET/CHE)) showed the highest mRNA expression both in cultured cells and in the mouse brain (Fig. 3d). These results suggest the possibility of using mRNA technology as a treatment for Alzheimer's disease and Huntington's disease [44]. As another material used to target the brain to treat many neurological disorders, polyplex micelles are prepared using poly(N'-(N-(2-aminoethyl)-2-aminoethyl) aspartamide and DET (PAsp(DET)). The co-delivery of Cas9 mRNA and single-guide RNA is an efficient strategy for editing the genome in the brain. Furthermore, the presence of Cas9 mRNA and PEG improved diffusion in brain tissues, which could ultimately be efficient for genome editing [45].

In addition, other organs such as the liver, lung, spleen, and kidney can be targeted by a genetic material delivery system. Fluorination was introduced into polyethyleneimine (PEI) to reduce cytotoxicity. In vitro delivery results proved that fluorination promoted siRNA delivery, and thus Cy5.5-labeled siRNA complexed with PEI was significantly accumulated in the lungs after intravenous injection. Notably, fluorination-induced alteration is an excellent strategy for controlling biodistribution [46]. PEGylation, as well as fluorination reduces the toxicity of cationic polymeric vehicles. To investigate the effect of PEGylation on the transfection efficiency, stability, and toxicity, polymers from the poly(amine-co-ester) (PACE) family were blended with PEG-conjugated PACE at different ratios. It was essential to optimize the PEGylation because the addition of low concentrations of PACE-PEG to PACE improved polyplex stability but prohibited transfection in vitro. By contrast, the effects of PACE-PEG in vivo were different depending on the delivery route, and low concentrations of PACE-PEG significantly increased inhalation delivery to the lungs [47]. Interestingly, in an attempt to deliver mRNA to the kidneys, nanomicelles were formed by the self-assembly of mRNA and PEG-polyamino acid PAsp(DET) block copolymers. After the administration of mRNA into mouse kidneys by renal pelvic injection, most of the injection bolus remained at the injection site without diffusing into circulation [48]. Besides cationic polymers, an anionic complex containing PEI, γ-polyglutamic acid, and mRNA exhibited stable and high in vitro protein expression without cytotoxicity. Notably, after intravenous administration of the anionic complex in vivo, high protein expression was observed mainly in the liver and spleen [49].

2.2.2 Applications for Cancer Therapy

Gene therapy is a powerful tool for cancer treatment because the delivery of certain genes can construct specific protein expressions or inhibit certain genes [50]. Numerous gene carriers have been studied for efficient and safe delivery of nucleic acids for cancer therapy. Specifically, charge-altering releasable transporters (CARTs) initially serve as oligo(α-amino ester) cations that can protect and deliver mRNA, then switch their properties during intramolecular rearrangement to promote the intracellular release of functional mRNA [51]. CARTs have been utilized to carry mRNA-encoding Ox40l-, Cd80-, and Cd866, which are well-known immunomodulatory molecules. In the A20 and CT26 models, CART–mRNA complexes showed local upregulation of proinflammatory cytokines, T-cell activation, and migration of immune cells, leading to delayed tumor growth and enhanced tumor regression (Fig. 4a) [52]. Recently, mRNA-encoding interferon regulatory factor 5 (IRF5), combined with its activating kinase IKKβ, formed complexes with cationic poly(β-amino ester) (PbAE) (Fig. 4b). In animal models

(a)

Ether backbone

PEG-poly(Glycidyl Butyl Amine)
(PEG-PGBA)

Amide backbone

PEG-poly(L-lysine)
(PEG-PLL)

Messenger RNA (mRNA)

Self-assembly
In aqueous conditions

mRNA-loaded PIC micelles
with PGBA core (mRNA/mPGBA)

mRNA-loaded PIC micelles
with PLL core (mRNA/mPLL)

(b)

hDD90-118

hC32-118

bPEI

Naked mRNA

Radiance (p/sec/cm²/sr×10⁵)

(c)

Naïve/m

NA/m

(d)

FLuc luminescence intensity (×10⁴)

$p < 0.05$

4 h   24 h

Naked mRNA   Lipo   CHE-Polyplex N/P=3   CHE-Polyplex N/P=5

of ovarian cancer, melanoma, and gliblastoma (GBM), M1-polarizing transcription factor 5 (IRF5) can reprogram immune-suppressive M2 macrophages into immune-stimulatory M1 macrophages. Therefore, the induction of

antigen-specific immunity via mRNA/polymeric nanoparticles is a prospective approach to treat cancer [53].

In prostate carcinoma xenograft mouse model, some modified PEIs have been used in cancer-medicine delivery systems. Specifically, tyrosine-modified PEIs were explored

◄**Fig. 3** Synthetic-polymer-based nanoparticles for improved delivery efficiency. **a** Preparation of mRNA/m after complexation with PEG-PGBA (mRNA/mPGBA) or PEG-PLL (mRNA/mPLL) (reproduced with permission from [39] Copyright 2020, Advanced Healthcare Materials). **b** Bioluminescence 24 h after inhalation of polyplexes; hDD90-118 vectors produced significantly higher radiance localized to the lung compared to hC32-118 and bPEI ($p < 0.001$, +SD, $n = 4$) (reproduced with permission from [40] Copyright 2019, Advanced Materials). **c** Representative images of in vivo luminescence imaging. Arrows represent naive mRNA/ PEG-PLys, NA/PEG-PLys injection site (NA: mRNA nanoassemblies) (reproduced with permission from [42] Copyright 2020, Biomaterials). **d** Quantitative evaluation of FLuc-mRNA expression level after intracerebroventricular administration of FLuc-mRNA/PAsp(DET/CHE) in mice (reproduced with permission from [44]. Copyright 2019, ACS Central Science)

with low or very low molecular weights for siRNA delivery. Tumor xenografts were established by injecting PC3 cells, followed by repeated systemic administration of siRNA complexes via intraperitoneal injection. Importantly, siRNA complexes led to the knockdown of a target gene in tumors [54]. Similarly, the combination of PEI-based nanoparticles with naturally occurring extracellular vesicles (ECVs) showed an increased ability to inhibit tumor cells. ECVs are beneficial in gene delivery systems because they protect their payload from degradation or renal clearance and deliver their cargo directly into the cytosol. In mice bearing PC3 prostate carcinoma xenografts, ECV-modified PEI/siRNA complexes significantly inhibited tumor growth [55].

The treatment of tumor cells with GBM-delivering siRNA is an approach recently receiving great interest. In a recent study, biodegradable PbAE incorporated with disulfide bonds successfully delivered siRNA to the cytosol by environmentally triggered degradation and demonstrated therapeutic efficiency both in vitro and in vivo. The polymeric nanoparticles showed the highest in vitro permeability and siRNA-knockdown in patient-derived GBM cells. Furthermore, in vivo analysis in mice inoculated with GBM1A cells verified significant knockdown in the inoculated GBM cells after crossing the BBB [56]. Likewise, five anti-GBM genes (Robo1, YAP1, NKCC1, EGFR, and survivine) were delivered via bioreducible PbAE-based nanoparticles as a platform for GBM treatment. In mice bearing luciferase-positive GBM1A cells, the in vivo imaging system (IVIS) results showed that tumors treated with co-delivery of five siRNA sequences were less luminescent than the controls [57].

2.2.3 Applications for Vaccination

In recent years, mRNA vaccines have played a major role in combating infectious pathogens. Therefore, the optimization of various mRNA vaccine delivery materials is crucial for safer and more efficient deliveries [58]. Besides lipid-based vehicles, other materials are being developed that can promote cargo storage and transportation.

As the mRNA vaccine targeted toward the SARS-CoV-2 delivery system, a polycationic polymer, polyglucin:spermidine conjugate (PGS), was used as a carrier (Fig. 4c). Moreover, mRNA was encoded with the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein because the main virus-neutralizing epitopes are concentrated in that domain. Mice were immunized with an mRNA-RBD-PGS vaccine to confirm their immunogenicity in vivo. Serum analysis revealed increased uptake by antigen-presenting cells and heightened immune responses with virus-specific antibodies [59].

Copolymers forming polyplex micelles have been studied as efficient and safe mRNA delivery systems. Interestingly, vitamin-E-succinate-modified polyethyleneimine copolymer (PVES) was synthesized for potential mRNA vaccine delivery. The in vitro results proved that modification with vitamin E significantly reduced the cytotoxicity of naïve PEI. Moreover, this self-assembled polymeric micelle delivery system containing SARS-CoV-2 RBD mRNA showed an increased amount of antigen-specific antibody IgG and substantial T-cell response against SARS-CoV-2 RBD antigen after administration in vivo [60]. In another study, the difference between extracellular and intracellular ATP concentration was utilized to achieve efficient cargo release from polyplex micelles. In particular, a PEG-polycation block copolymer consisting of PEG and poly(β-benzyl-L-aspartate) was synthesized with 4-carboxy-3-fluorophenylboronic acid (FPBA) (PEG-PAsp(DET/GlcAm) to obtain ATP responsiveness (Fig. 4d). As the formulation was stabilized by installing cholesterol moieties into the mRNA and ω-end of the block copolymers, in vivo measurement of the blood circulation profile in mice indicated upregulation of intact mRNA in the blood circulation [61].

3 Peptide-Derived Nanoparticles

Cell-penetrating peptides (CPPs) are short cationic peptide sequences that mediate the intracellular delivery of numerous biological cargos [62]. It is difficult for large protein complexes, genetic materials, and other molecules to penetrate cellular membranes; however, CPPs, a class of 5–30 residue peptides, can pass through membranes in an energy-dependent or independent manner [63]. In this regard, peptide-based delivery platforms have been studied as mRNA carriers of genetic materials for intracellular delivery.
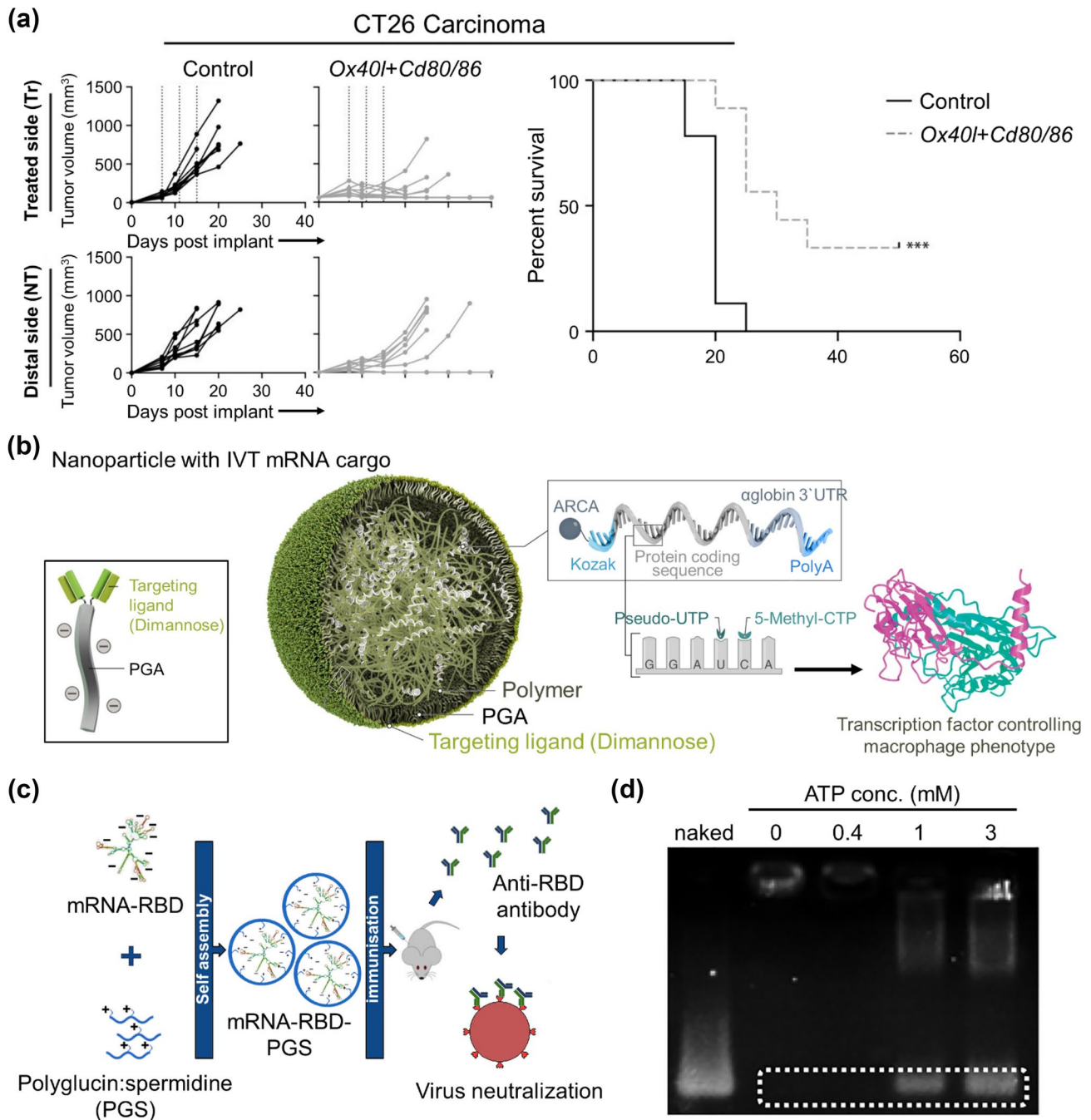
Fig. 4 Synthetic-polymer-based nanoparticles for cancer therapy and vaccination. **a** Mice harboring two CT26 colon carcinoma tumors were treated intratumorally in one of the tumors with either Ox40l + Cd80/86 mRNA-CARTs or control mRNA-CARTs (Reproduced with permission from [52] Copyright 2019, Cancer Research). **b** Design of macrophage-targeted polymeric NPs based on poly(beta-amino ester) formulated with mRNAs encoding key regulators of macrophage polarization (Reproduced with permission from [53] Copyright 2019, Nature Communication). **c** Graphical abstract of polyglucin:spermidine conjugate as a carrier of an mRNA-RBD vaccine encoding the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein (reproduced with permission from [59] Copyright 2021, Vaccines). **d** Responsivity of PMs (PEG-PAsp(DET/GlcAm) to ATP. Gel electrophoresis was performed after incubation of PMs with several concentrations of ATP (reproduced with permission from [61] Copyright 2021, Journal of Controlled Release)

3.1 Efforts to Improve Delivery Efficiency

To facilitate mRNA transfection efficiency, a single histidine was added to four branched histidine–lysine (HK) peptides to form H3K(+H)4b peptides. To assess the ability of these peptides to carry mRNA-expressing luciferase
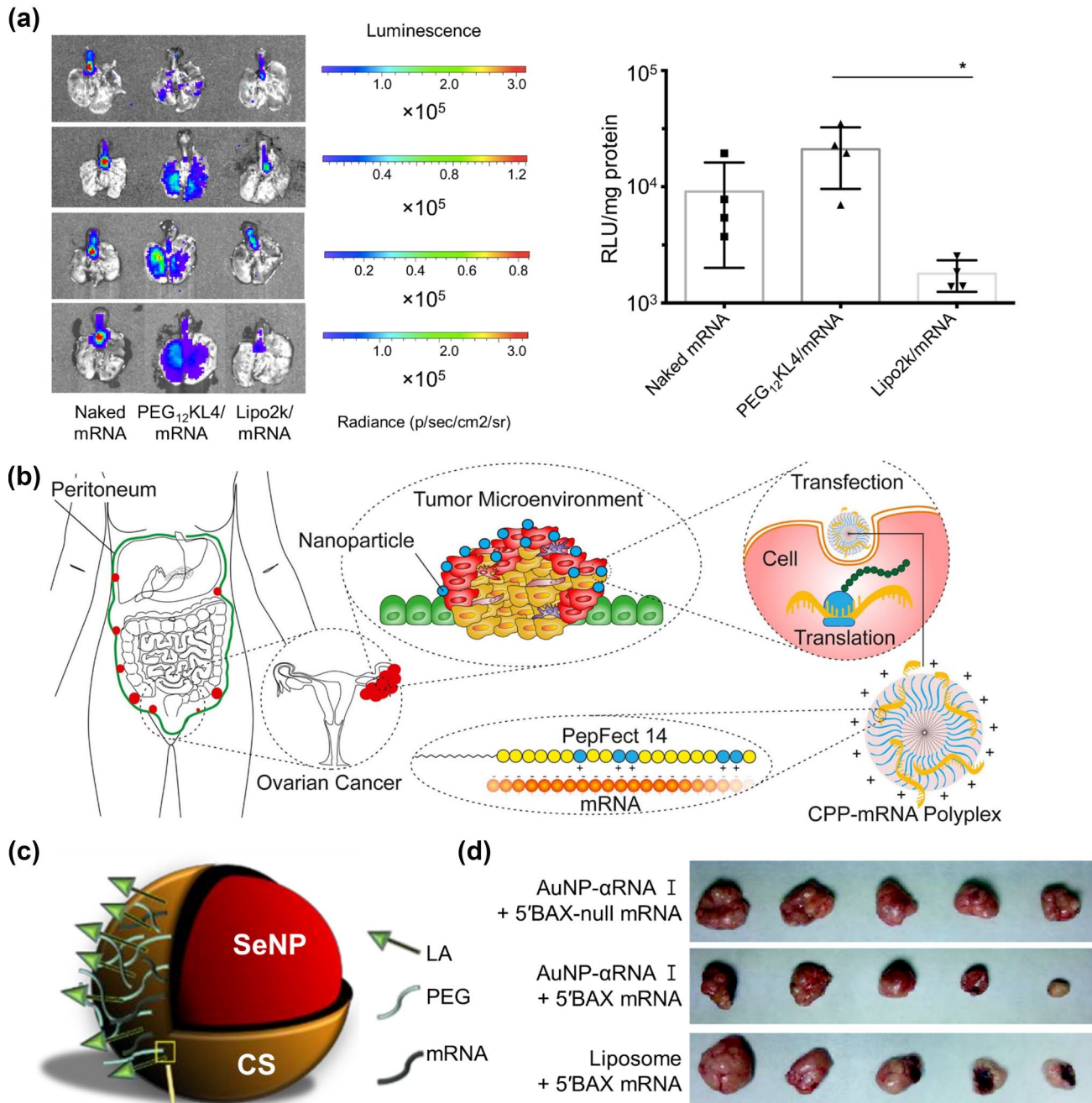

Fig. 5 mRNA delivery systems formulated with peptide-derived and inorganic nanoparticles. **a** Pulmonary delivery of mRNA formulations with different transfection agents (naked mRNA, PEG-KL4/mRNA, and lipoplex/mRNA). At 24 h post-administration, the lungs were isolated for bioluminescence imaging, and luciferase protein expression of lung tissues were measured (Reproduced with permission from [1] Copyright 2019, Journal of Controlled Release). **b** Graphical abstract of cell-penetrating peptide (CPP) PepFect 14 (PF14)-mRNA nanoparticles in models of ovarian cancer (Reproduced with permission from [67] Copyright 2019, European Journal of Pharmaceutics and Biopharmaceutics). **c** Illustration of the fully functionalized SeNP (Reproduced with permission from [74] Copyright 2021, Pharmaceutics). **d** Actual sizes of representative tumors after xenograft implantation followed by injection of 5 nM AuNP-αRNA I-5′BAX mRNA in mice (reproduced with permission from [75] Copyright 2013, PLOS ONE)

to MDA-MB-231 cells, researchers transfected the cells with H3K(+H)4b-mRNA polyplexes, which resulted in luciferase expression levels tenfold greater than those of controls. Stability and intracellular uptake, as well as transfection efficiency, also increased. Taken together, these histidine-rich H3K(+H)4b peptides played an essential role in enhancing mRNA delivery in vitro [64].

Imitating the structure of peptides is another format for peptide-based delivery systems. For example, the KL4 peptide, which mimics pulmonary surfactant protein B (SP-B), is utilized as the mRNA carrier with a monodisperse linear PEG attachment. Indeed, PEG-KL4/mRNA complexes showed better transfection efficiency than naked mRNA or lipoplexes in the lung region of mice after intratracheal administration (Fig. 5a). Importantly, this synthetic KL4 peptide provides a novel formulation of inhalable dry powder for mRNA pulmonary delivery [1]. In another research, a library of peptide-like oligoaminoamides composed of synthetic amino acids with the beneficial properties of PEI was investigated. Furthermore, various modifications, such as histidine and bioreducible disulfide blocks, were controlled to promote mRNA transfection efficiency [65].

3.2 Applications for Cancer Therapy

In a recent study, mRNA delivery systems based on CPP PepFect 14 (PF14) nanoparticles and LNPs were compared to measure detectable reporter protein expression in a xenograft model of ovarian cancer which is one of the most common cancers in women [66]. mRNA-PF14 nanoparticles could endure the in vivo environment of the abdominal cavity where ovarian cancer is usually confined, whereas LNPs could not. Therefore, this study demonstrates the potential of a CPP-based delivery system by intraperitoneal injection to induce specific proteins in ovarian cancer (Fig. 5b) [67]. In an effort to treat castration-resistant prostate cancer, amphiphilic phospholipid peptide dendrimers (AmPPDs) were formulated as an effective siRNA delivery system. Notably, DSPE–KK$_2$ AmPPD bearing a less hydrophilic moiety facilitated efficient intracellular uptake and endosome release of siRNA complexes. Furthermore, these complexes caused a more potent gene knockdown and exhibited anti-cancer effects both in vitro and in vivo [68].

Most pancreatic adenocarcinomas (PDACs) are driven by the activation of KRAS, a proto-oncogene family of RAS proteins. In an attempt to target KRAS, a peptide-based p5RHH nanoparticle-condensing oligonucleotide was formed to deliver KRAS-specific siRNA. Delivery of the siRNA/p5RHH system to an in vivo model containing KPC-1 pancreatic cancer cells significantly reduced KRAS expression and inhibited pancreatic cancer growth [69].

Furthermore, a novel chimeric peptide supramolecular nanoparticle delivery system was also developed to deliver plectin-1 (PL-1)-targeted PDAC-specific miR-9. In detail, PL-1/miR-9 nanocomplexes could increase the anti-cancer effect of doxorubicin, giving rise to the chemosensitivity of PDAC cells to doxorubicin. PL-1/miR-9 nanoparticles significantly inhibited autophagy and induced apoptosis in PDAC cells in a pancreatic tumor model established from patient-derived xenografts, highlighting the possibility of synergic therapies against PDACs in the near future [70].

4 Inorganic Nanoparticles

Inorganic materials uniquely possess ideal characteristics such as non-toxicity, hydrophilicity, biocompatibility, and high stability. Gold nanoparticles (AuNPs) and selenium nanoparticles (SeNPs), in particular, have been widely explored for diagnosis, imaging, and drug delivery because they are biocompatible and chemically inert [71]. Unfortunately, neither inorganic nanomaterials for mRNA delivery nor lipid- or polymer-based vehicles are well developed, and yet requires much progress. However, these underdeveloped fields have shown promising results in recent years.

4.1 Efforts to Improve Delivery Efficiency

To improve transfection efficacy, cationic materials such as PEI or polyamidoamine (PAMAM) dendrimers were employed to coat the surface of inorganic nanoparticles with mRNA. PEI- and PAMAM-dendrimer-coated NPs showed high transfection activities owing to their branched structures and multiple amine groups [72, 73]. Another attractive material, SeNPs possess lower toxicity, higher bioavailability, biodegradability, and antioxidant activity. One study explored the novel use of SeNPs as liver-targeted mRNA-based nanocarriers by functionalizing SeNPs with CS, PEG, and lactobionic acid (LA) (Fig. 5c). Among LA-CS-SeNPs, PEG-CS-SeNPs, and dual-targeted PEG-LA-CS-SeNPs, LA-targeted nanocomplexes were primarily taken up by HepG2 cells. Therefore, functionalized SeNPs suggest the possibility of future liver-targeted immunotherapy [74].

4.2 Applications for Cancer Therapy

Interestingly, DNA oligonucleotides were conjugated to gold nanoparticles to form AuNP-DNA, which served as efficient vehicles for mRNA delivery. mRNA molecules hybridized to AuNP-DNA conjugates could be protected

from attack by cellular RNase, which is why mRNA delivered by the AuNP–DNA complex exhibited greater longevity than that delivered by the liposome-based agent. BAX mRNA loaded onto AuNPs–DNA was injected into xenograft tumors in mice, resulting in the synthesis of biologically functional BAX protein, which could ultimately inhibit tumor growth by inducing apoptosis (Fig. 5d) [75]. In another study, among core–shell type biphasic materials, SeNPs were selected as drug carriers owing to their antioxidant and biocompatible properties. SeNPs formed core–shell structures coated with CS, mRNA, and a folate-targeting moiety. The functionalized surface was able to safely and stably deliver mRNA cargo in vitro and showed significant uptake in folate receptor cells. Therefore, CS-coated SeNPs have the potential for application in tumor vaccination and immunotherapy [76].

5 Hybrid Nanoparticles

Many efforts have been made to construct an ideal gene delivery platform. Focusing on only one type of component and more than two types of materials can maximize delivery efficiency. Specifically, hybrid nanoparticles using polymers, peptides, lipids, or inorganic materials are currently employed as gene carriers to promote biocompatibility and efficiency and reduce toxicity.

5.1 Efforts to Improve Delivery Efficiency

Metal–organic frameworks (MOFs) are extremely porous crystalline hybrid compounds that connect with metal ions and organic ligands to form 3D structures. MOFs possess a networking structure with organic ligands, containing voids and a wide range of surface areas, making them ideal candidates for biomedical applications [77]. These networks can be modulated to incorporate various ligands and metal ions [78, 79].
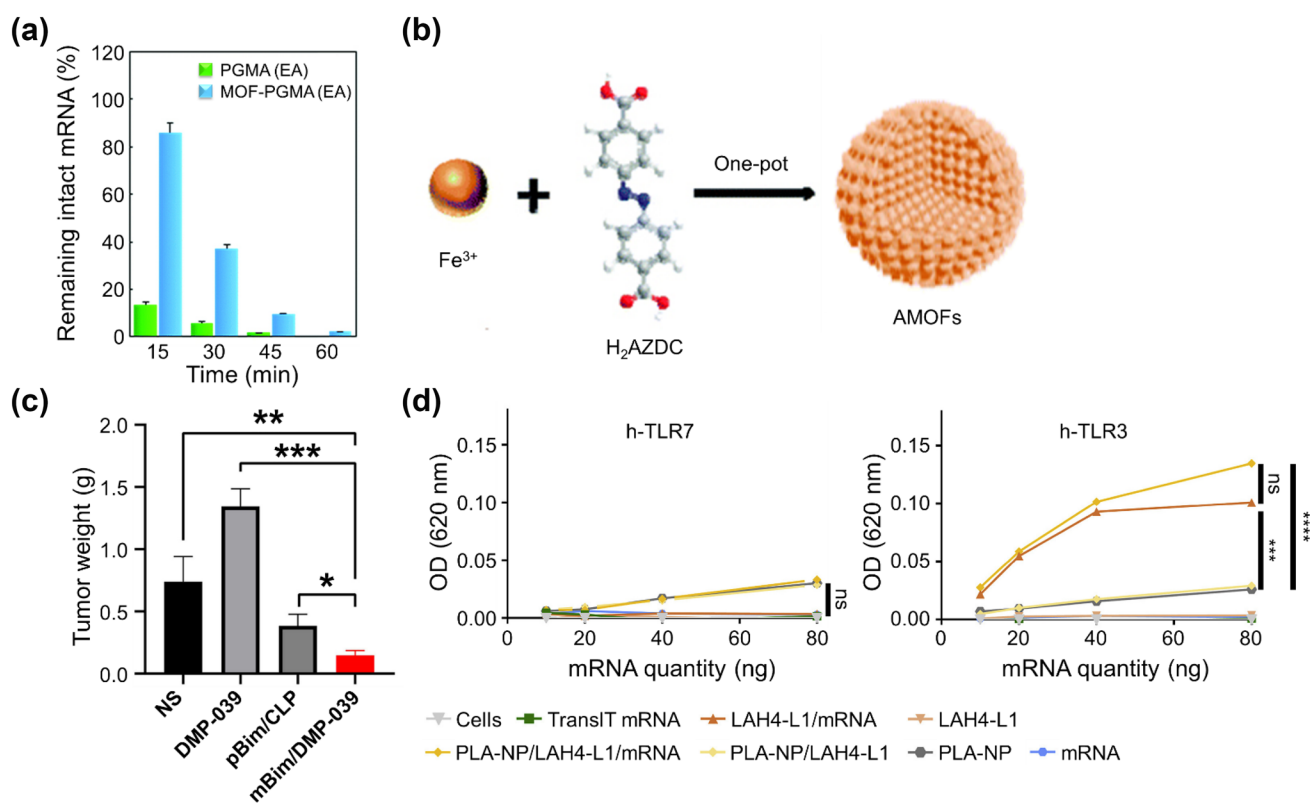
Fig. 6 Efficient mRNA delivery by hybrid nanoparticles. **a** The tolerability of the mRNA encapsulated in MOF-PGMA(EA) or PGMA(EA) complexes (at an N/P ratio of 1.5) in the presence of 10% FBS (reproduced with permission from [80] Copyright 2018, Chemical Communication). **b** Synthesis of the azobenzene-unit-containing metal–organic-framework-based nanoprobe (AMOF@MBs) (reproduced with permission from [82] Copyright 2019, Analyst). **c** mBim/DMP-039 complex suppressing the C26 subcutaneous xenograft model in vivo (reproduced with permission from [88] Copyright 2021, International Journal of Nanomedicine). **d** mRNA complexes stimulate innate immunity through PRR activation. Evaluation of PRR activation via the TLR7 and TLR3 pathways in HEK-Blue hTLR7 and HEK-Blue hTLR3 cells with mRNA formulations (reproduced with permission from [90] Copyright 2019, Biomaterials)

In a recent study, zirconium (Zr)-based MOFs coated with cationic polymers promoted the condensation of mRNA and improved colloidal stability and protection of encapsulated mRNA payloads from enzymatic degradation (Fig. 6a) [80]. In the case of delivering molecular beacons via Zr-based MOFs, selectively lighting up endogenous RNA targets have been shown by double-layer MOFs even without specific treatments [81]. Similarly, Liu et al. investigated VEGF mRNA by monitoring MOFs under hypoxia in living cells. This novel fluorescent nanoprobe system based on azoreductase-responsive functional MOFs (AMOFs) was engineered to visualize VEGF mRNA (Fig. 6b). Owing to a specific reduction reaction in the ligand moiety, the nanoprobe was released from the MOFs and combined with VEGF mRNA in its presence [82].

Polymers and lipids commonly form hybrid nanoparticles for mRNA delivery. Similarly, cationic lipids, 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), and cationic biopolymer protamine can be combined to form an mRNA delivery platform. As proven in both in vitro and in vivo studies, the most beneficial aspect of combining polymers and lipids is that much higher transfection can be achieved compared to when using a sole carrier [83]. This is due to the fact that hybrid systems based on polymer-lipid conjugates can improve circulatory half-lives dramatically [17]. Thus, the integration of a polymer into a lipid enhances structural stability, allows for controlled release, and prolongs protein expression [84, 85].

5.2 Applications for Cancer Therapy

Even though there are few papers that directly address mRNA delivery using MOFs, MOFs carriers have shown to exhibit remarkable cell uptake and lysosomal escape effect for encapsulated or adsorbed cargo. With specific post-modification and a precise understanding of the disease mechanism, MOF-based delivery systems are promising for both RNA delivery and combination tumor therapy. Chunbi et al. found that Zr-based MOFs that protect siRNA from nuclease degradation can enhance siRNA cellular uptake and promote siRNA escape from endosomes to silence MDR genes, increasing the effect of combination therapy against in vitro ovarian cancer cells [86]. MOFs created from another metal source, zinc (Zn)-based MOFs, have also demonstrated the possibility of being developed into potent delivery nanomaterials. The miRNA, which is known to have low stability and delivery efficacy in vivo, when adsorbed onto Zn-based MOFs, was able to effectively suppress tumor growth in a triple-negative breast-cancer mouse model after being successfully released in the cytoplasm [87].

CPP can enhance membrane penetration and cellular internalization. As an example of a peptide-functionalized hybrid delivery platform, the DMP nano-backbone was synthesized via the self-assembly of a cationic lipid (i.e., DOTAP) and copolymer, methoxy PEG-poly(ε-carprolactone) (mPEG-PCL). To promote cellular uptake, they fused two CPPs of R9 with cRGD and introduced them to the DMP backbone, producing DMP-R9-cRGD. As a result, modification of the cRGD-R9 peptide significantly promoted mRNA internalization into cancer cells and anti-cancer solid effects in numerous colon-cancer models (Fig. 6c) [88].

5.3 Applications for Vaccination

In one hybrid carrier designed for the co-delivery of mRNA and adjuvant, poly(lactic-co-glycolic acid) (PLGA) composed the core for loading of toll-like receptor (TLR)7 adjuvant, while lipids composed the shell for conjugation with mRNA. In vitro results demonstrated enhanced antigen expression and dendritic cell (DC) maturation. After intravenous administration of the hybrid nanovaccine, the expression levels of OVA-mRNA were high in the spleen, and strong immune responses were caused by dense antigen-presenting cells in vivo. Specifically, adjuvant-loaded hybrid mRNA stimulated a stronger OVA-specific $CD4^+$, $CD8^+$ T-cell immune response. Importantly, the co-delivery of antigens and adjuvants via a hybrid delivery platform can be used as a nanovaccine [89].

DCs have been used as targets for mRNA-based vaccine strategies because they connect innate and adaptive immune responses and are major regulators of cytotoxic and humoral adaptive responses. In this regard, poly(lactic acid) (PLA) and cationic CPP, which are membrane-active and efficient at mRNA translation in the cytosol, were synthesized together to deliver antigen-coding mRNAs into the DC cytosol. CPP was able to successfully condense mRNA and associate it with PLA nanoparticles. In all, in vitro results showed strong protein expression in DCs and the promotion of innate immunity activation (Fig. 6d) [90].

6 Conclusion and Perspectives

In this brief overview of RNA delivery systems, naturally derived carriers, synthetic polymer-based nanoparticles for numerous purposes, inorganic materials, and hybrid nanoparticles were introduced. The most crucial aspect of mRNA or siRNA delivery for biomedical applications is that genetic materials should reach the targeted site as efficiently and safely as possible. To promote delivery efficiency, numerous techniques for encapsulating genetic materials have been investigated. These carriers can protect the cargo from harsh in vivo environments, such as enzymatic degradation and recognition by the innate immune system with biocompatibility and long-term stability [67]. Ideal carriers should also

induce efficient transfection into cells and produce high levels of specific proteins. In this regard, the nanoparticle has been attractive in the biomedical field due to their useful physiochemical properties [91].

Currently, LNPs carrying mRNA have received considerable attention owing to the COVID-19 pandemic. However, they have faced problems related to the potential toxicity of the components and their decreased interaction with endosomal membranes [92]. Up to this point, the feasibility of polymers or low-cytotoxicity of naturally derived materials and peptides has been advantageous for RNA delivery. Controlling various parameters such as stiffness, the level of branching, hydrophobicity, surface modification, and the steric structures of the cargo can affect delivery efficiency. Moreover, other therapeutic purposes, as well as vaccination, can be achieved through mRNA therapy. For example, numerous tumor models, such as ovarian cancer, glioma, and prostate cancer, have been tested using gene therapy. Gene therapy has many potential applications in protein replacement therapy and gene-editing techniques.

Applications in cancer immunotherapy, gene editing, and cell reprograming require protein expression for only a desired duration as well as high carrier stability, moderate binding force, efficient cellular uptake, and endosomal escape [93, 94]. Studies related to gene delivery systems have attempted to solve immunogenicity problems, the short duration of protein expression, rapid enzymatic degradation, and limited cellular uptake [95]. However, there still remains challenges to be addressed. First, detailed mechanisms of the nanoparticle-mediated delivery processes need more study to improve endosomal escape and transfection efficiency. Next, different nucleic acid medicines should achieve in vivo targeted delivery to the same immune cell of interest. Although the medicines were effective in animal studies, they may not be applicable to humans due to the differences in the immune systems between human and animal models [16].

In this regard, future research should concentrate on better understanding many aspects related to cellular internalization, intracellular trafficking pathways, and formulation design. Collaboration between biologists, biomaterial scientists, and doctors are critical in this endeavor [96]. Interestingly, there has been extensive focus on combining nanotechnology, chemistry, physics, and biology to develop biomaterials [97]. In conclusion, in addressing mentioned challenges, mRNA nanomedicines will solve various medical problems that are intractable using conventional approaches and constitute a promising strategy in biomedical research to treat serious diseases [95, 98].

Declarations

Conflict of interest The authors declare that they have no known competing financial interests.

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