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REVIEW ARTICLE

Nanocarrier-Mediated Delivery of MicroRNAs for Fibrotic Diseases

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Abstract
MicroRNAs (miRNAs) are endogenous noncoding RNAs that mediate the fibrotic process by regulating multiple targets. MicroRNA-based therapy can restore or inhibit miRNA expression and is expected to become an effective approach to prevent and alleviate fibrotic diseases. However, the safe, targeted, and effective delivery of miRNAs is a major challenge in translating miRNA therapy from bench to bedside. In this review, we briefly describe the pathophysiological process of fibrosis and the mechanism by which miRNAs regulate the progression of fibrosis. Additionally, we summarize the miRNA nanodelivery tools for fibrotic diseases, including chemical modifications and polymer-based, lipid-based, and exosome-based delivery systems. Further clarification of the role of miRNAs in fibrosis and the development of a novel nanodelivery system may facilitate the prevention and alleviation of fibrotic diseases in the future.

Key Points

MicroRNAs regulate the occurrence of fibrotic disease.

A nanocarrier can mediate delivery of microRNAs for fibrotic diseases.

1 Introduction

Fibrotic diseases are mediated by chronic pathophysiological processes in which fibrous connective tissue accumulates excessively during injury or inflammation, leading to scarring or organ failure [1]. Fibrosis is considered to result from abnormal repair after organ damage. A typical repair process involves regeneration, in which damaged tissue is replaced by cells of the same type without evidence of damage. However, uncontrolled injury results in remodeling of the extracellular matrix and the establishment of an abnormal fibrotic state (Fig. 1) [2]. Continued deterioration of the fibrotic state leads to organ failure and can even lead to death in severe cases. Worldwide, organ fibrosis is the leading cause of death and disability resulting from many diseases. Despite the continuous development of medical technology and the decreasing trend in fibrotic diseases, nearly half of the fatalities from diseases in developed countries can be attributed to tissue fiber hyperplasia [3, 4].

Antifibrotic drug treatment and elimination of the cause of injury, for example, by inhibiting inflammation, repairing damage, promoting extracellular matrix degradation, and altering collagenase activity, can effectively alleviate the development of fibrotic diseases [5, 6]. However, the antifibrotic effects of a single-agent treatment is limited owing to the low permeability, poor targeting, and inevitable side effects of the drug [7]. Increasing the drug dose to achieve a therapeutic effect may produce irreversible effects on the target organs, and the single-agent treatment approach does not guarantee specific delivery to the target cells allowing the suppression of disease development [8]. Thus, searching for specific small-molecule drugs that regulate the key signaling pathways of fibrosis may provide the optimal therapeutic option for fibrotic diseases.

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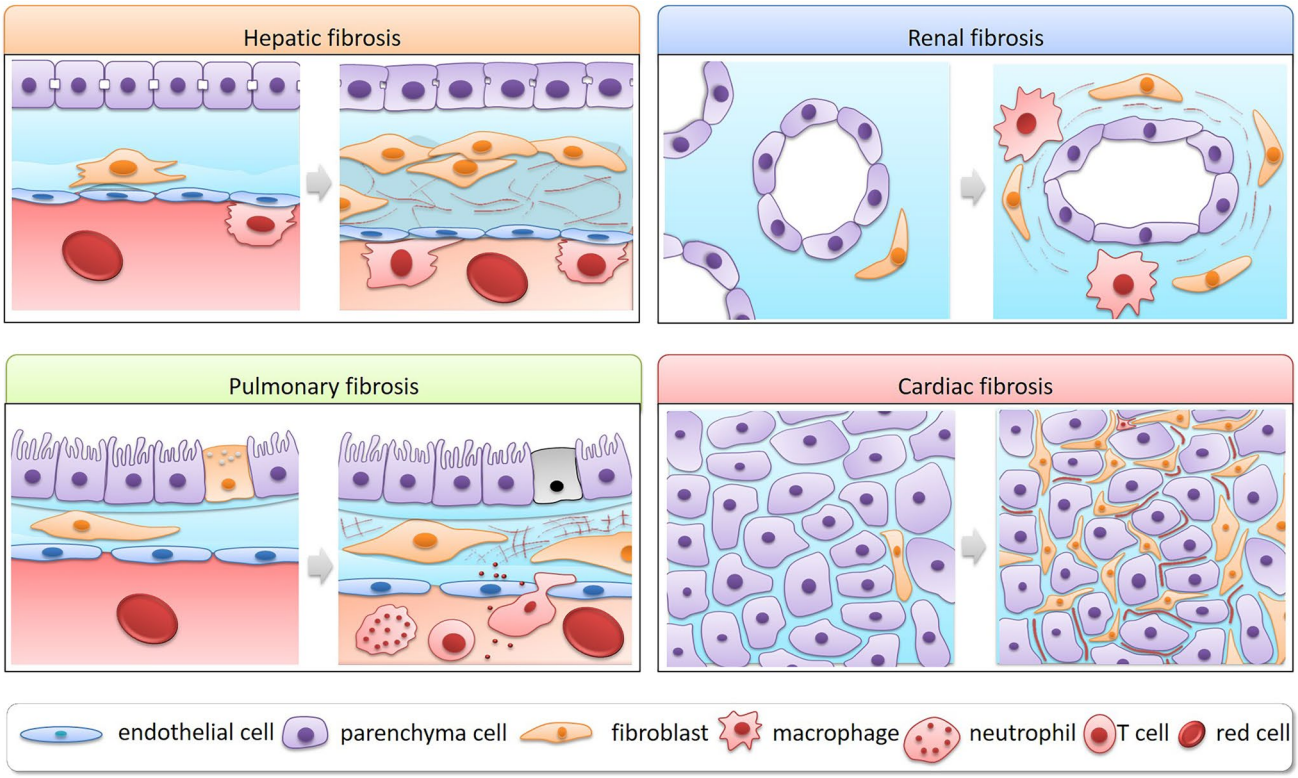

Fig. 1 Common fibrotic diseases involving liver fibrosis, renal fibrosis, pulmonary fibrosis, and myocardial fibrosis

2 MicroRNAs

2.1 Biogenesis of MicroRNAs

MicroRNAs (miRNAs) are single-stranded noncoding RNAs that contain approximately 18–25 nucleotides and are encoded by an endogenous gene [9, 10]. They contain a seed sequence complementary to the 3′ untranslated region (3′UTR), the 5′UTR, or the coding sequence of the target messenger RNA (mRNA) and regulate cell proliferation, apoptosis, differentiation, and metabolism by inhibiting the translation process or enhancing mRNA cleavage [11–14].

After DNA transcription, the primary transcript, called a primary miRNA can be processed to produce a precursor miRNA, which is released into the cytoplasm for cleavage into a double-stranded miRNA by RNase III. Mature single-stranded miRNAs can interact with the RNA-induced silencing complex to regulate mRNA translation [15]. A single miRNA can regulate multiple target genes, and multiple miRNAs can regulate a single target gene. This precise regulatory network diversifies the complement of miRNA regulatory mechanisms [16]. As important components of epigenetics, miRNAs participate in various physiological and pathological processes and regulate the occurrence and progression of fibrosis [17].

2.2 MicroRNAs in Fibrosis

MicroRNAs can mediate the development of fibrotic diseases by regulating the expression of target genes [18, 19]. The transforming growth factor (TGF)-β/Smad signaling pathway can be triggered by miRNAs to mediate the development of liver and lung fibrosis and scleroderma [20]. In addition, miRNAs can participate in the SIRT1/p38 signaling pathway to regulate the process of renal fibrosis and can target the TGF-β/Smad and PI3K/Akt/mammalian target of rapamycin signaling pathways to alleviate myocardial fibrosis and keloids [21–23]. The regulatory mechanisms of miRNAs provide a theoretical basis for the treatment of fibrotic diseases [24, 25].

2.3 MicroRNA-Based Therapies

In fibrotic diseases, miRNAs can be upregulated as a fibrosis marker or downregulated as an antifibrotic factor [26–28]. When miRNAs are suppressed in fibrotic diseases, alternative therapies are suitable. Synthesized miRNA mimics instead of endogenous miRNA can interact with the RNA-induced silencing complex, which regulates the translation process and restore the expression of profibrotic miRNAs downregulated in fibrotic diseases. If a miRNA

is overexpressed in fibrotic diseases, miRNA suppression therapy is used. Therapeutic approaches using miRNA-specific antagonists or synthetic anti-miRNA oligonucleotide chains to bind to the target miRNA can rescue the function of target genes and promote their antifibrotic function in vivo.

Using overexpressed miRNA mimics, anti-miRNA oligonucleotides or miRNA antagonists that modulate the function of miRNAs, has been suggested as a possible effective therapeutic strategy for human fibrotic diseases [29, 30]. Therefore, it is possible that miRNA can be used as a drug and a target to suppress the fibrotic process.

2.4 Major Obstacles in MicroRNA Delivery

MicroRNAs are negatively charged and easily degraded by nucleases; thus, their tissue-specific delivery and passage through the cell membrane are difficult to achieve [31]. The dilemma of targeted delivery involves challenges such as maximizing cell uptake and tissue-specific delivery and minimizing off-target effects [32]. To improve the efficiency of miRNA delivery, viral and nonviral vectors have been developed to deliver genetic material to target cells to perform the corresponding functions [33]. However, because of the low loading capacity of viral vectors, their propensity to induce inflammatory reactions, and their toxicity and side effects that limit their delivery capabilities, the development of liposomes, polymer systems, nanoparticles, and other nonviral vectors is trending [34]. Nanocarriers have the advantages of improved resistance to enzymatic degradation and high affinity and are therefore very promising for application in treating fibrotic diseases.

2.5 Nanocarriers for MicroRNA Delivery

Nanomedicine is expected to play a role in the targeted delivery of miRNAs for treating fibrosis. Nanodrug delivery carriers have high affinity and stability and can increase the solubility and reduce the toxicity and side effects of drugs [35]. Moreover, the addition of targeting groups such as polypeptides can endow nanodrug carriers with active targeting potential [36, 37].

Because the current outcome for patients with fibrotic diseases is not optimistic, developing new treatment strategies with an understanding of the mechanism of fibrosis development is urgently needed. Because of the unique properties of nanomaterials, increasing attention is being paid to the therapeutic application of nanomedicine in fibrotic diseases. Here, we review the miRNA delivery nano-systems useful for the treatment of fibrotic diseases (Table 1).

3 MicroRNA Delivery Approaches

3.1 Chemical Modifications and Oligonucleotide Conjugates

Chemical modifications of miRNA include locked nucleic acids (LNAs), peptide nucleic acids (PNAs), backbone modifications, and ribose 2′-OH group modifications, which can increase the stability and reduce the off-target effects of miRNAs [38–40].

3.1.1 Locked Nucleic Acids

Locked nucleic acids are a class of oligonucleotide derivatives that contain a methylene linkage between the 2′-oxygen and the 4′-carbon. Due to their unique structure, LNAs have high binding power. Locked nucleic acid-modified DNAzymes can target regulatory RNAs, and LNAs can also modify small-interfering RNAs (siRNAs) to disrupt gene expression [41]. In addition, LNAs have strong antisense activity and participate in reverse regulation of targeted miRNAs. The target site blockers seal specific binding sites of miRNA to the mRNA, and block the binding of normal miRNA to the target gene mRNA by occupying the binding site in vivo and in vitro [42–46].

MiR-29b1 can regulate liver fibrosis. The hedgehog inhibitor GDC-0449 and miR-29b1 cooperate to inhibit hepatic stellate cell activation and extracellular matrix production in mice subjected to common bile duct ligation [47, 48]. LNA-miR-29b1, phosphorothioate (PS-miR-29b1), 2′-O-methyl-phosphorothioate (OMe-miR-29b1), and N,N′-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine (ZEN-miR-29b1) can be used to chemically modify the antisense strand of miR-29b1. These chemical modifications can significantly improve the stability of miR-29b1 in medium containing 50% fetal bovine serum. However, among the modified miRNAs tested, LNA-miR-29b1 was less stable than OMe-PS-miR-29b1 [47]. Therefore, the delivery of LNA-miR-29b1 needs further optimization as a promising therapeutic strategy for liver fibrosis.

Several siRNAs corresponding to miRNAs modified by LNA have been shown to alleviate organ fibrosis in mouse models. LNA-anti-miR-132 was promising for the treatment of liver fibrosis [49]. The LNA-modified anti-miR-34a, miR-21, and miR-320 can prevent heart enlargement and fibrosis [50–53]. LNA-anti-miR-150 reduces pro-inflammatory M1 and M2 macrophages polarization via the SOCS 1/JAK 1/STAT 1 pathway[54–56]. The target site blockers were used to block miR-9 binding to the 3′UTR of anoctamin 1 to increase its activity, thus compensating for the lack of transmembrane conductance modulators in cystic fibrosis (CF) [57].

Table 1 Nanocarriers for miRNA delivery in fibrotic diseases

Delivery system	Target miRNA	Therapeutic mechanism	Fibrosis type	Ref.
Chemical modifications and oligonucleotide conjugate				
Locked nucleic acid	miR-29b1	PI3K/AKT and adhesion-related pathways	Liver fibrosis	[47]
Locked nucleic acid	miR-132	Fibrogenesis and inflammatory	Liver fibrosis	[49]
Locked nucleic acid	miR-34a/21/320	Inflammatory response and fibrosis	Myocardial fibrosis	[50–53]
Locked nucleic acid	miR-150	Reduces proinflammatory M1 and M2 macrophage polarization via the SOCS 1/JAK1/STAT1 pathway	Renal fibrosis	[54, 55]
Locked nucleic acid	miR-9	Block miRNA binding to the 3′UTR of anoctamin 1 to increase its activity	Cystic fibrosis	[57]
Locked nucleic acid	miR-509-3p	Post-transcriptional regulation of the *CFTR* gene	Cystic fibrosis	[65]
Peptide nucleic acid	miR-145-5p/101-3p	Synergistic enhancement of the expression of the CFTR gene	Cystic fibrosis	[60, 67]
Peptide nucleic acid	miR-33	Enhances macrophages autophagy and improves mitochondrial homeostasis	Pulmonary fibrosis	[68]
Peptide nucleic acid	miR-33	Increasing the expression of factors involved in FAO and reducing the development of fibrosis	Renal fibrosis	[69]
Oligonucleotide	miR-29b mimic, remlarsen (also called MRG-201)	TGF-α1 and cell adhesion pathway	Cutaneous fibrosis	[71, 72]
Oligonucleotide	miR-21 inhibitors (RCS-21)	Reverses the pathological activation of macrophages and prevents lung dysfunction and fibrosis	Pulmonary fibrosis	[73]
Inorganic delivery system				
Gold nanoparticle	miR-133b	Inhibits the transformation of myofibroblasts	Scar	[78]
Gold nanoparticle	miR-155	Decreasing inflammation	Myocardial fibrosis	[80]
Mesoporous silica nanoparticle	miR combo (miR-1, 133, 208, and 499)	Reprogram cardiac fibroblasts for cardiac regeneration	Myocardial fibrosis	[82]
Polymer-based delivery system				
Polyethylenimine	miR-146a	Smad4/TGF-α1 & TRAF6/NF-κB pathways	Renal fibrosis	[86]
Polyethylenimine	miR-126	Resulted in significant knockdown of TOM1	Cystic fibrosis	[87]
Polyethylenimine	muscle-specific miRNAs (miR-1 and miR-133a)	Reprogram the adult human cardiac fibroblasts into cardiomyocyte-like cells	Myocardial fibrosis	[88]
Poly(lactic-co-glycolic acid)	miR-17	Decreasing inflammation	Cystic fibrosis	[93]
Poly(lactic-co-glycolic acid)	miR-19b-3p	Decreasinginflammation	Cystic fibrosis	[94]
Poly(lactic-co-glycolic acid)	miR-21	TGF-α1/Smad3 and kinases/MAPK pathway	Renal fibrosis	[95]
Poly(lactic-co-glycolic acid)	miR-519c	Decreases inflammation	Pulmonary fibrosis	[96, 97]
Chitosan	miR-29b	TGF-β1/Smad3 pathway	Achilles tendon injury	[100]
Chitosan	miR-126	Resulted in significant knockdown of TOM1	Cystic fibrosis	[87]
Chitosan	miR-21	Promotes gingival fibroblast adhesion, proliferation, and increased expression of genes related to extracellular matrix	Gingival fiber hyperplasia	[101]
Hyaluronic acid	miR-21	Decreases inflammation,	Cardiac fibrosis	[104]
Hyaluronic acid	miR-24-3p	reduces fibrosis and macrophage activation	Corneal stromal fibrosis	[105]

Table 1 (Continued)

Delivery system	Target miRNA	Therapeutic mechanism	Fibrosis type	Ref.
Lipid-based delivery system				
Cationic lipid	miR-122	Regulates autophagy,	Liver fibrosis	[109]
Cationic lipid	miR-21	inhibits myofibroblast differentiation, reduces extracellular matrix synthesis, and inhibits fibrotic progression	Cardiac fibrosis	[110]
Exosome-based delivery system				
Menstrual blood-derived stem-cell exosome	let-7	LOX1/NLRP3/caspase 3 pathway	Pulmonary fibrosis	[116]
Placenta-derived mesenchymal stem-cell exosome	miR-29c	TGF-b/SMAD3 pathway	Myocardium and diaphragm fibrosis	[117]
Adipose stem-cell-derived exosome	miR-126	VEGF pathway and MAPK and PI3K pathway	Cardiac fibrosis	[119–122]
Bone marrow mesenchymal stem cell-derived exosome	miR-21a	Attenuates glycolysis by targeting ATP-dependent 6-phosphofructokinase fibrosis	Renal fibrosis	[125]
Bone marrow mesenchymal stem cell-derived exosome	miR-214	Inhibiting the IL-33/ST2 axis	Skin fibrosis	[126]
Embryonic stem cells	miR-17	Targeting thrombospondin-2	Pulmonary fibrosis	[127]
Hepatocyte-derived exosome	miR-146a	Suppresses the EMT process in hepatic stellate cells	Liver fibrosis	[128]
Hepatocyte-derived exosome	miR-99a	Targeting BMPR 2 promotes hepatocyte apoptosis	Liver fibrosis	[129]
Satellite cell-derived exosome	miR-23a/27a/26a	Ameliorates renal tubulointerstitial fibrosis	Renal fibrosis	[130]
M2-polarized macrophage-derived exosome	miR-381	Attenuates the activation of urethral fibroblasts through YAP/gls 1-regulated glutaminolysis	Renal fibrosis	[131]
Urinary exosome	miR-615-3p/3147	Associated with Inflammation and fibrosis in diabetic nephropathy	Renal fibrosis	[132]
Plasma exosome	miR-125a	Regulates T-lymphocyte subsets, promoting silica-induced pulmonary fibrosis by targeting TRAF6	Pulmonary fibrosis	[133]
Exosomes loaded in a soluble microneedle array	miR-141-3p	Relief of hypertrophic scar in the ear of rabbits	Hypertrophic scar	[137]
Microneedle patches loaded with exosomes	miR-29b	Prevented cardiac fibrosis in a mouse myocardial infarction model	Cardiac fibrosis	[138]

BMPR 2 bone morphogenetic protein receptor type II, *CFTR* cystic fibrosis transmembrane conductance regulator, *combo* combination, *EMT* epithelial–mesenchymal transition, *FAO* fatty acid oxidation, *IL-33* interleukin-33, *MAPK* mitogen-activate protein kinase, *miRNA* microRNA, *ND* no data, *Ref.* reference, *TGF* transforming growth factor, *UTR* untranslated region, *VEGF* vascular endothelial growth factor

3.1.2 Peptide Nucleic Acids

Peptide nucleic acids are negatively charged nucleic acid analogs whose sugar-phosphate backbone is replaced by a polypeptide backbone. Peptide nucleic acids are very stable structural analogs of delivered miRNAs [58–60].

Cystic fibrosis is caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene that result in reduced or altered CFTR functions [61]. Several miRNAs downregulate the expression of CFTR, thereby causing or aggravating the symptoms of CF [62]. Modification of miRNAs with PNAs facilitates the protection of specific sequences in the 3′UTR of the CFTR mRNA [63, 64]. PNAs synthesized by the addition of two tetrapeptides (Gly-SerP-SerP-Gly) at the two C-termini can specifically bind to miR-509-3p as a target miRNA-binding agent, blocking the effect of this miRNA on CFTR mRNA activity [65]. In A549 cells co-transfected with the pLuc-CFTR-3′UTR vector and different combinations of PNAs, longer PNA constructs restored up to 70% of the luciferase activity, suggesting that the appropriate use of PNAs can counteract the decrease in CFTR expression and is expected to alleviate the symptoms of CF [5].

MiR-145 is another miRNA that targets and inhibits CFTR expression [66]. Conjugation of the octoglycine (R8) carrier peptide at the N-terminus of the PNA chain can block the regulatory effect of miR-145-5p and miR-101-3p, enhance the expression of the *CFTR* gene, and suppress the

development of CF [60, 67]. Another study suggested that inhibition of miR-33 in macrophages by administration of anti-miR-33 PNA attenuates fibrosis in mouse pulmonary fibrosis models in vivo and ex vivo [68]. Because PNAs have high affinity for nucleic acid targets, they regulate gene expression with outstanding efficacy and are an effective means of inducing pharmacologically mediated changes in gene expression in vivo and in vitro.

Abnormal fatty acid oxidation may have an important effect on the progression of kidney disease. The lack of miR-33 as an important regulator of lipid metabolism can partially prevent fatty acid oxidation in fibrotic kidneys and reduce lipid accumulation. Using low pH insertion peptides as a carrier can allow the delivery of PNA miR-33 inhibitors to the kidney and other acidic microenvironments, which can effectively promote the expression of fatty acid oxidation mediators and reduce the development of fibrosis [69]. These findings suggest that delivery of PNA miR-33 inhibitors may be an attractive therapeutic approach for chronic kidney disease.

3.1.3 Oligonucleotide Conjugate Modifications

MiR-29 is downregulated in multiple fibrotic organs, including the skin and lungs, and negatively regulates fibrosis [70]. A miR-29b oligonucleotide was synthesized via standard phosphoramidite solid-phase synthesis, and the sense strand of the miR-29b oligonucleotide mimic was conjugated to cholesterol at the 3′ end. Intravenous injection of this synthetic oligonucleotide increased miR-29 levels in vivo. In bleomycin-induced pulmonary fibrosis in mice, treatment with a miR-29b oligonucleotide mimic restored the function of endogenous miR-29, thereby reducing collagen expression and in turn blocking and reversing pulmonary fibrosis [71]. Another trial was conducted to evaluate the pharmacodynamic activity of a second development-stage miR-29b mimic, remlarsen (also called MRG-201) [72]. Remlarsen was shown to regulate the expression of miR-29b in skin wounds of mice, rats, and rabbits, as well as in cultured human skin fibroblasts. In this intrasubject controlled clinical trial (ClinicalTrials.gov ID NCT02603224), remlarsen inhibited the expression of collagen and the development of fibrosis in incised skin wounds. Currently, a carbohydrate-conjugated miRNA oligonucleotide drug, RCS-21, which is a miR-21 inhibitor, is being developed to deliver inhaled oligonucleotides efficiently and selectively to lung macrophages. RCS-21 reverses the pathological activation of macrophages and prevents lung dysfunction and fibrosis after acute lung injury in mice. RCS-21 effectively prevents exaggerated inflammatory responses in human lung tissue infected with SARS-CoV-2 in vitro [73]. These results suggest that miR-29b oligonucleotide mimics may effectively prevent fibrotic pulmonary and skin conditions such as hypertrophic scars, keloids, and scleroderma.

3.2 Inorganic Delivery Systems

Compared with conventional drugs, chemotherapy, and radiotherapy, inorganic nanomaterials used as drug carriers can enhance the targeted transport, controlled release, and sustained release of drugs [74]. Common inorganic nanomaterials include gold nanoparticles (AuNPs), mesoporous silica nanoparticles, carbon nanomaterials, and magnetic nanoparticles, but the use of these nanomaterials in miRNA delivery to treat fibrotic diseases needs to be further explored [75].

Gold nanoparticles are precious metal colloids with a particle diameter that ranges from 1 to 100 nm. These colloids exhibit secondary electron emission, and an ion resonance effect can be produced through the interaction of incident light with free electrons in AuNPs to achieve drug delivery (Fig. 2) [76]. They are excellent carriers for delivering small molecule drugs and biomolecules. MicroRNAs delivered into cells via AuNPs can specifically bind to mRNA sequences to exert inhibitory effects on their target genes [77]. For example, miR-133b can inhibit the transformation of myofibroblasts [78]. A nanocomposite made of AuNPs and miR-133b was loaded onto the surface of a corneal collagen membrane and on the inside of the collagen membrane [79]. The properties of the collagen membrane did not change, although the cornea was rapidly epithelialized and the corneal transparency remained constant. In addition, only a low level of fibrosis was observed in the corneal stroma. These results suggest that the AuNP/miR-133b complex can achieve rapid corneal repair and inhibit scarring.

Diabetic cardiomyopathy is a common disease in postmenopausal women, in whom the lack of estrogen aggravates its pathology. Compared with diabetic mice, ovariectomized diabetic mice exhibited increased ROS accumulation, apoptosis, myocardial hypertrophy, and fibrosis. miR-155 is a potentially effective promoter of type 1 proinflammatory (M1) macrophage polarization, and its expression can be further enhanced in macrophages and heart tissue by ovariectomy [80, 81]. miR-155 AuNPs were injected into the tail vein, the thiol-modified antagomiR-155 was covalently bound to AuNPs, and the nucleic acid was preferentially delivered to macrophages via phagocytosis [80]. By increasing the proportion of anti-inflammatory type 2 (M2) macrophages and decreasing inflammation, in vivo administration of antagomiR-155 reduced apoptosis and restored cardiac function. The recovery effect of miR-155-AuNP was far superior to that of general depletion of macrophage clones. Obviously, an imbalance in the M1/M2 ratio led to aggravation of cardiomyopathy in ovariectomized diabetic

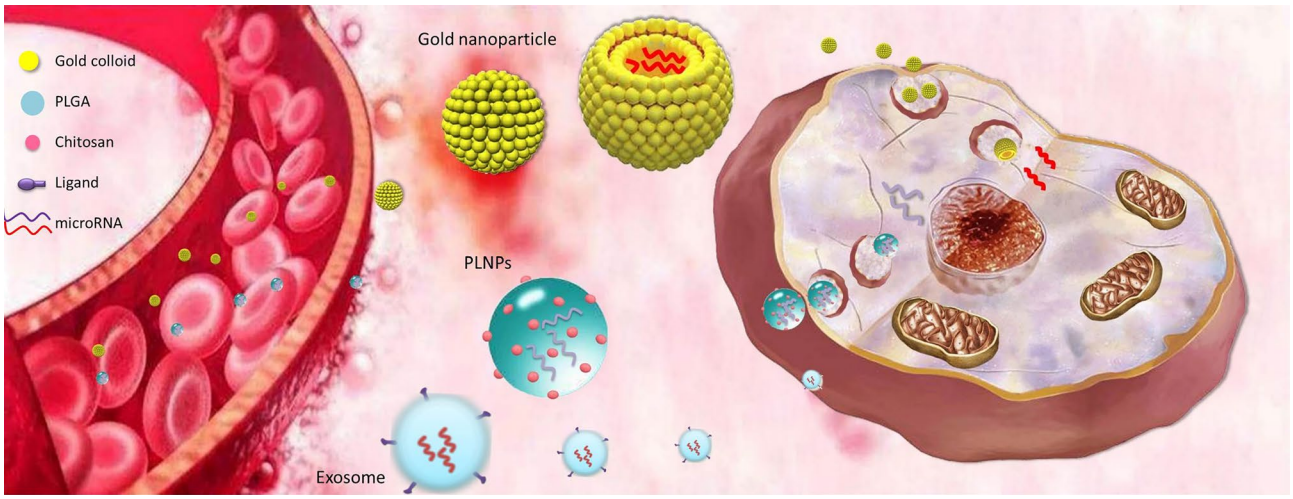

Fig. 2 Schematic of the structure of gold nanoparticles, poly(lactic-glycolic acid) [PLGA] graft chitosan, and exosome delivery microRNA. *PLNPs* PLGA nanoparticles

mice. Thus, AuNP-mediated inhibition of miR-155 in macrophages is a promising strategy for improving cardiac function.

Another non-viral biomimetic system was constructed by coating the FH peptide-modified neutrophil membrane on mesoporous silicon nanoparticles loaded with miR Combo (miR-1, 133, 208, and 499). In a mouse model of myocardial ischemia/reperfusion injury, intravenous injection of nanoparticles successfully delivered miRCombo into fibroblasts, which was used to reprogram cardiac fibroblasts for cardiac regeneration after myocardial injury, thereby reducing fibrosis and improving cardiac function[82].

3.3 Polymer-Based Delivery Systems

Polymers have good biosimulation characteristics, biocompatibility, and a wide range of structural changes therefore they are widely used in drug-delivery systems [81]. Polymer nanodrugs are nanopreparations that connect polymers and drugs through chemical bonds. Upon entry into the body, the conjugate responds to exogenous or endogenous changes to break the chemical bond and release the drug at the target site. Polymers are classified as synthetic or natural. Synthetic polymers are composed of mainly polyethyleneimines (PEIs) and poly (lactic-glycolic acid), while natural polymers include peptides, proteins, and polysaccharides [83].

3.3.1 Synthetic Polymers

3.3.1.1 Polyethyleneimines Polyethyleneimines are a class of cationic synthetic polymers that contain multiple amino groups within their linear or branched structure [84]. The positively charged amino groups in PEIs and the negatively charged phosphate groups in nucleic acids undergo polycondensation through electrostatic interactions to form nanoparticles, not only preventing the degradation of nucleic acids during delivery but also improving the cell uptake efficiency because of their high transfection efficiency [85]. Polyethyleneimines are considered the gold standard for nonviral vectors. Currently, studies based on PEIs as delivery vehicles are increasing; moreover, PEI-based delivery of miRNA is being explored for the treatment of fibrotic diseases.

Polyethyleneimine nanoparticles were shown to effectively deliver miR-146a and significantly enhance its expression in obstructive kidney disease while reducing the area of renal fibrosis, the expression of alpha-smooth muscle actin and the infiltration of F4/80-positive macrophages into the obstructed area [86]. These effects may occur because miR-146a polyethyleneimine nanoparticles can inhibit the TGF-β/Smad and tumor necrosis factor receptor-associated factor 6/nuclear factor kappa B signaling pathways. These results indicate that miR-146a delivery alleviates renal fibrosis by inhibiting profibrotic and inflammatory signaling pathways.

Polyethyleneimine-based nanoparticles also significantly promote miR-126 entry into human F508del CF transmembrane conductance regulator bronchial epithelial (CFBE41o) cells [87]. The low nitrogen/phosphate ratio of PEI-premiR-126 nanoparticles resulted in significant knockdown of target of Myb1 (TOM1), a known target of miR-126. The reduction in TOM1 expression was most pronounced (66% reduction) in CFBE41o cells with an nitrogen/phosphate ratio of 1:1.

Polyethyleneimine forms a stable PEI-miRNA complex via electrostatic interactions. These complexes are immobilized on an electrospun smooth porous scaffold to achieve continuous delivery of two muscle-specific miRNAs (miR-1 and miR-133a). These dual miRNA scaffold systems proved to be a good formulation, and the delivered dual miRNAs

contributed to the precise control of the cardiac fibroblast fate to alleviate myocardial fibrosis [88].

However, the main limitations of PEIs as nanocarriers are their low biodegradability inside cells and their propensity to form aggregates with negatively charged proteins in cells, resulting in dose-dependent cytotoxicity. To reduce their cytotoxicity and improve their transfection and targeting efficiency, chemical structural modifications of PEIs have been widely studied, yielding constructs such as polyurethane-grafted PEI, poly-L-lysine-modified PEI co-polymers, poly(1,8-octanediol-citric acid)-grafted PEI, and poly(lactic acid)-grafted PEI copolymers [89]. These PEIs with modified chemical structures and loaded with miRNAs constitute a future therapeutic option for fibrotic diseases.

3.3.1.2 Poly (Lactic-Co-Glycolic Acid) Poly(lactic-co-glycolic acid) [PLGA] is a safe, biocompatible, and biodegradable polymer. Poly(lactic-co-glycolic acid) can be hydrolyzed into non-toxic lactic acid and glycolic acid monomers, which are metabolized by the body without any side effects [90]. Nanoparticles prepared from PLGA polymers can capture biologically active molecules and escape from the lysosome into the cytoplasm, inducing sustained release of the transported substance in the cell and achieving the goal of slow release. The PLGA delivery system can be subjected to a variety of surface modifications and is currently used for miRNA delivery to treat fibrotic diseases (Fig. 3) [91, 92].

miR-17 mimics were encapsulated in poly(lactic acid) graft-based particles, which were phagocytosed by bronchial epithelial cells, and possibly improved CF [93]. The PLGA microparticle system encapsulating premiR-19b-3p can deliver mature miR-19b-3p to macrophages in vitro with high efficiency. Indeed, the level of secretion leucoprotease inhibitor, the target gene of miR-19b-3p, was still observed to be significantly reduced 72 hours after delivery [94]. As macrophages are key inflammatory cells, they are essential mediators of chronic inflammatory lung diseases such as CF. MiRNA-coated PLGA particles may be delivered to CF tissues by inhalation, thus providing a new treatment paradigm for delivery to macrophages.

However, the ability of anionic PLGA nanoparticles to transfect genetic materials into cells is poor, and their encapsulation efficiency is low, which limits their application. To overcome these limitations, positively charged low-molecular-weight chitosan can be added to the PLGA system. Low-molecular-weight chitosan may facilitate the encapsulation of negatively charged substances such as nucleic acids, thereby improving the packaging efficiency of PLGA. By coupling miRi (a miR-21 inhibitor) with PLGA and low-molecular-weight chitosan, Geng et al. prepared small cationic miRi-low-molecular-weight chitosan-modified PLGA nanoparticles [95]. The easily degradable miRi was encapsulated in these PCNPs, thereby preventing its degradation by nucleases. In vitro and in vivo assays showed that PCNPs have good biocompatibility, a high cell uptake efficiency, and selective kidney-targeting ability. Moreover, the therapeutic effect of miRi-PCNPs on renal fibrosis was much higher than that of miRi alone. The tubule injury index and tubulointerstitial fibrosis area in the miRi-PCNP group were 2.5 times lower than those in the saline and miRi groups. miRi-PCNPs mainly suppress the TGF-β1/Smad3 and extracellular signal-regulated kinase/mitogen-activated protein kinase signaling pathways by inhibiting the expression of miR-21 [95]. Therefore, miRi-PCNPs with specific renal targeting and strong antifibrotic therapeutic effects may constitute a promising basis for the design and development of treatments for renal fibrosis.

Porous polymer particles made from PLGA satisfy unique characteristics required for drug delivery to the lungs, such as aerodynamic density, an adaptive aerodynamic diameter, a porous surface, sustainable release, and enhanced lung deposition effects, which can promote access by or weaken the phagocytosis of lung macrophages. Their good atomization characteristics in the lungs have been exploited to deliver miRNAs to the lungs [96, 97]. Doxorubicin and miR-519c were encapsulated in porous PLGA particles via the water-oil-water emulsion solvent evaporation method and were used for pulmonary administration [98]. These two-component porous PLGA particles had a stronger inhibitory effect on cell proliferation than porous PLGA particles containing a single component (doxorubicin or miR-519c). Therefore, porous PLGA particles can enhance antifibrotic effects and reduce side effects and can be used as sustained-release carriers for the treatment of idiopathic pulmonary fibrosis.

3.3.2 Natural polymers

3.3.2.1 Chitosan Chitosans (CS) are a family of biodegradable, nontoxic, linear cationic polysaccharides consisting of repeating D-glucosamine and N-acetyl-D-glucosamine units connected by 1,4-glycosidic bonds. They are produced by partial deacetylation of chitin isolated from crustacean shells [99]. Chitosans alone can prevent tendon adhesion during tendon healing. In addition, Chen et al. found that CS can increase the sliding distance of repaired tendons and reduce the content of collagen fibers [100]. This effect of CS may occur through the promotion of miR-29b and P21 expression in fibroblasts with a concurrent reduction in the levels of TGF-β1 and Smad3.

MiR-21 promotes fibroblast proliferation and collagen formation in tissue fibrosis, and CS/TPP/hyaluronic acid (HA) nanoparticles are coated with a smooth Ti surface, which promotes gingival fibroblast adhesion, proliferation, and increased expression of genes related to the extracellular matrix [101]. Chitosans were used

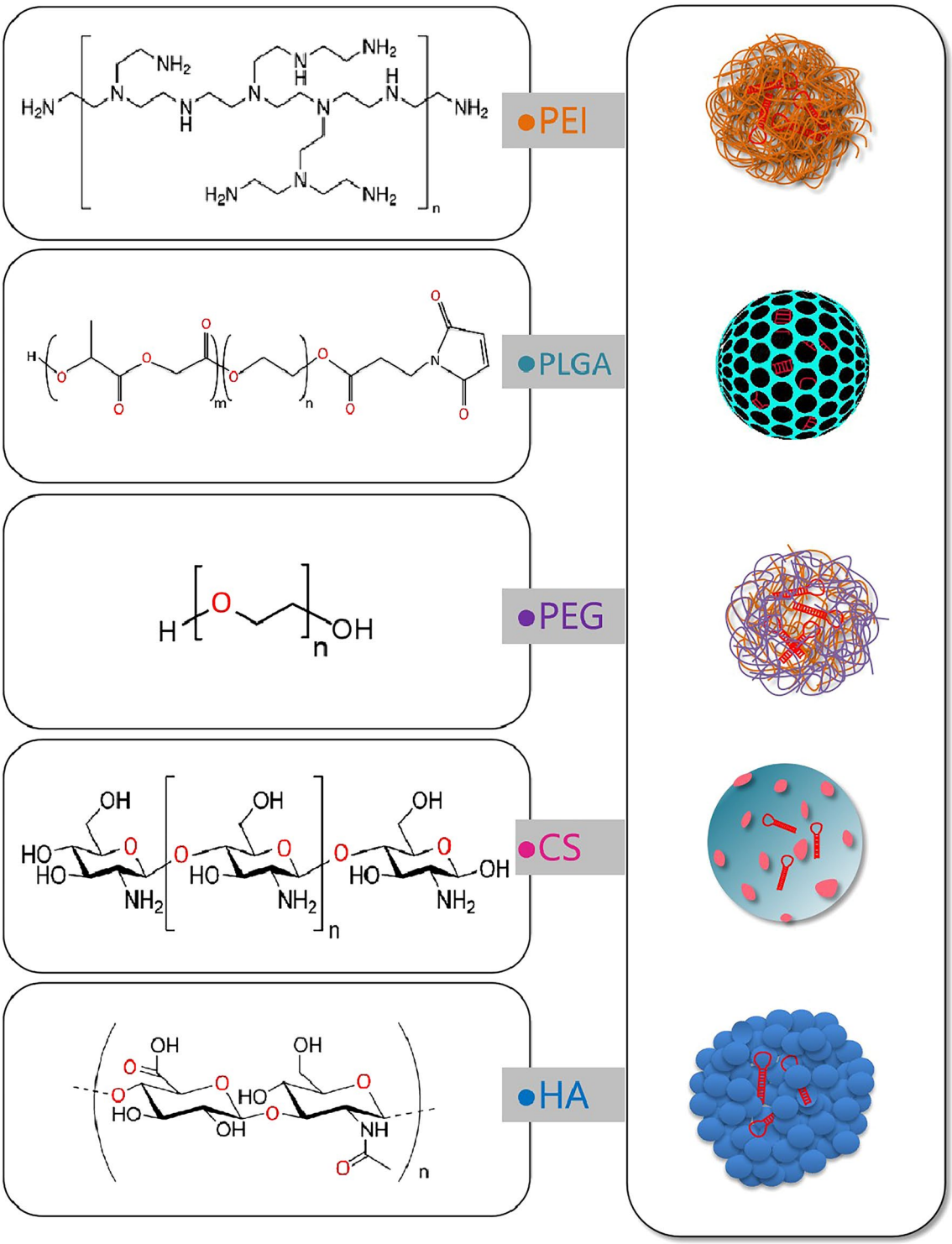

Fig. 3 Chemical structures of synthetic and natural polymers and their constructed microRNA delivery systems. *CS* chitosans, *HA* hyaluronic acid, *PEG* polyethylene glycol, *PEI* polyethyleneimines, *PLGA* poly(lactic-glycolic acid)

to prepare CS-tripolyphosphate-miR-126 (CS-TPP-miRNA) nanocomposites, and the nanoparticles showed relative non-toxicity. However, compared with that of the PEI-miRNA nanodelivery system, the ability of the CS-TPP-miRNA system to deliver miR-126 was poor (Fig. 3) [87]. Therefore, the CS-loaded miRNA system needs further optimization.

3.3.2.2 Hyaluronic Acid Hyaluronic acid sulfate is an anionic nanocarrier that can deliver the reversible complex of miRNA or siRNA with calcium ions in solution to produce an anionic complex [102]. Compared with unmodified HA, sulfated HA is not easily degraded, has enhanced stability during transport, and contains multiple functional groups that can be used for targeted ligand binding and other functions [103]. Hyaluronic acid sulfate can deliver miRNA to inhibit the development of fibrosis. Nanoparticles assembled from miR-21 mimics, calcium bridge channels, and HA sulfate can be delivered to cardiac macrophages after a myocardial infarction (Fig. 3) [104]. MiR-24-3p-rich exosomes functionalized di(ethylene glycol) monomethyl ether methacrylate-modified HA hydrogels reduces corneal stromal fibrosis and activation of macrophages activation, suggesting its potential utility in cell-free therapy for corneal epithelial regeneration [105]. These nanoparticles can induce a switch from a proinflammatory to a reparative phenotype, regulate angiogenesis, and reduce fibrosis in the distal myocardium.

3.4 Lipid-Based Delivery Systems

Lipids, including cationic lipids, ionizable lipids, and auxiliary lipids, are one of the most widely used vehicles for nucleic acid delivery [106]. Cationic lipids have been developed for use in lipid-based nanoparticles to deliver siRNA and miRNA [107]. However, less attention has been devoted to "helper lipids". The addition of the unsaturated fatty acid oleic acid to LNP formulations significantly improved the mRNA delivery efficiency [108]. MiR-122 is a biospecific miRNA associated with many liver diseases, including liver fibrosis [109]. Lipid-based nanoparticles containing oleic acid delivered miR-122 more effectively than the commercial transfection agent Lipofectamine 2000. The expression of mature miR-122 increased 1.8-fold, and the target of miR-122, Bcl-w, was significantly downregulated. Compared with Invivofectamine®, another commercial transfection agent designed specifically for liver delivery, lipid-based nanoparticles containing oleic acid showed considerable liver accumulation and delivery efficiency in vivo. Yan et al. designed a lung-targeted cationic liposome preparation to encapsulate anti-miR-21, and cationic liposome-miR-21 was delivered to inhibit myofibroblast differentiation, which reduced extracellular matrix synthesis, and inhibited fibrotic progression [110]. These findings demonstrate the importance of the "helper lipid" component in LNP preparations for enhancing the miRNA uptake and transfection efficiency [111]. Lipid-based nanoparticles containing OA are promising nanocarriers for miRNA-based therapy in liver fibrosis.

3.5 Exosome-Based Delivery Systems

Exosomes are nanosized (40–100 nm) vesicular particles secreted by cells and exist in various body fluids, such as plasma, saliva, and urine [112]. The membrane is composed of lipid raft domains containing proteins and lipids, which protects the cell-specific proteins, lipids, and nucleic acids (mRNAs, miRNAs, and lncRNAs) carried within from RNase-mediated degradation [113]. Exosomes can participate in immune regulation, cell migration and differentiation, angiogenesis, and proteolysis. Cells can selectively transport noncoding RNA-containing exosomes to adjacent or distant cells, and exosomes have an active sorting mechanism, which plays an important role in cellular information exchange [114]. Interestingly, stem cell-derived exosomes have been found to alleviate fibrosis by delivering miRNAs (Fig. 2) [115].

Cy3-labeled let-7 mimics and antagomiR-let-7 were shown to be delivered to alveolar epithelial cells and lung tissue through menstrual blood-derived, stem-cell secreted exosomes. Let-7 in exosomes can reduce reactive oxygen species levels, alleviate mitochondrial DNA damage, and activate NLRP3 to relieve pulmonary fibrosis [116]. Exosomes secreted from placenta-derived mesenchymal stem cells can express high levels of miR-29c, and were shown to deliver miR-29c from exosomes to myofibroblasts in a co-culture system [117]. Placental-derived mesenchymal stem cells were found to reduce the degree of fibrosis in the myocardium and diaphragm [118]. Adipose stem cell-derived exosomes contain high levels of miRNA, and exosomal miRNA can reduce the expression of fibrosis-related proteins and relieve myocardial fibrosis after an acute myocardial infarction [119–122]. Exosomes derived from adipose-derived mesenchymal stem cells inhibit the proliferation of keloid fibroblasts and promote angiogenesis through miR-181a and miR-7846-3p [123, 124]. Bone marrow mesenchymal stem cell-derived exosomal miR-21a-5p attenuates glycolysis by targeting ATP-dependent 6-phosphofructokinase, thereby alleviating renal fibrosis [125]. Inhibiting the interleukin-33/ST2 axis by delivering miR-214 thereby relieves skin fibrosis in systemic sclerosis [126]. Exosomal miR-17 derived from human embryonic stem cells prevents pulmonary fibrosis by targeting thrombospondin-2 [127].

In addition to stem cells, exosomes secreted by differentiated cells also mediate the progression of fibrosis. Hepatocyte-derived exosomal miR-146a-5p suppresses the epithelial–mesenchymal transition process in hepatic stellate cells [128]. The exosome-associated miR-99a-5p targeting BMPR 2 promotes hepatocyte apoptosis during liver fibrosis [129]. Satellite cell-derived exosome-mediated

delivery of the miR-23a/27a/26a cluster ameliorates renal tubulointerstitial fibrosis in diabetic nephropathy in mice [130]. Exosomal miR-381 derived from M2-polarized macrophages attenuates the activation of urethral fibroblasts through YAP/gls 1-regulated glutaminolysis [131]. Urinary exosomal miR-615-3p and miR-3147 are highly expressed and associated with inflammation and fibrosis in diabetic nephropathy [132]. Plasma exosomal miR-125a-5p regulates T-lymphocyte subsets, promoting silica-induced pulmonary fibrosis by targeting tumor necrosis factor receptor-associated factor 6 [133].

Obviously, stem cell-derived exosomes are very promising for the development of new materials for miRNA nanodelivery systems; more importantly, the development of artificial exosomes for miRNA delivery has been attempted [134–136]. The miR-141-3p-functionalized exosomes were loaded in a soluble microneedle array for the treatment of hypertrophic scars on rabbit ears [137]. Microneedle patches loaded with exosomes containing miR-29b prevented cardiac fibrosis in a mouse myocardial infarction model [138]. The delivery of miRNA via exosomes may prevent and alleviate fibrotic diseases.

4 Prospects and Conclusion

RNA interference-based drugs have been used in therapy, and oligonucleotide mimics constructed using miRNAs have been clinically tested in fibrotic diseases [72]. Various nanomaterials as new vehicles for miRNA delivery, offer a potentially effective therapeutic strategy for fibrotic diseases and combination drug therapy. Various miRNA delivery pathways have been explored and are showing promise. The preparation method of liposomes is simple, safe, and non-toxic. Unfortunately, the synthesis of liposome molecules is complex and expensive. Polymer nanodelivery systems have significant advantages, such as high therapeutic efficiency and good biocompatibility; however, designing multifunctional nanodelivery systems still faces many challenges. Inorganic nanoparticles can protect miRNA from nuclease degradation and regulate the expression of cellular target genes, which is promising, but their biodegradable properties need to be further improved. Stem cell-derived exosomes provide new strategies and methods for tissue repair and antifibrosis of miRNA; however, the methods to isolate and purify exosomes needs improvement [139–141].

However, the delivery of miRNAs to specifically regulate target genes is still the greatest challenge to be overcome [142] and to achieve this, two details need to be clarified. First, miRNAs undoubtedly have high specificity and low immunogenicity, but their biological function and the mechanism by which they regulate fibrosis still need to be further understood. Second, the liposome molecular synthesis process is still relatively complex; moreover, although the inorganic nanoparticle preparation method is simple and these nanoparticles have good biocompatibility and stability, their biodegradability remains an issue, and their metabolic kinetics still need to be clarified in vivo [143, 144].

With the developments in nanomaterials and biomedicine, as well as the clarification of the mechanism underlying miRNA-mediated fibrosis, the development of nanocarriers with different structures and functions is expected to solve the problems of effective miRNA delivery. The safety, efficiency, and stability of miRNA nanodelivery for fibrosis treatment is expected to be improved.

Declarations

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Consent to Participate Not applicable.

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Authors' Contributions LL and JS contributed to the conception. YG, HW, RL, JW, and TW performed the experiments. LL and JS wrote the original draft. All the authors have reviewed the final manuscript.

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