Asian Journal of Pharmaceutical Sciences 18 (2023) 100779

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/AJPS

Review

Insights on drug and gene delivery systems in liver fibrosis

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ARTICLE INFO

Article history:
Received 6 August 2022
Revised 12 January 2023
Accepted 12 January 2023
Available online 29 January 2023

Keywords:
Liver fibrosis
Gene delivery system
Nano drug delivery system
Cirrhosis
Targeted delivery system
Vector

ABSTRACT

Complications of the liver are amongst the world's worst diseases. Liver fibrosis is the first stage of liver problems, while cirrhosis is the last stage, which can lead to death. The creation of effective anti-fibrotic drug delivery methods appears critical due to the liver's metabolic capacity for drugs and the presence of insurmountable physiological impediments in the way of targeting. Recent breakthroughs in anti-fibrotic agents have substantially assisted in fibrosis; nevertheless, the working mechanism of anti-fibrotic medications is not fully understood, and there is a need to design delivery systems that are well-understood and can aid in cirrhosis. Nanotechnology-based delivery systems are regarded to be effective but they have not been adequately researched for liver delivery. As a result, the capability of nanoparticles in hepatic delivery was explored. Another approach is targeted drug delivery, which can considerably improve efficacy if delivery systems are designed to target hepatic stellate cells (HSCs). We have addressed numerous delivery strategies that target HSCs, which can eventually aid in fibrosis. Recently genetics have proved to be useful, and methods for delivering genetic material to the target place have also been investigated where different techniques are depicted. To summarize, this review paper sheds light on the most recent breakthroughs in drug and gene-based nano and targeted delivery systems that have lately shown useful for the treatment of liver fibrosis and cirrhosis.

1. Introduction

The liver is an integral part of the body and is truly considered a storehouse of the human body. Disturbances in normal liver functioning can lead to severe fatalities. Chronic liver disorders (CLD) are major liver-related complications comprised of persistent chronic parenchymal injury, long-lasting inflammatory response as well as prevailing liver fibrosis [1]. Liver fibrosis is characterized by progressive aggregation of a variety of extracellular matrix (ECM), comprising different types of collagens like I, III, and V, elastin, tenascin, and fibronectin [2,3]. Normal ECM in healthy liver consists of collagen IV and VI in space of Disse which are replaced during fibrosis [3]. Liver fibrosis can result from enduring liver damage while cirrhosis is the term used for prolonged liver fibrosis which is eventually converted to hepatocellular carcinoma and leads to liver damage and ultimately death. Moreover, there is a possibility of tumorigenic nodules forming directly from

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Peer review under responsibility of Shenyang Pharmaceutical University.

https://doi.org/10.1016/j.ajps.2023.100779

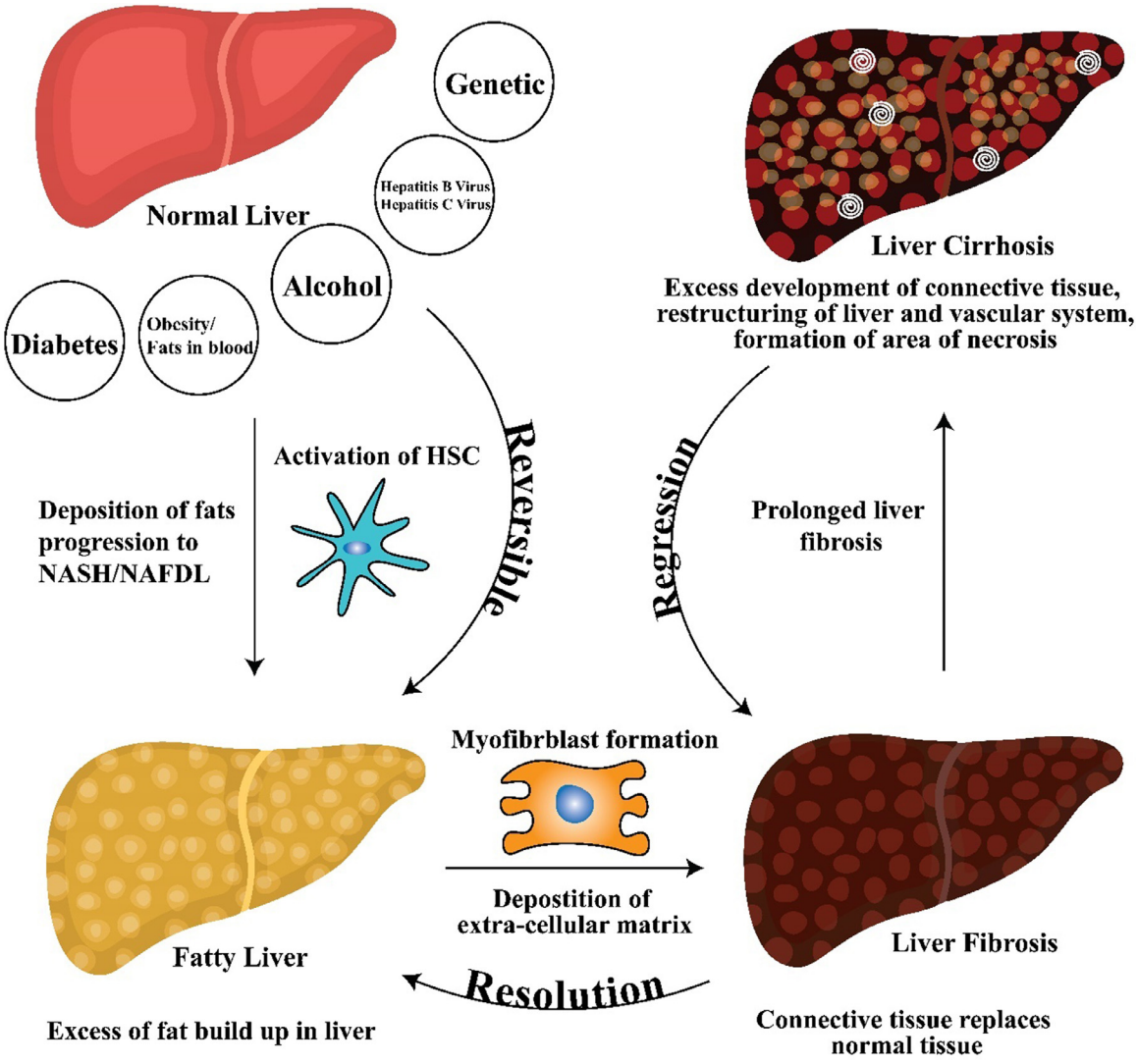

Fig. 1 – Schematic diagram of the progression of liver fibrosis.

hepatic fibrosis by generating a favourable microenvironment, however, the process is currently being debated by experts [4]. Unfortunately, the occurrence of liver inflammation and fibrosis has reached pandemic proportions over the world, resulting in cirrhotic patients or liver failure and millions of fatalities per year, which are observed in end-stage liver disease. Cirrhosis is a disorder in which scar tissue replaces normal tissue and regenerative nodules with fibrous bands form as a result of the damage [5]. Cirrhosis does not have any direct links. As seen in Fig. 1, cirrhosis develops across a number of phases.

Cirrhosis is the 11th most common cause of death the in the world accounting for almost 1 million death per year in the world including 170,000 deaths per year in Europe [6]. The USA has reported almost 0.27% which relates to 633,323 cases of cirrhosis amongst the population but the real-world scenario is different since many patients remain asymptomatic while some are diagnosed during the premortem stage. Along with this approximately 69% were oblivious about their liver damage [7].

Cirrhosis develops from chronic liver illness by inflammation, activation of hepatic stellate cells (HSCs), and subsequent fibrogenesis, angiogenesis, and parenchymal extinction lesions produced by vascular blockage. Histological signs of advanced fibrosis are taken into consideration today when trying to diagnose cirrhosis. Cirrhosis is characterized by capillarization of the sinusoids and perisinusoidal fibrosis, arterial thrombosis and obliterative lesions in the portal tracts and hepatic veins, and under-perfusion of the lobular parenchyma with resulting tissue hypoxia. The issue with treating fibrosis is a financial hardship, which most people cannot afford and which exacerbates the situation. In reality, the liver has a significant regenerating ability, so eliminating those toxic elements may prevent the future advancement of fibrosis and, in some circumstances, reverse the condition [5]. Fibrosis, on the other hand, is unavoidable when regeneration outnumbers destruction. After roughly 80 to 90 percent of the parenchyma has been damaged, clinical indications of liver failure generally occur.

1.1. *Pathophysiology of liver cirrhosis*

Pathophysiology of liver cirrhosis can vary depending on underlying aetiology which created a need for developing new specific treatments along with solving the new challenges arising in cirrhosis treatment. Hepatitis B virus (HBV) and hepatitis C virus (HCV) related chronic liver disease, diabetes, alcoholic steatohepatitis (ASH), and non-alcoholic fatty liver disease (NAFLD) or its advance form non-alcoholic steatohepatitis (NASH), as well as autoimmune diseases such as primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), autoimmune hepatitis (AIH) and genetic diseases including Wilson's disease, haemochromatosis, and α1-anti-trypsin deficiency, are common liver cirrhosis aetiology [8]. Amongst this HBV infections are considered the primary factor causing almost more than half of deaths due to cirrhosis [9].

The progression of liver fibrosis is based on multiple mechanisms including excessive wound healing response, inflammation/immune response, and excessive ECM deposition [10]. Subsequent progression of any normal liver into the cirrhotic liver can take approximately 15–20 years of continuous hepatic damage. This is the time when ECM accumulation has increased to six folds in any damaged liver. Major toxic agents like hepatitis virus, alcohol, or bile agents when exposed for a long duration to patients can induce apoptosis or hepatic cell damage. In response to this activation of hepatic myofibroblasts takes place by the infiltration of inflammatory/immune response which in turn activate transdifferentiation of HSCs into collagen-producing myofibroblasts [11]. HSCs are the major ECM-producing cells in the liver and are the most likely to induce and differentiate into hyperproliferative myofibroblasts. Portal myofibroblasts and bone marrow-derived myofibroblasts both have significant contributions to liver fibrosis.

HSC activation takes place either by reactive oxygen species (ROS) associated release or by fibrogenic mediators and is regulated by immune cells through cytokines and chemokines [12]. Hepatic fibrogenesis is developed by the stimulation of both inflammatory and pro-fibrogenic cells. Certain structural changes happen as a result of cirrhosis development, including extensive capillarization of the liver sinusoids and the formation of intrahepatic vascular shunts. Along with this, there can be endothelial dysfunction, which is characterized as a functional abnormality. Structural and functional issues can cause portal hypertension, which is a critical consequence of liver cirrhosis and causes complications such as hepatic encephalopathy, renal failure, and excess abdominal fluid [13]. Liver damage is persistent in cirrhosis, and one loses the ability to perform physiological tasks, ultimately resulting in liver failure. Moreover, the major risk associated is contracting hepatocellular carcinoma, which increases up to 80% in patients suffering from fibrosis or cirrhosis.

1.2. *Diagnosis of liver cirrhosis*

Liver diseases are asymptomatic until cirrhosis takes over which leads to clinical decomposition. There are a series of complications like sepsis, ascites, encephalopathy, and jaundice can occur. Currently, ultrasound imaging, CT, and MRI have shown promise in fibrosis diagnosis [14]. These are considered older techniques while new techniques include MR elastography, T1 mapping of the liver, and real-time elastography-Hi-RTE or SE. Generally, to confirm aetiology in uncertain cases liver biopsy can provide a solid diagnosis [15]. A liver biopsy is considered the best invasive detection method for cirrhosis. To date, hepatic venous pressure gradient (HVPG) is identified as the best diagnostic tool. Different pressure gradients indicate different aetiology. Imagining techniques can give a false or wrong diagnosis during early cirrhosis due to overt symptoms and patient negligence. So non-invasive markers of fibrosis are used extensively. Newly developed non-invasive serum fibrosis biomarkers include procollagen type III N-terminal peptide (PIIINP), YKL-40, sH2a + ALT, and tissue inhibitors of metalloproteinases (TIMP). There is considerable 80% increase is seen in the detection of advanced fibrosis or early cirrhosis but none is a liver-specific marker and thus they can be sometimes altered by hepatic inflammation. To measure accurate damage of liver fibrosis the AST/ALT, AST-platelet ratio index and fibrosis-4 (FIB-4) index are the most validated models [15]. In most cases, cirrhosis and even fibrosis can be prevented but due to the patient's lack of knowledge, it is diagnosed at a very late stage when significant lifestyle changes or aetiology is observed and hence prevention becomes difficult. Despite such advances, there is a further need to develop nanotechnology-based biomarkers to improve the diagnosis of liver fibrosis to target the diagnostic marker.

1.3. *Treatment of liver cirrhosis*

The primary therapy for cirrhosis should focus on eradicating aetiological causes that promote liver disease, which can eventually reduce portal hypertension, and decomposition, and increase patient compliance with cirrhosis, and this is presently the only effective antifibrotic method [16]. Even after curing aetiology patients having decompensated cirrhosis, generally do not have a consistent result. These studies have established that fibrosis regression in cirrhosis is feasible and may vary between patients as some patients may not be treatable. Fibrosis regression is a very slow process that can be achieved in as little as one year and can last up to five to seven years, depending on the patient's aetiology. Cirrhosis is now considered a systemic disease, due to its association with other organs in pathology. Current treatment includes rifaximin, long-term albumin (improve circulatory functioning), statins (anti-inflammatory action), and non-selective beta-blockers (targeting portal hypertension) [17]. New antiviral drugs such as entecavir, epclusa, and vosevi are tried for liver fibrosis [18]. These have demonstrated good effectiveness in treating liver fibrosis, but they possess problems such as toxicity, side effects, non-selective targeting, and poor pharmacokinetics. Another method is liver transplantation is the most effective treatment for cirrhosis, especially decompensated cirrhosis, and is usually used as a secondary or final treatment due to a

Table 1
Clinical Trials of various drugs for CLD.

Drug	Target	Disease condition	Phase	Clinical trial number
Selonsertib	Apoptosis signal-regulating kinase 1 inhibitor	NASH	3	NCT03053063
Hydronidone	Inhibitor of TGF-β	Chronic hepatitis B	2	NCT02499562
Hydronidone capsules	Inhibitor of TGF-β	Liver Fibrosis	3	NCT05115942
Pirfenidone	Inhibitor of TGF-β	Advanced cirrhosis	2	NCT04099407
Simtuzumab	LOXL2 antibody	NASH-related cirrhosis	2	NCT01672879
Candesartan	angiotensin II type 1 receptor blocker	Alcoholic liver fibrosis	2	NCT00990639
Losartan	angiotensin II type 1 receptor blocker	Liver fibrosis (F2-F3) with chronic HCV infection and NASH	4	NCT00298714; NCT01051219
Moexipril	angiotensin I blocker	PBC	2	NCT00588302
GR-MD-02	Galectin-3 inhibitor	NASH	2	NCT02462967
GB1211	Gelectin-3 inhibitor	NASH	1b/2	NCT04607655
Farglitazar (GI262570)	PPARγ agonist	Liver fibrosis with chronic HCV infection	2	NCT00244751
Tropifexor	FXR agonist	NASH	2	NCT03517540
Obeticholic acid	FXR agonist	PBC	3	NCT02308111; NCT01473524
MGL-3196 (Resmetirom)	thyroid hormone receptor-β	NASH	3	NCT03900429
Warfarin	Anticoagulant	Liver fibrosis	2	NCT00180674
Glycyrrhizin	Antioxidant	Chronic hepatitis C and F2/F3 liver fibrosis	3	NCT00686881

shortage of donor organs and there is also the problem of immunological rejection. Outcomes and effectiveness are normally excellent with exceptionally high survival time and rate.

Currently, there are several drugs in clinical trials for the prospective treatment of cirrhosis, as depicted in Table 1. Even after the potent activity of antifibrotic drugs, only minor effects are observed *in vivo*, so there is a need to develop effective antifibrotic treatment. A major problem is the lack of liver- or fibrosis-specific drugs, which leads to a vague accumulation of drugs in cells. This creates a need to develop targeted drug delivery systems for the liver. Currently, nanotechnology-based delivery systems are considered the best of all. They have been studied extensively and have a proven record of helping people with fibrosis. Furthermore, HSC's targeted delivery system plays an important role in fibrotic therapy but has problems such as poor solubility and low efficiency [19]. Researchers are working hard to find new ways to properly target and inactivate HSCs, which might help alleviate hepatic fibrosis. All of these approaches are believed to be traditional and have been studied for a long time. Gene therapy and gene delivery systems were born out of the necessity to replace these outdated procedures with something more current. Many diseases are claimed to have genetic treatments, and liver fibrosis is no exception. Loading gene material that has a significant influence on fibrosis onto a carrier and delivering it precisely into the liver is a new approach that has shown remarkable promise in the treatment of hepatic fibrosis. In this review, we summarize different types of delivery systems and recent advances in drug delivery in cirrhosis. This primarily includes nano-based drug delivery systems, targeted drug delivery systems, and the recently developed gene delivery system.

2. Nanoparticulate delivery system

Nanotechnology is by far considered the most reliable and advanced technique for drug delivery. Nanoparticles (NPs) are the widely used delivery system, and their design and application not only in treatment but also in diagnostic methods play a crucial role. They are broadly classified into organic and inorganic NPs based on their characteristics. They are further classified as metal oxide NPs (NO, SiO_2, Fe_2O_3), metal NPs (Ag, Au), lipid NPs, polymer NPs, and protein NPs [20]. Major benefits include good biocompatibility, the feasibility of incorporation, better bioavailability, reduced side effects, controlled release, active targeting, and less systemic toxicity. Certainly, along with the benefits, there are some drawbacks, including poor biodegradation and the accumulation of NPs in the liver, which causes more liver damage. Further, the benefits and drawbacks of various nanoparticulate systems are discussed in Table 2 [21–23]. For optimization of properties like surface charge, shape, and size in any nanocarrier, active targeting and passive targeting strategies are put to use. Particle size plays a significant role and normally affects pharmacokinetic properties like excretion, distribution, and circulation in tissues, organs, and cells. NPs with a diameter greater than 400 nm can be easily trapped in RES, while those smaller than 200 nm have increased circulation and reduced RES uptake. The process of NP uptake has been depicted in Fig. 2.

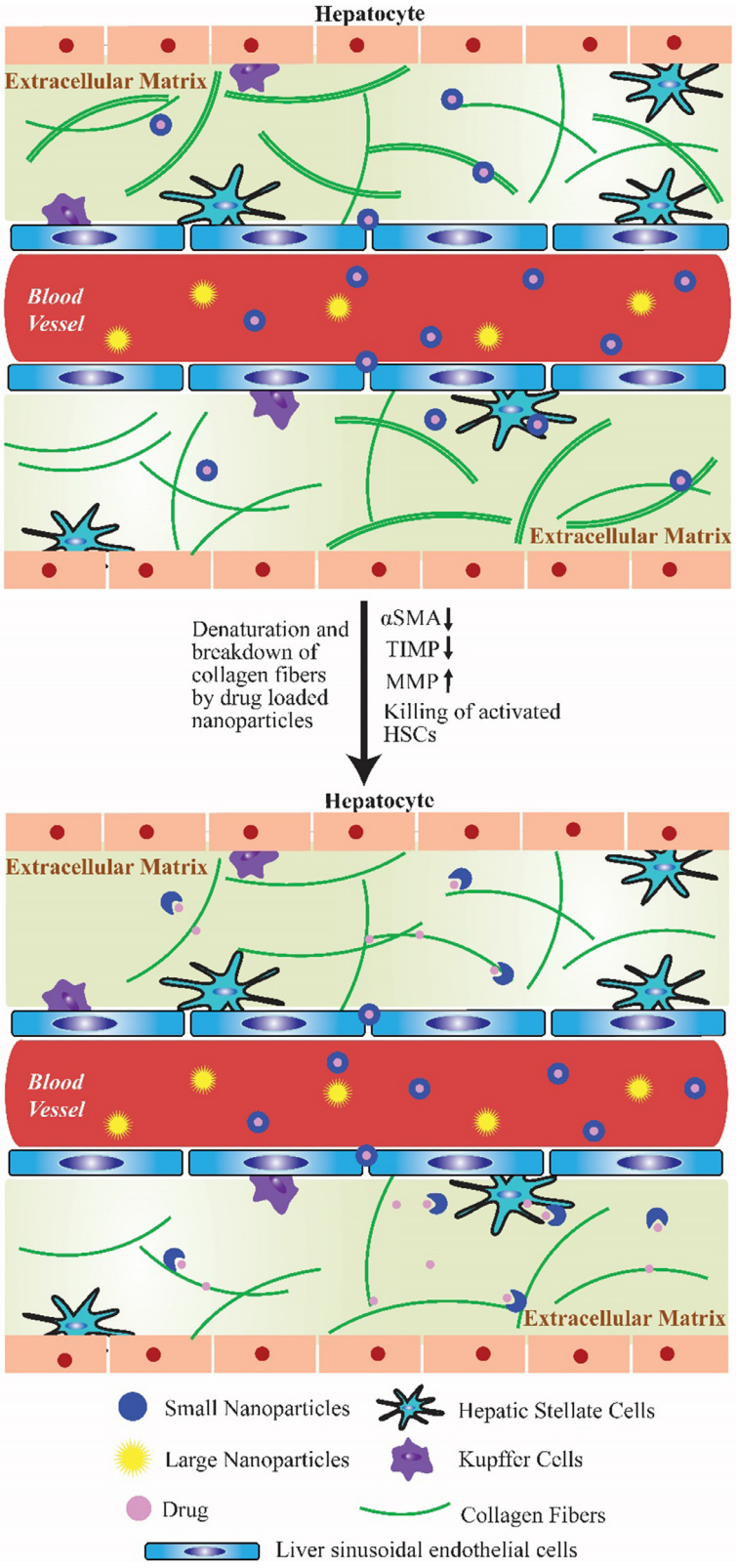

Fig. 2 – Different types of NPs are delivered into the Disse space through the liver sinusoids, thereby targeting HSCs and hepatocytes, and destroying collagen fibers, thus, resulting in the treatment of liver fibrosis.

Table 2
Advantage and disadvantage of multiple NPs.

NPs	Advantages	Disadvantage
Magnetic NPs	Compatible with high range of sizes	Low efficacy when magnet is removed
	Targeted delivery	
	Low cost of production	High cost
	Higher stability	Requires specialized microspheres and magnets
	Good biocompatibility	
AuNPs	Biocompatible	High Cost
	Particle size control	Non-biodegradable
	Easy to synthesis	High aggregation
Polymeric NPs	Excellent stability	Toxicity with I.V and non-biodegradable polymers
	Good biocompatibility	
	Low leakage of drug	
	Sustained release pattern	
	Flexibility of administration	
Liposome	Biodegradable and biocompatible	Low stability
	Compatible with both polar and non-polar agents	May be allergic
		High agglomeration
SLNs	Avoiding the use of organic solvents	Poor encapsulation
	Improved drug stability	Limited loading capacity
	Sustained drug release	Drug degradation
Carbon NPs	Ease of synthesis	Poor water solubility
	Large surface area	Non-biodegradable
	High protection of entrapped drug	Toxicity
	Control of particle size	Poor PK

2.1. *Inorganic nanoparticles*

This type is most well known for its special optical and magnetic properties, which are the core of developing a highly sustainable nano-drug delivery system. These properties exert a broad therapeutic benefit in the delivery of various drugs for treating fibrosis. In general, the tendency of inorganic NPs to accumulate in the liver, spleen, and lymph nodes is put to use [24]. Active phagocytosis responses from reticuloendothelial and Kupffer cells (KCs), which take up NPs, make the accumulation of NPs possible. This response can cause them to be retained in the body for about 6 months. This seems like a long time and would undermine the applicability of inorganic NPs because of possible immunogenicity, cytotoxicity, or organ dysfunction due to excessive accumulation. Despite these challenges, therapeutic applicability is dependant on chronic exposure to NPs at the site of action. Inorganic NPs are classified as metal oxide NPs and metal NPs, which possess a core with a protective layer on their surface. They are non-biodegradable and can accumulate in different parts of the body according to their sizes. A size between 20 and 250 nm is considered ideal for accumulation in the spleen and liver. The convenience of changing shapes is also one of the key features of inorganic NPs. Moreover, selective biodistribution and the continual build-up of inorganic NPs can trigger liver-specific therapy without disturbing other non-targeted tissues. Silica NPs (SiO_2NPs), titanium dioxide NPs (TiO_2NPs), and gold NPs (AuNPs) have supported this notion *in vivo* studies due to their physical characteristics and route of administration [25–27]. There are reports suggesting an extensive accumulation of these NPs in the liver compared to other organs. Inorganic NPs have demonstrated advances in diagnosis and treatment strategies. They are put to work as contrast agents in MRI and CT, and these NPs are generally made up of iron oxide and ferrites [28]. Superparamagnetic iron oxide nanoparticles (SPIONs) are the most utilized NPs in MRI [29]. Magnetic properties attributed to maghemite (Fe_3O_4) and magnetite (-Fe_2O_3) enable SPIONs to align with the magnetic field during MRI. Furthermore, an *in vivo* study was conducted in which dextran-stabilized SPIONs (D-SPIONs) were used for liver fibrosis detection. The size of SPIONs (12 nm) was modified with high molecular weight dextran, which yields a particle of about 50 nm. It was found that there was a 55% decrease in pixel intensity. This increased the difference between fibrotic tissue and the rest of the hepatic tissues at the fibrosis stage. Despite this, they cause particle buildup and deterioration of SPIONs, which include hazardous by-products. SPIONs can still be considered promising contrast agents for the diagnosis of cirrhosis if proper research is carried out.

AuNPs are preferably used for nano-drug delivery, and they help regulate cellular processes. AuNPs also possess an anti-inflammatory effect, as proved by a study in which chlorogenic acid (CGA)-induced AuNPs (CGA-AuNPs) were

prepared. CGA possesses anti-inflammatory and antioxidant effects, which will help reduce the inflammatory response. *In vivo* and *in vitro* tests were conducted on mice to check anti-inflammatory efficacy, and the result suggested a decrease in proinflammatory cytokines [30].

The most commonly found fibrosis is CCl_4-induced liver fibrosis, and plenty of NPs are used for its treatment. This notion has been supported by various *in vivo* studies, including AuNPs [31], manganese oxide NPs (Mn_3O_4NPs) [32], Cerium oxide NPs (CeO_2NPs) [33], and Iron oxide NPs (IONPs) [34]. CCl_4-induced liver fibrosis is very common, and studies suggest that it can be greatly reduced by downregulating HSC and KCs using silymarin-coated AuNPs. An *in vivo* study was conducted on male Wistar rats, and the results exhibited a marked decrease in several fibrosis markers. Furthermore, after the completion of treatment, there was also a reduction in α-smooth muscle actin (α-SMA) expression, which indicated a decreased fibrosis level [31]. Citrate-functionalized Mn3O4NPs administered *in vivo* to mice have shown a better protective effect against CCl_4-induced chronic hepatitis, and this is generally mediated through their pH-dependant anti-oxidant activity [32]. Treatment for CCl_4-induced liver fibrosis was also suggested by a study involving CeO_2NPs. Hepatic and systemic effects of CeO_2NPs were evaluated in an *in vivo* study and administered intravenously in rats for about 8 weeks over a prolonged period. mRNA expression of inflammatory cytokines and mediators for oxidative stress was observed and identified a marked regression in hepatic fibrosis levels. Moreover, after treatment, histology examinations such as macrophage infiltration, α-SMA expression, and apoptotic studies were carried out, which exhibited further reductions in inflammation, portal hypertension, and apoptosis [33]. IONPs are also proven effective treatments for CCl_4-induced liver fibrosis. The effect of SPIONs on CCl_4-induced hepatic injury was studied in a mouse model. This experiment was done by conjugating fibroblast growth factor 2 (FGF2) with SPIONs. FGF2 helps inhibit overexpressed HSCs and does the same by primarily interacting with FGFR1. FGF2 faces problems like a poor systemic half-life and instability, leading to enzymatic degradation. *In vitro* results suggested that the therapeutic efficacy of FGF2 in transforming growth factor-β (TGF-β) activated HSCs can be greatly improved when treated with FGF2-SPIONs. Moreover, an *in vivo* study suggested an increased efficacy in CCl_4-induced hepatic injury in contrast to standalone GF2, which showed less or no effect in treatment [34]. The liver and spleen are the chief organs where IOPNs are accumulated and also where oxidative stress can begin.

SiO_2 is also another widely used inorganic NP. They have a large void volume, and a fixed surface area, and are stable and biocompatible. They can provide profibrogenic effects by easily entering KCs and HSCs. A study was conducted in which SiO_2NPs were developed and administered intravenously to 8-week-old ICR mice. Activation of the TGF-β1/Smad3 signalling pathway occurred, which leads to hepatic fibrosis and helps in the induction of cellular apoptosis and oxidative stress [35]. Treatment for thioacetamide-induced liver fibrosis was shown in an *in vivo* study containing zinc oxide nanoparticles (ZnONPs). The result showed inhibition in hepatic lipid peroxidation, growth in antioxidant protection, and a decline in inflammation levels [36].

2.2. *Organic nanoparticles*

Biodegradable and nonbiodegradable are two major categories of organic NPs. Biodegradable NPs include lipid-based NPs (LNP) and polymer-based NPs, while non-biodegradable NPs consist of carbon NPs [37]. Drug molecules, nucleic acids, antibodies, and specialized targeted moieties can be efficiently delivered using liposomes and numerous polymers. Stimuli-sensitive nanoparticulate systems have gained much popularity in the treatment of liver fibrosis. Organic nanoparticles have an edge in terms of better encapsulation efficiency and a high loading capacity, along with good biocompatibility, increased bioavailability, and decreased systemic side effects and toxicity. They are considered the primary choice in the therapy of cirrhosis when a sustainable site-specific delivery system has to be developed.

LNP has established itself as an emerging field for treating liver fibrosis. LNPs mainly consist of stable nucleic acid LNPs (SNALPs), liposomes, and solid lipid NPs (SLNs) [23]. LNPs are non-viral vectors used not only for therapeutic agent delivery but have also emerged in gene delivery owing to their ease of formulation and high versatility. Simple surface modification properties enable targeted drug delivery in LNPs. Their composition, particle size, and surface characteristics help in the evaluation of *in vivo* studies. Liposomes are one of the major LNPs, and there is a growing research interest in both preclinical and clinical stages for site-specific delivery in liver fibrosis. They are potent carriers, and liposome-based delivery is considered the best amongst its peers for carrier-mediated delivery in liver fibrosis. Prominent properties include entrapment of both hydrophilic and hydrophobic drugs, bioavailability, biocompatibility, and low toxicity. This in contrast comes at a high price for production, low solubility, and chances of having a leakage, which hinders its further development [23]. The therapeutic potential of liposome-carried drug delivery was proven by several researchers, in whom delivery of dexamethasone incorporated into liposomes in mice showed a marked reduction in liver fibrosis as well as liver inflammation. A reduction in T cells in the liver and polarization of hepatic macrophages were evident [38]. In another research project, naringenin (Nar)-loaded albumin self-modified liposomes (NaAlLs) were prepared and evaluated. Nar is a specific Smad3 inhibitor that blocks the TGF-β/Smad3 signalling pathway. The albumin adjusted to the outer layer of NaAls and incredibly diminished the accumulation of liposomes, drug leakage, and stability of liposomes. More importantly, the take-up of NaAlLs by enacted HSCs was 1.5 times higher than that of Nar-stacked liposomes (NaLs), recommending that NaAlLs explicitly enhanced targeting of activated HSCs via albumin and SPARC-dependant pathways. True to form, NaALS was more successful in treating liver fibrosis [39].

SLNs are another candidate for the delivery of drugs in the treatment of cirrhosis. Because of their different features from other NPs, SLNs have evolved quickly. They are distinguished from other carriers by their regulated drug release and increased drug content as compared to other NPs. SLNs are known for their high biocompatibility and the ability to include both hydrophilic and hydrophobic drugs, as well

as genes. Owing to their lipidic outer surfaces, more complex lipid-based drugs can be easily encapsulated. Recently, bioinspired and reconstituted from natural LDLs, cationic solid lipid nanoparticles (CSLNs) were prepared and applied to target specific systemic delivery connective tissue growth factor siRNA (siCTGF) for treating cirrhosis. A nuclease-resistant stable nano-complex containing siRNA was created and was well absorbed into cells, resulting in targeted gene silencing in the presence of serum with minimal cytotoxicity. An intravenous injection of the CSLN/siCTGF complex was given, which in turn targeted the liver. The target-specific transport and accumulation of CSLN/siCTGF complexes in the liver tissues were validated in a bio-distribution investigation using fluorescence bioimaging and single-photon emission computed tomography (SPECT). An *in vivo* study suggested that changes in collagen content and pro-fibrogenic markers such as tumour necrosis factor alpha (TNF-α), TGF-β, interleukin-6 (IL-6), and CTGF were associated with a remarkable improvement in pathophysiological symptoms [40]. Despite their superior qualities, SLNs have not been thoroughly explored in clinical trials for the treatment of liver fibrosis.

Polymeric NPs are the second-most widely used biodegradable NPs. Researchers are becoming interested in polymeric NPs for the delivery of drugs, nucleic acids, and other therapeutic components. For the treatment of liver fibrosis and cirrhosis, several polymeric-based NPs have been shown to transport drugs and other therapeutic compounds to distinct liver cells. They are considered safe, effective, biocompatible, and biodegradable. Recently, curcumin-incorporated polymeric NPs were prepared and evaluated against CCl_4-induced subacute hepatotoxicity in Wistar rats. PLGA and PVA NPs were prepared as polymers and stabilizers, respectively, having sizes less than 220 nm. After giving the NPs orally, it was observed that curcumin-loaded NPs give much stronger hepatoprotection than curcumin, according to behavioural observation, biochemical analysis of serum using Erba test kits, and histological inspection of the liver of experimental animals [40]. HSC activation, inhibition, or control of inflammatory cytokine release by HSCs might be an effective treatment for liver fibrosis or cirrhosis. An *in vitro* study was conducted that included docetaxel (DTX) incorporated into pegylated carboxymethylcellulose for selective targeting of activated HSCs and abrogating their fibrogenic properties. Furthermore, in a therapeutically relevant hepatic cell carcinoma (HCC) model associated with underlying liver fibrosis, these NPs reduced CCl_4-induced hepatic fibrosis and decreased HCC development [41].

Biodegradable NPs have demonstrated their ability to target, but non-biodegradable NPs aren't far behind. C_{60} fullerenes have recently received a lot of attention as a potential treatment for liver fibrosis. Antioxidant, anti-inflammatory, and high-accumulation qualities are believed to be present [42]. They protect hepatocytes and inhibit fibrogenesis, according to a study that included an *in vivo* evaluation of C_{60} fullerene on an experimentally generated liver fibrosis model [43]. Carbon nanotubes (CNTs) are another possibility that has been investigated. CNTs may easily infiltrate various tissues, causing hepatic cell necrosis and macrophage destruction in certain livers [44]. Recent studies have discovered that chiral single-cell walled CNTs may be readily employed in multi-drug and gene delivery. They've also shown promise in terms of reducing liver fibrosis by targeting inflammation. This approach has been demonstrated to be effective in the treatment of NASH [45]. Nanomicelles have been popular as drug delivery carriers in recent years due to their tiny particle size, good stability, and wide drug encapsulation range [46]. Hepatic fibrosis patients may benefit from them. To show its effect on hepatic fibrosis, silibinin-loaded hyaluronic acid (SLB-HA) micelles were prepared and evaluated. The *in vivo* testing was done on a liver fibrosis-induced Sprague–Dawley rat model. It was demonstrated that HA micelles had a more selective absorption in HSC cells, leaving the rest of the cells unaffected. It clearly showed increased liver targeting efficiency in hepatic fibrosis. Furthermore, SLB-HA micelles demonstrated the ability to selectively kill activated HSCs while also having a great anti-hepatic fibrosis impact *in vivo* and a considerable sustained release effect, as well as good biological safety and biocompatibility [47]. Such progress in the pre-clinical phase of NP-based delivery systems has resulted in the creation of clinical trials of organic NPs, as shown in Table 3.

3. Targeted drug delivery

Drugs intended to treat fibrosis should concentrate largely on activated HSCs since they primarily release ECM. Drugs can be linked to carrier molecules that are tailored for selective absorption by HSCs to achieve HSC-specific uptake. Such carriers may be attracted to receptors expressed on activated HSC. Many delivery systems have shown promise in antifibrotic treatment, and drugs transported by them have demonstrated a significant antifibrotic impact *in vivo*. Using the autocrine and paracrine functions of numerous cytokines and chemokines, HSCs can extensively act on KCs, hepatocytes, liver sinusoidal endothelial cells (LSECs), and immune cells [48]. TGF-β1, insulin-like growth factor I (IGFI), platelet-derived growth factor (PDGF), ROS, and endothelin-1 are some of the mediators that might activate dormant HSCs. Despite advancements in fibrosis treatment, there are still issues since HSCs make up just 5%–8% of total liver cells, making it difficult to reach them with antifibrotic drugs in fibrotic livers. Furthermore, increased ECM and the closing of endothelial fenestrae prevent antifibrotic drugs from reaching the liver. The limited space of Disse also reduces antifibrotic drug delivery to HSCs. The effective treatment of liver fibrosis requires targeted delivery of therapeutic drugs to HSCs. In inactivated HSCs, several receptors are overexpressed, including the type I retinol-binding protein (RBP) receptor, the platelet-derived growth factor receptor (PDGFR), synaptophysin, the insulin-like growth factor-II receptor (IGFIIR), and the cluster of differentiation 44 (CD44), as shown in Fig. 3. Several ligands have been developed particularly for the delivery of antifibrotic drugs, and the ligand-receptor binding process makes it simple to reach the target cells. Different phases of HSC have been depicted in Fig. 3. This section summarizes a few HSC-specific indicators that might be used to deliver antifibrotic drugs to fibrotic livers with precision

Table 3
NP based delivery system under clinical trials for liver related diseases.

NPs	Drug	Disease condition	Phase	Clinical trial number
LNP	BMS-986,263 LNP delivering small interfering RNA designed to degrade HSP47 Mrna	Advance Hepatic Fibrosis	2	NCT03420768
LNP	ND-L02-s0201 Vitamin A-coupled LNP Containing siRNA Against HSP47	Hepatic Fibrosis	1b/2	NCT02227459
Polymeric	PEG-Interferon Alfa combined with Tenofovir Disoproxil Fumarate	HBV related Liver Fibrosis	4	NCT04640129
Polymeric	BMS-986,036 Pegbelfermin (PEGylated sfibroblast growth factor 21)	NASH and Liver Cirrhosis	2b	NCT03486912
Polymeric	SCH 54,031 PEG-Intron plus Rebetol treatment	Chronic HCV with Liver fibrosis	3	NCT00049842

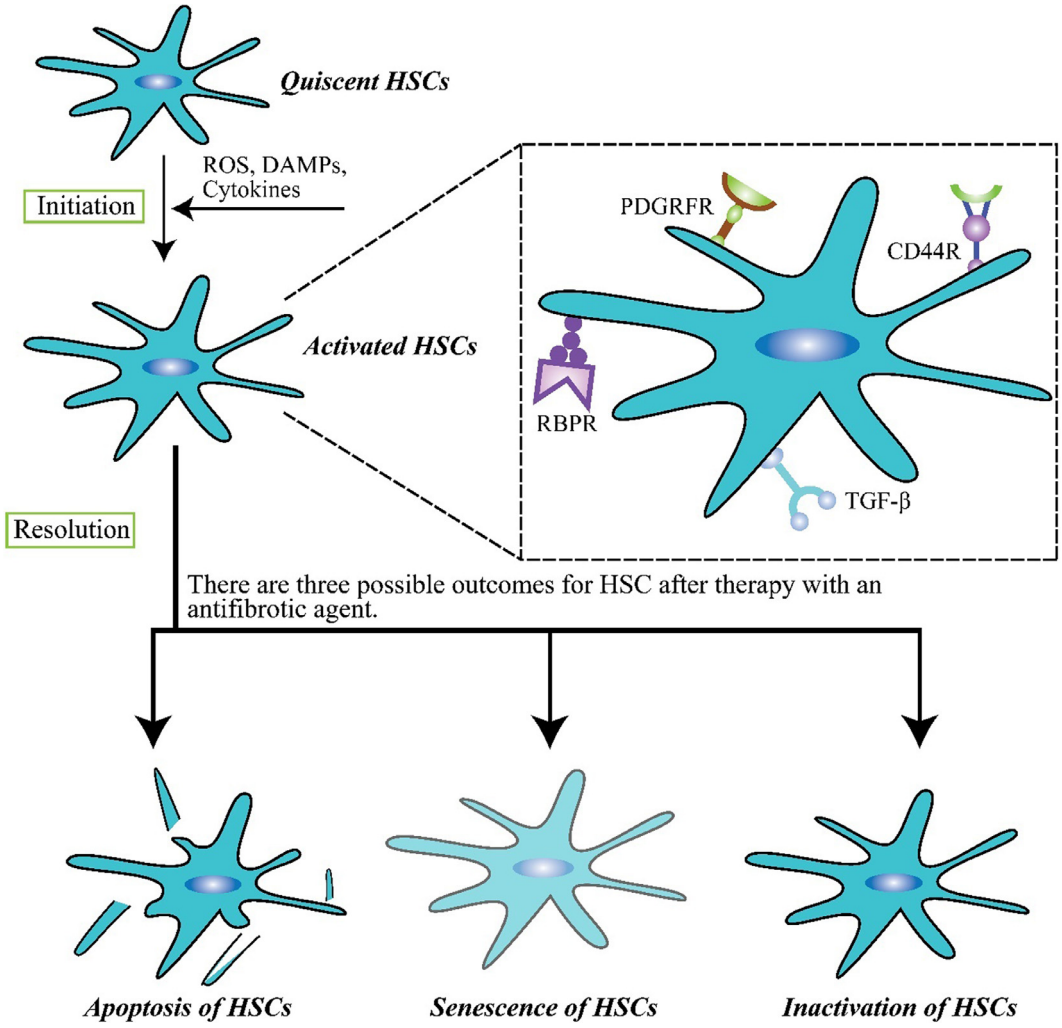

Fig. 3 – The activation and resolution of HSCs. Following liver injury, the activation of HSCs is triggered by a number of signals, including damage-associated molecular patterns (DAMPs), ROS, and cytokines and chemokines generated by injured hepatocytes. Quiescent HSCs transdifferentiate to their activated HSCs during the initiation phase. Several receptors are expressed during the activation phase of HSCs. Following the antifibrotic therapy when the damage has subsided, the HSCs go through apoptosis, senescence, or return to an inactive state that is more sensitive to repeated damaging stimuli.

3.1. *Retinol binding protein (RBP)*

The liver is known as the storehouse of retinol. Hepatocytes absorb freshly absorbed vitamin A from food, which is then carried in chylomicron leftovers via blood circulation. Retinol is then transferred to HSC, which stores vitamin A. HSCs store retinyl esters, which make up about 80 percent of the total retinol in the body, in their cytoplasmic lipid droplets. HSCs are known for their ability to store vitamin A. They play an important role in retinoid metabolism and regulation. HSCs govern the dynamic equilibrium between the accumulation and mobilization of systemic liver retinol deposits. Circulating retinol is linked to plasma RBP. Furthermore, active HSCs absorb vitamin A more effectively than dormant HSCs. As a result, ligands for HSC-specific delivery, such as vitamin A and RBP, have been developed [49].

Novel albumin and RBP fusion-based technology were developed to deliver RBP to HSCs. To fuse albumin with RBP, domain III of albumin was employed. R-III was the name given to the whole system, and this fusion was tested *in vivo* on HSCs. They inhibited retinoic acid (RA) signalling, which is important in the activation of HSCs. The anti-fibrotic effects of R-III and albumin were then reduced by RA receptor agonists and retinaldehyde dehydrogenase overexpression, respectively. Furthermore, *in vivo* research on rats revealed that CCl_4-induced liver fibrosis was reduced by using R-III protein fusion [50].

In another investigation, silibinin was employed as the active ingredient in lipid NPs containing retinol. In this research, the nanocarrier's biodistribution in real time after IV administration was studied in an *in vivo* rat model. It was found that NPs collected quickly in the liver and spleen. The inclusion of retinol in NPs allows for active targeting of the liver via the RBP. The surface charge of NPs was also considered, and it was discovered that the negatively charged formulation containing retinol had better absorption and retention in the liver than the other formulations. The addition of silibinin to NPs reduced lung deposition while increasing liver absorption. Retinol-loaded anionic nanocarriers provided greater liver-specific selectivity. To maximize hepatic targeting, the formulation of the lipid NPs must be optimized [51]. Another approach based on NPs was tested *in vivo* on a rat fibrosis model caused by CCl_4 and bile duct ligation (BDL). The system consists of retinol-conjugated polyetherimine (RcP). RBP-4 was recruited as one of the system's key protein components. RBP was discovered to bind retinol and route the antisense oligonucleotide-laden RcP carrier to HSC, which is important in the advancement of hepatic fibrosis. The NP binds to RBP in the serum and is taken up by HSCs exclusively through the RBP receptor. The NPs successfully decreased type I collagen expression in the liver [52].

3.2. *Platelet-Derived growth factor (PDGF)*

During fibrosis, PDGF can easily trigger fibroblast proliferation. PDGF is important for the onset and development of hepatic fibrogenesis. They are dimeric and made up of A and/or B chains. Their isoforms bind to two structurally distinct receptors and act on target cells. It affects HSC migration, proliferation, and survival via binding to PDGFR. PDGFR is up-regulated in activated HSCs in animals and human fibrotic livers [53]. The PDGF- α receptor binds to both the A and B chains with great affinity, however, the PDGF-β receptor binds only to the B chain. On activated HSCs, PDGFR- β is drastically overexpressed, and its expression is substantially greater than on other PDGFR-β positive cells [54]. Rapid PDGFR-β induction is a key feature of HSC activation. As a result, numerous PDGFR-β targeted delivery strategies for antifibrotic drugs have been studied.

To further comprehend PDGFR-β targeting, a novel natural cyclopeptide called destruxin A5 was used, which has demonstrated inhibitory properties in PDGF-BB-induced PDGFR-β signalling in a BDL-induced mice model. Destruxin works differently than conventional tyrosine kinase inhibitory mechanisms, owing to its non-binding ability to the ATP-binding pocket of PDGFR-β. Several methods showed that destruxin A5 preferentially targets the PDGF-B/PDGFR-β interaction interface to inhibit this signalling. Furthermore, the inhibitory impact of destruxin A5 on PDGF-BB/PDGFR-β signalling was validated using *in vitro, ex vivo*, and *in vivo* models, in which destruxin A5 successfully reduced the amount of liver fibrosis [55].

Dihydroartemisinin (DHA), an active molecule derived from the Chinese herb artemisinin, was evaluated in a BDL-induced liver fibrosis Sprague-Dawley rat model. DHA may have alleviated liver fibrosis by targeting HSCs via the PDGF-R/ERK pathway. In the fibrotic rat liver, there was an improvement in the liver's histological architecture and less collagen deposition. DHA has been shown *in vitro* to suppress the growth of HSCs. Along with them, the cell cycle was halted during the S checkpoint, which was accomplished by modifying multiple cell-cycle regulatory proteins. Furthermore, DHA lowered the protein expression of collagen, α-SMA, α1 (I), and fibronectin, which has been linked to interference with the PDGFR-β-mediated ERK pathway. This suggests that DHA has the potential to be a therapeutic antifibrotic drug for the treatment of hepatic fibrosis [56].

In a recent *in vivo* study, genetic models with varied PDGFR activity for the treatment of human cirrhosis were evaluated [57]. The effect of either PDGFR-β deletion or constitutive activation on the CCl_4 or BDL-induced mouse model was investigated. Deregulated pathways were identified and their relationship with prognostic gene profiles in human cirrhosis was assessed using genome-wide expression profiling from isolated stellate cells that expressed or lacked PDGFR-β. PDGFR-β depletion in HSCs showed a reduction in liver fibrosis, whereas activation accelerated the process. Furthermore, in HCV-related cirrhosis, genomic profiling indicated ERK, AKT, and NF-B pathways, as well as a component of a previously reported 186-gene prognostic signature, as being downstream of PDGFR-β in HSCs. It was also noted that the PDGFR-β signature was not connected with the formation of HCC in the human population, but was strongly associated with a worse outcome in HCV cirrhosis. This clearly states that PDGFR-β is a critical modulator of hepatic damage and fibrogenesis *in vivo* and that it plays a role in human cirrhosis prognosis [57].

PDGFR-β is undeniably a competent receptor for inducing fibrosis regression, however, PDGFR-α signalling has also been linked to liver fibrosis, albeit to a lesser level. To demonstrate the influence of PDGFR-α, researchers used a CCl_4-induced cirrhotic mouse model. The involvement of PDGFR-α in the expression of HSCs was investigated. The findings demonstrated that overexpression and activation of PDGFR-α are required for liver fibrosis and that inhibiting PDGFR–α specific signalling pathways in HSCs might give therapeutic advantages to patients with cirrhosis [58]. The expression of both receptors increases during liver fibrogenesis, with PDGFR-α being generated by myofibroblasts in fibrous septa. They are also highly upregulated in HSCs during fibrosis. PDGFR-α colocalization in CCl_4 and BDL-induced mice was studied. Using the PDGFR-α specific inhibitory monoclonal antibody olaratumab, researchers investigated the effect of PDGFR on proliferation, profibrotic gene expression, and migration in primary human HSCs. Olaratumab was effective in reducing HSCs and preventing cell migration. Phospho-specific studies have indicated that olaratumab reduces phosphorylation of extracellular signal-regulated kinase 1 and attenuates PDGFR-α activation in response to PDGF-BB. All of this supported the idea that PDGFR signalling plays a unique role in HSC proliferation and migration. Furthermore, it showed that inhibiting PDGFR signalling can affect the aetiology of hepatic fibrosis [59].

PDGF signalling is a key driver of liver fibrosis when used together. PDGFR-β signalling, in particular, appears to be a viable target for anti-fibrotic treatment. However, we are unaware of any clinical trials focusing on PDGF in the treatment of liver fibrosis.

3.3. *Cellular targeting*

3.3.1. *Liver sinusoidal endothelial cells (LSECs)*

LSECs make up the wall of the hepatic sinusoids and account for 15–20 percent of liver cells [60]. LSECs are a permeable barrier that allows tiny or soluble molecules to go back and forth between the blood and the space of Disse, the space between hepatocytes and LSECs where HSCs are stored [60]. LSECs also collaborate with hepatocytes and HSCs to regulate protein, lipid, and glucose metabolism [61]. When liver damage occurs, LSECs produce several angiocrine signals to maintain liver homoeostasis by balancing liver regeneration and fibrosis. In the normal liver, LSECs are maintained by nitric oxide (NO), which is induced from LSECs by the paracrine synthesis of vascular endothelial growth factor (VEGF) from hepatocytes and HSCs. Upregulation of VEGF by LSECs during liver damage stimulates the production of hepatocyte growth factor (HGF), which leads to liver regeneration. Chronic injury, on the other hand, activates the fibroblast growth factor receptor 1 (FGFR1) in LSECs, resulting in a profibrotic angiocrine response to HSCs and fibrosis [62]. As liver fibrosis proceeds, damaged LSECs lose fenestration with the basement membrane, leading them to become capillarized, which is one of the primary pathological abnormalities of liver fibrosis. Capillarized LSECs lose their hepatoprotective properties as well as their ability to inactivate HSCs, leading to intrahepatic vasoconstriction and liver fibrosis [61]. Activated HSCs shrink and store a large quantity of ECM in the Disse space, resulting in endothelial fenestration loss and LSEC failure. HSCs and LSECs are therefore linked and stimulated in a self-perpetuating cycle, which contributes to hepatic fibrosis. Experiments have shown that cross-talk between cultured LSECs and HSCs is important in controlling each other's phenotypic. HSCs quiescence is maintained by healthy LSECs, but it is lost by capillarized LSECs [60,63].

This alteration in endothelial phenotype that accompanies capillarization and progressive fibrosis has been connected to changes in signalling via the Hedgehog gene family [64]. and cause vasoconstriction and increased intrahepatic vascular resistance because of decreased NO generation by LSECs. Recent studies have revealed that modifications in LSECs are required to restore normal functioning. A therapeutic has been proposed in the form of a soluble guanylate cyclase activator, which restores fenestrations and has been connected to fibrosis regression in early cirrhosis induced in a rat model using thioacetamide [63]. Modifications are also conceivable by utilizing GATA4-mediated cellular reprogramming to restore the differentiated phenotype of LSECs and facilitate fibrosis resolution [65]. Similarly, treatments that restore normal Hedgehog signalling enhance capillarization regression and the emergence of fenestrations, implying a potential mechanism for fibrosis reversal and lipid transport restoration.

According to current research, properly differentiated LSECs operate as fibrosis gatekeepers by preserving HSC quiescence and supporting the reversal of activated HSCs to quiescence through a VEGF-stimulated-NO-dependant/independent route [66]. In NASH, LSECs are key mediators of liver inflammation, and as a result, they induce hepatic fibrosis. LSECs, for example, overexpress VAP-1 during inflammation, which is directly engaged in HSC activation in addition to its pro-inflammatory roles in NASH. VAP-1 inhibition or deficiency reduces liver fibrosis in mice fed a methionine- and choline-deficient diet or a high-fat diet. The absence or inhibition of functional VAP-1 decreased inflammatory cell migration to the liver and mitigated fibrosis [67]. Given the cellular importance of LSECs in HSCs, LSEC protection might be an effective method for slowing the advancement of fibrosis.

3.3.2. *Immune cells*

Immune cells play a variety of roles in the liver, including maintaining homoeostasis, antimicrobial defence, and correct metabolism. Immune signalling pathways have been found to play a key role in hepatic fibrogenesis in several investigations. Immune cells create inflammatory cytokines or chemokines in response to inflammatory or metabolic stimuli, which influence liver damage directly or indirectly. Importantly, the aetiology of liver fibrosis is influenced by bidirectional interactions between immune cells and HSCs. Immune cells play an important role in inducing HSC death, and numerous pathways have been implicated in the apoptosis of activated HSCs. Some of these pathways are caspase 3 and caspase 8, death receptor-mediated pathways (FAS or TRAIL), overexpression of pro-apoptotic proteins (such as p53 and BAX), and activation of liver-associated NK cells and NKT cells [68].

The functional cells in immune cell targeting are Natural Killer (NK) and Natural Killer T (NKT) cells. The majority of liver lymphocytes are NK cells, which are found in the sinusoid of the liver. Many studies have shown that in mice and humans, NK cells have an antifibrotic activity by destroying activated HSCs and generating interferon-γ (IFN-γ) [69]. Activation of the liver-associated NK cell population may reduce liver fibrosis by eliminating activated HSCs. This occurs as a result of direct cell contact with HSC natural killer group 2, member D (NKG2D) receptors. This results in the downregulation of cytotoxic peptides into the cytoplasm, or in the activation of the death receptors FAS, TNFR1, and TRAIL receptors on HSCs by their respective ligands, triggering apoptosis via a caspase 3-dependant and caspase 8-dependant pathway [68].

Another important NK activating receptor produced by NK cells is NKp46. The absence of NKp46 can directly aggravate hepatic fibrosis by killing primary mice and human HSCs. A recent study was undertaken to demonstrate the anti-fibrotic action of the NKp46 receptor. CCl_4-induced NKp46-deficient mice were used for *in vivo* and *in vitro* testing. The expression of the NKp46 ligand was marked in mice and human HSCs using fusion proteins made up of the extracellular regions of murine and human NKp46 receptors linked to human IgG1. It was discovered that HSC expresses NCR1, a ligand of the NKp46 receptor. This NCR 1 reduced liver fibrosis *in vivo* and destroyed mouse HSC in an NCR1-dependant way *in vitro*. Human HSC has been proven to express a ligand for the human NKp46 receptor, and death of human HSC is NKp46 dependant [70].

Ultimately, this research showed that, in addition to NKG2D, NKp46/NCR1 plays an important role in the suppression of liver fibrosis. This demonstrates that fibrosis can be better controlled by modulating NKp46 activity.

Another type of cell is NKT cells which are a diverse subset of T cells that exhibit characteristics of both T cells and NK cells. Many of these cells identify the antigen-presenting molecule, which binds both self and foreign lipids and glycolipids. In general, NKT cells are classified into two subpopulations: type I NKT cells (express semi-invariant T cell receptors (TCRs) and account for 95 percent of liver NKT cells) and type II NKT cells (which express more varied TCRs). NKT cells have a variety of roles in liver fibrogenesis. NKT cells, for example, reduce liver fibrosis by reducing HSC activation, but they can also induce liver fibrosis by increasing liver inflammation and damage [71]. A recent study was undertaken to investigate the therapeutic effectiveness and probable mechanisms of interleukin (IL) 30 as antifibrosis treatment in mice liver fibrosis models to examine the influence of NKT cells. Immunophenotyping and deletion experiments revealed that IL-30 attracts NKT cells to the liver, considerably reducing activated HSCs and ameliorating liver fibrosis. Furthermore, flow cytometric and antibody-mediated neutralization investigations revealed that liver NKT cells up-regulate the NKG2D ligand and interact with the NKG2D ligand, RA early inducible 1, as well as positively activated HSCs, to alleviate liver fibrosis. In addition, the adoptive transfer of liver NKT cells in T-cell-deficient animals has shown a reduction in fibrosis after administering with IL-30 [72].

There are primarily two types of T cells: helper T cells (Th) and cytotoxic T cells. Th, as the name implies, 'assist' other immune system cells. T helper cells are classified as Th1, Th2, Th3, Th17, or TFH. These T helper cells can aid in the reduction of HSC activation. This impact was demonstrated in a study that employed CCl_4-induced mice to assess the effect and frequency of Th22 cells, Th17 cells, Th1 cells, and IL-22. Recombinant IL-22, in combination with an increase in the frequency of Th cells, can reduce HSC activation and down-regulate inflammatory cytokine levels, ultimately alleviating hepatic fibrogenesis [73]. A similar study was carried out on C57BL/6 mice to assess the involvement of CD4+ T lymphocytes in the prevention of fibrosis. Th17 and Th22 levels climbed and then declined. In mice, hepatic infiltration of Th17 cells is required for the onset of NASH and the development of fibrosis, and this represents an infiltration of Th22 cells. Th22 cells defend against NASH-induced liver fibrosis [74].

3.3.3. *Kupffer cells (KCs)*

KCs, also known as stellate macrophages and Kupffer–Browicz cells, are specialized cells found in the liver's sinusoids that adhere to the endothelial cells that make up the blood vessel walls. KCs make up approximately 80% of the total macrophages of the body [75]. They are connected to the sinusoidal endothelium layer and can be triggered by circulating blood stimuli, secreting cytokines such as TGF-β1, TNF-α, MCP-1, chemokines (*e.g.*, CCL_3 and CCL_5), and other soluble mediators, evoking a physiological response in other liver cells [76].

Currently, there is significant evidence that KCs govern the activation of HSCs bilaterally. In a recent study, Lignans, the principal bioactive components of Schisandra chinensis that have an anti-liver fibrosis effect, were utilized to identify the targets in KCs and discover the underlying anti-fibrotic mechanism using RAW264.7, co-cultured HSCs, and CCl_4-induced liver fibrosis $CB2^{-/-}$ mice. According to *in vitro* and *in vivo* studies it was found that Schisandrin B alleviated CCl_4-induced liver fibrosis in KCs by inhibiting the nuclear factor-kappa B (NF-kB) and p38 mitogen-activated protein kinases (MAPK) pathways and targeting the CB2 receptor [77]. The inflammasome NOD-like receptor protein 3 (NLRP3) was shown to be expressed in schistosomiasis-induced liver fibrosis (SSLF). In a recent study, the NLRP3 inflammasome inhibitor MCC950 was tested in a BALB/c mice fibrosis model. The influence of SSLF phenotype on liver fibrosis, KCs, and HSCs was investigated. In result, it was found that both the NLRP3 inflammasome and liver fibrosis-associated markers were elevated in primary KCs and HSCs separated from infected mice. Interestingly, the NLRP3 inflammasome was found to be implicated in liver fibrosis caused mostly by KCs. As a result, it was proposed that inhibiting NLRP 3, which is mostly generated by KCs, might be a viable route in avoiding SSLF [78].

KCs are a prominent source of ROS and have been linked to the development of liver fibrosis in NASH and ASH patients with chronic hepatitis. Targeting NASH and ASH can help prevent fibrosis, as evidenced by a recent study that used KCs to target nano antioxidants. Polythiolated and mannosylated human serum albumin (SH-Man-HSA) was employed, which

has mannose receptor C type 1 as its target, which is expressed on KCs. The study used a NASH and AASH-mimickinghepatic fibrosis mouse model, and the results indicated that SH-Man-HSA significantly increased survival and inhibited liver fibrosis in the experimental model. Furthermore, SH-Man-HSA reduced hepatic oxidative stress, reducing the number of apoptotic cells. These findings suggest that repeated treatment of the KC targeting nano antioxidant, SH-Man-HSA, improves liver fibrosis in mice by decreasing oxidative stress and a portion of inflammation, and may have a therapeutic impact against NASH and ASH [79]. Recent research has also suggested that treating NASH-induced fibrosis with proinflammatory and profibrotic phenotypic changes found in iron-rich KCs can aid in fibrosis reduction. Iron accumulation is more in KCs, and this increases the KCs due to which it activates transcription factors MiT/TFE. In murine and human NASH, activation of MiT/TFE transcription factors in KC results in the formation of crown-like shapes (CLSs). CLSs are structures in which KCs surround and scavenge the detritus of deceased hepatocytes, causing inflammation and fibrosis. Furthermore, iron chelation was shown to reduce liver fibrosis in a mouse NASH model [80].

Activated KCs are also known to release cytokines and chemokines such as TGF-β, TNF-α, and IL-1β that have been linked to HSC activation [81]. These variables, together with the creation of ROS and lipid peroxidation, stimulate HSCs to transdifferentiate into myofibroblasts and lead to liver fibrosis.

3.4. *TGF-β*

TGF-β is triggered from ECM deposits after acute and chronic liver damage and expressed to activated HSCs, where it plays an important role in attenuating fibrosis in the liver. Several cell types, including KCs, release them. HSC is an important TGF target because they are activated and transdifferentiated to myofibroblasts, which involves the elimination of intracellular vitamin A droplets, the adaptation of a fibroblast shape, and the formation of a contractile, proliferative, and migratory phenotype [82]. TGF-β signalling is regarded as the primary fibrogenic mechanism that promotes HSC activation and ECM formation [83]. There is a total of 33 members in the TGF-β family including TGF-βs, activins, and bone morphogenetic proteins. There are generally three isoforms of TGF-β proteins namely TGF-β1, 2, and 3. TGF-β1 is the most commonly and intensively studied isoform in hepatic fibrogenesis [83].

Despite strong evidence of reversibility, there is currently no direct antifibrotic therapy for liver fibrosis, and it is a fact that removing the fibrosis-inducing components is often difficult, if not impossible, especially in the growing CLD such as NAFLD and ASH. Thus, direct antifibrotic treatment aims to try to reduce scar development or accelerate scar clearance processes [48]. Because TGF-β plays such an important role in liver fibrogenesis, various research has focused on its suppression to find antifibrotic treatments that have a significant impact. Previous approaches to target TGF-β include using soluble TGF-β type II receptor (TβRII), which competes for TGF-β binding with membrane-anchored TβRII [84]. TβRII antifibrotic effectiveness was recently investigated in a study that employed truncated TβRII with His-SUMO fusion protein. His-SUMO-tTβRII was the name given to the fusion. A CCl_4-induced liver fibrosis C57BL/6 mice model was used for the *in vivo* investigation. The results revealed that fibrosis-related Collagen I and α-SMA protein expression was inhibited. Along with these effects, tβTRII inhibited the phosphorylation of SMAD2/3, which partially suppressed TGF-β1-mediated signalling [85]. In line with the previous findings, a novel PDGFβR-binding peptide BiPPB modified tTβRII (BiPPB-tTβRII) was synthesized by cleaving SUMO-BiPPB-tTβRII using SUMO-specific protease. *In vivo* experiments on CCl_4-induced liver fibrosis in mice revealed BiPPB-tβTRII specific targeting of HSCs and fibrotic liver tissue, as well as dramatically inhibiting the protein levels of fibrosis-related genes in TGF-β1-induced HSC-T6 cells. The findings showed that the target protein BiPPB-tRII, with its high specific fibrotic liver-targeting capacity and increased anti-fibrotic action in liver fibrosis, might be a promising treatment for liver fibrosis [86].

Many natural substances with antifibrotic action have been shown to interact with the TGF-β signalling pathway at some point. As previously said, schisandra chinensis is a Chinese herb that improves liver fibrosis and liver protection, however, the active component that aids in antifibrotic activity has yet to be identified. To discover this molecule, a schisandra chinensis extract called schisantherin A (SCA) was tested against a thioacetamide-induced mice liver fibrosis model. SCA was shown to greatly improve the degenerative alterations in liver tissue caused by thioacetamide by lowering the expression of α-SMA and collagen1A1. SCA suppressed the proliferation and activation of HCS-T6 cells produced by TGF-β1, lowered TNF-α and IL-6 levels, and inhibited TAK1 activation induced by TGF-β1, as well as the production of MAPK and NF-$_k$B signalling pathway-related proteins. This indicated that SCA can reduce liver fibrosis by blocking TGF-β1 driven TAK1/MAPK and signalling pathways [87].

Another research looked at the antifibrotic impact of a novel pharmacological extract called levo-tetrahydropalmatine, which operates by modulating the TGF-β1/Smad pathway. *In vivo* testing was performed using C57 mice models of CCl_4 and BDL-induced hepatic fibrosis. The findings demonstrated that levo-tetrahydropalmatine reduced liver fibrosis by preventing the development of ECM. *In vivo* and *in vitro*, levo-tetrahydropalmatine suppressed HSC activation and autophagy by regulating the PPAR/NF-$_k$B and TGF-β1/Smad pathways [88].

Similarly, apigenin a dietary flavonoid has been screened in CCl4 and BDL-induced C57 mice models to confirm its antifibrotic effect. Apigenin was shown to lower serum liver enzyme levels, restrict ECM production, prevent HSC activation, alter the balance of MMP2 and tissue inhibitor of metalloproteinase (TIMP1), diminish autophagy-linked protein expression, and inhibit the TGF-β1/Smad3 and p38/PPARα pathways. Apigenin was shown to relieve liver fibrosis by suppressing HSC and autophagy via the TGF-β1/Smad3 and p38/PPARα pathways [89]. Caffeine is one more such compound that has benefits in hepatic fibrosis and a study was conducted to evaluate its effect on NASH. Thioacetamide induced rat model was used and results stated that Caffeine may lower the hepatic collagen level and the fibrotic area in the liver. Caffeine slowed the advancement of liver fibrosis by lowering TGF-β, CTGF, and

α-SMA expression as well as suppressing MAPKs activation and Smad3 phosphorylation. As a result, caffeine may be a promising treatment option for fibrotic liver disorders [90].

Unfortunately, antifibrotic therapies based on TGF-β signalling have still not been tested on humans. The reason for this is the pathway's intricacy, as well as the highly dynamic, context and cell-type-dependant result of TGF-β signalling. Another reason for this is the cytokine's pleiotropic actions. Then also new branches, regulatory mechanisms, and transcriptional targets of the TGF-β pathway are constantly being identified, some of which are associated with HSC activation and liver fibrogenesis.

3.5. CD44

Hyaluronic acid (HA), a nonsulfated glycosaminoglycan found in the ECM, is made up of repeating disaccharides of $\alpha-1,4$-D-glucuronic acid and $\beta-1,3$-N-acetyl-D-glucosamine [91]. HA interacts with CD44, the major HA receptor on the cell surface, to perform a variety of biological actions such as cell proliferation, differentiation, migration, survival, angiogenesis, chemical release, and protease docking at the cell membrane [92]. HA has been exploited to deliver antifibrotic drugs to HSCs. For example, in one research, curcumin-encapsulated hyaluronic acid–polylactide nanoparticles (CEHPNPs) were created and utilized to treat liver fibrosis. CEHPNPs might bind to CD44 and be successfully absorbed by endocytosis, releasing curcumin. Thus, CEHPNPs could improve drug efficiency while simultaneously targeting activated HSCs. It was demonstrated by a liver biopsy that CEHPNPs dramatically lowered serum aspartate transaminase/alanine transaminase (ALT/AST) and inhibited tissue collagen synthesis and cell proliferation. Finally, the benefits of improved biosafety and good therapeutic impact indicate that CEHPNPs have a high potential for treating hepatic fibrosis [91].

CD44 inhibition may potentially help with NASH-related liver fibrosis. A recent investigation on $CD44^{-/-}$ mice was undertaken to demonstrate the role of CD44 in the development of NASH and liver fibrosis. Fibrosis was shown to be significantly reduced in $CD44^{-/-}$ mice. CD44 deficiency increased M2 polarisation while drastically lowering macrophage activation. Finally, it was proposed that blocking CD44 partly corrects NASH, making it a possible treatment option [93]. NASH contributes largely to hepatic fibrosis, hence identifying a precise treatment target is essential. Recently, research was conducted on the role of CD44 in the development of NASH-related hepatic fibrosis by utilizing Male Fischer rats. HA was produced and deposited in liver tissue. CD44 mRNA and protein expression were both considerably elevated. CD44 protein was found in part of the bile duct epithelium, which was surrounded by HA, and these bile duct lesions corresponded to the region of hepatic fibrosis. As a result, CD44 expression in the bile duct epithelium may be a therapeutic target for NASH-related liver fibrosis [94]. NO has been shown to successfully promote HSC apoptosis. However, there is an issue with the systemic administration of NO, which is inefficient and might result in serious consequences such as hypotension. The researchers employed a newly developed NP with a core encased in mesoporous silica shells and modified with HA. HA molecules identify and bind to CD44 proteins, which are overexpressed on activated HSCs. Exposure to a 980-nm NIR laser can result in the efficient release of NO within the HSCs. As a result, CD44 receptor-targeted release strategies may be more effective therapeutic options for treating hepatic fibrosis [95].

Given that targeted delivery of antifibrotic drugs has shown promising outcomes in several animal research, we believe that using HSC-specific ligands in antifibrotic therapies might significantly boost their success rate in clinical trials.

4. Gene therapy and delivery systems

Gene therapy based on the liver has been utilized to reduce or block the production of defective genes, deliver therapeutically active genetic materials, and avoid allograft rejection [96]. Based on probable alterations in the liver during fibrosis, genetic modification seems to be able to alter myofibroblasts and enhance liver regeneration [96]. The prospective applicability of gene therapy procedures to human cirrhosis will be dependant on the effective and tissue-specific delivery of therapeutic genes to fibrotic livers [97]. Gene therapy goes through several stages before arriving at personalized gene delivery. In the gene therapy procedure, the defective gene is first identified and characterized; next, the healthy and natural gene is taken and mass manufactured; and last, this gene is inserted into cells and given to target cells, as depicted in Fig. 4. There is also an anti-fibrotic mechanism illustrated in Fig. 4 that works by lowering ECM depositions and inactivating HSCs by producing variations in protein levels.

Several approaches, including clustered regularly interspaced short palindromic repeats (CRISPR), transcription activator-like effector nucleases (TALEN), and zink finger nucleases (ZFN), are widespread genome editing techniques that are regarded as advances in genetic engineering and medical sciences [96]. CRISPR is a group of DNA sequences present in the genomes of prokaryotic species like bacteria and archaea, whereas ZFN and TALEN contain two domains that attach to DNA and can edit any DNA. For gene therapy and targeted delivery, there are now various delivery mechanisms and innovative approaches available. DNA- and RNA-based delivery, ultrasound-targeted microbubble-mediated gene therapy, matrix metalloproteinase (MMP) targeted gene therapy, and vector-based delivery systems are amongst them. They are sophisticated, and precise, and can benefit the regression of liver fibrosis.

4.1. DNA and RNA

4.1.1. DNA

Nucleic acids are a significant source not only for understanding the underlying foundation of human existence but also for the creation of a new class of treatments. One of the major benefits of DNA-based drugs over currently available pharmaceuticals is their selective identification of molecular targets and pathways, which results in great specificity of action. Plasmids, which contain transgenes,

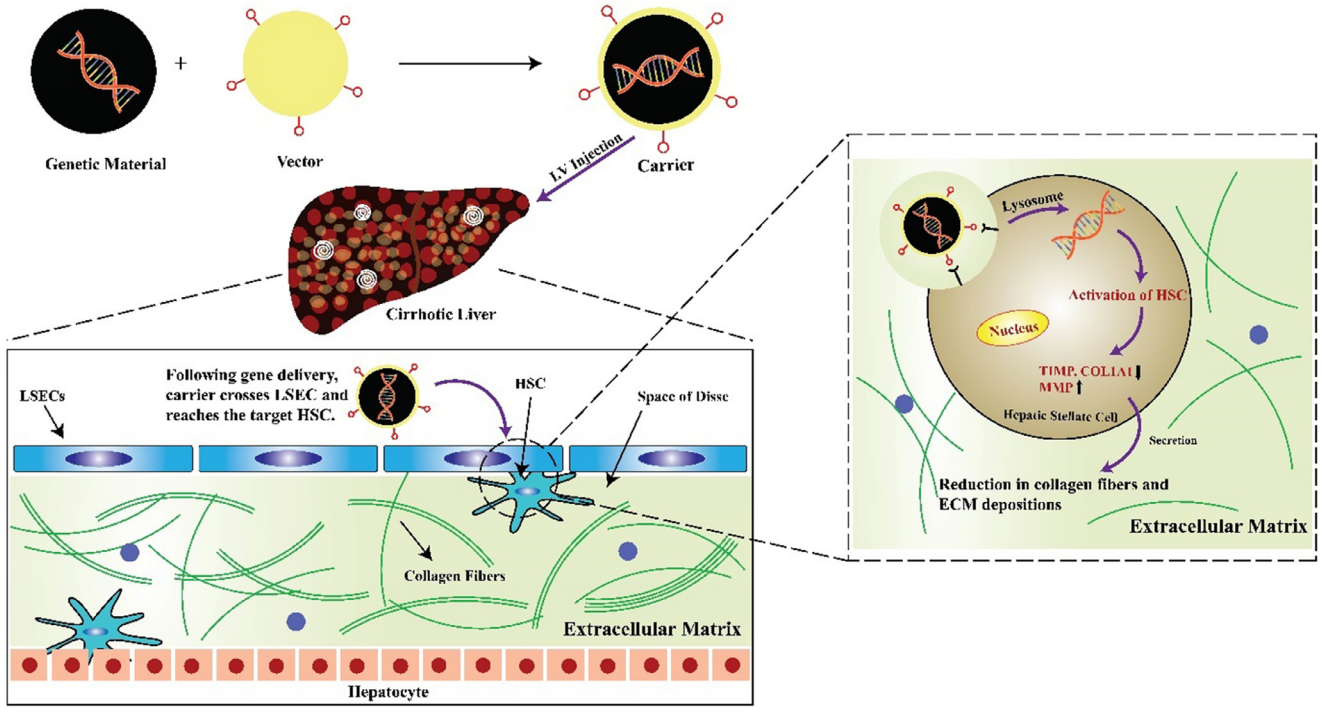

Fig. 4 – The use of gene-based targeted delivery in the treatment of liver fibrosis. It was observed that the expression of liver fibrosis-related genes was downregulated in a synergistic manner to alleviate liver fibrosis.

antisense and antigene oligonucleotide applications, aptamers, DNAzymes, and ribozymes are examples of DNA-based therapies [98]. High transmission efficiency, nontoxicity with high immunity, good biocompatibility, pharmaceutical formulation stability, and ease of manipulation are some of the desired qualities of a DNA delivery system for medicinal uses [96].

Antisense oligonucleotides (ASOs) are small single-stranded DNA segments that, when internalized by the cell, can selectively suppress the production of a single protein. They suppress gene expression at the post-translational stage. ASOs attach to their target mRNA by reverse complementarity and either limit translation by steric blockage of mRNA regions critical for translation or decay mRNA via RNase H, an endonuclease abundant in the cytoplasm that cleaves just the mRNA component of RNA: DNA fusions. As a result, each ASO has the ability to hybridize and destroy numerous RNA molecules. ASOs have been designed to have a high selectivity towards the liver. The uptake capacity of ASOs is between 50 and 95 percent following parenteral delivery. Hepatocytes, macrophages, and HSCs are primary cells, all of which are taken up by optimized ASOs [99]. The ability of ASOs to hybridize with mRNA is determined by their physicochemical and thermodynamic characteristics. To form a stable duplex with mRNA, a minimum of 12–15 bases are necessary. Chemical modification of ASOs may help to improve their stability, efficiency, and target specificity. Recent research has attempted to cure NASH-related liver fibrosis by employing ASOs. GalNAc3-conjugated ASO-mediated silencing of patatin-like phospholipase domain-containing 3 (PNPLA3), which is encoded with a 148 M protein sequence variant in a mouse model, can be beneficial in NASH. PNPLA3 ASO treatment can enhance all aspects of NAFLD, including liver fibrosis, and reduce the expression of a powerful intrinsic genetic risk factor, Pnpla3 148 M, thus opening the door to a precision medicine strategy in NASH [100]. The Signal transducer and activator of transcription 3 (STAT3) is a transcription factor implicated in the pathophysiology of liver fibrosis. STAT3 is thought to be a possible therapeutic target, although there are no particular therapeutic candidates for STAT3. In recent research, exosomes, like mesenchymal stem cells, were designed to transport ASO that targets STAT3. ASO-STAT3 demonstrated improved STAT3 targeting efficiency. ASO-STAT3 therapies dramatically improved liver function and decreased STAT3 levels and ECM deposition in mice with developed hepatic fibrosis. It improved the efficiency with which the liver functioned. These findings point to a new anti-fibrotic strategy based on direct targeting of STAT3 with exosomes that have immediate translational prospects [101].

Plasmid DNA is promising in safety and efficiency, but it has a relatively limited transfection capacity. To overcome the transfection issue, novel gene carrier NPs with low toxicity and high transfection efficiency were created and tested for possible biological functions using molecularly controlled release and transfection assays. The L-tyrosine polyurethane NP, which has proven therapeutic effects in liver fibrosis, was employed. The results showed that L-tyrosine polyurethane NPs encapsulated with plasmid DNA-linear polyethyleneimine had improved cellular uptake and high transfection efficiency in LX2 cell lines [102]. In recent research, CRISPR/ associated protein 9 (Cas9) plasmids were employed, which demonstrated their usefulness in complex

and varied genome editing scenarios. This plasmid was administered using high aspect ratio cationic gold nanorods. The study suggested how nanomaterials with a specific structure contribute to genome editing activity and proposes a new technique for the effective delivery of CRISPR/Cas9 plasmids. Intracellular delivery mediated by high aspect ratio cationic nanorods enables Cas9-mediated genome editing and dCas9-mediated transcriptional activation, as well as *in vivo* delivery of CRISPR/Cas9 plasmid-targeting. Fas-mediated by cationic gold nanorods effectively protects mice from liver fibrosis [103].

4.1.2. RNA

Several screening programs for liver fibrosis research and clinical trials have revealed a great number of coding RNA (mRNA) and non-coding RNA (miRNA & siRNA) in recent years. Treatment with mRNA, as opposed to gene therapy based on plasmid DNA, is a novel method that is still in its early stages [104].

RNA interference (RNAi) is a comparatively recent technique that uses small interfering RNAs (siRNAs) of 21–23 nucleotides, short hairpin RNA (shRNA), and micro RNA (miRNA) to precisely inhibit target genes [105]. Numerous membrane receptor signalling pathways, nuclear receptor signalling pathways, and transcription factors are dysregulated in HSCs as liver fibrosis progresses [106]. Utilizing mRNA for gene therapy has various benefits over using DNA. For starters, mRNA functions in the cytoplasm and is unaffected by the breach of the core membrane, which is the principal intracellular barrier for DNA gene therapy [107]. Second, because of its location, mRNA treatment does not need genomic integration, which reduces the risk of an internal mutation. Furthermore, mRNA gene therapy is more attractive since the manufacturing technique, raw material production, and mRNA product quality compared to DNA may all be readily tweaked [108].

SiRNA is a potential therapeutic candidate to reverse hepatic fibrogenesis due to its high specificity and effectiveness in downregulating genes related to liver fibrogenesis. siRNAs can specifically down-regulate target mRNA in a sequence-specific recognition way [109]. Recently, four siRNA therapeutics, ONPATTRO1 (patisiran) and GIVLAARI1 (givosiran), OXLUMO® (Lumasiran), and LEQVIO® (Inclisiran) were given FDA approval in 2018 and 2019, respectively [110,111]. Various siRNA delivery technologies, which include cationic liposomes, polymeric NPs, and micelles, have been developed in prior decades to deliver antifibrotic siRNA to activated HSCs [112]. Furthermore, because of the decreased perisinusoidal space and flow exchange in the fibrotic liver, ligands that may precisely target active HSCs have been found to improve siRNA delivery to the fibrotic liver [113]. Recently, a poly(rC)-binding protein 2 (PCBP2) siRNA has been found to reverse fibrogenesis in activated HSCs. A novel technique for fabricating a multicomponent nanocomplex employing a siRNA/peptide nucleic acid (PNA) hybrid instead of chemically linked siRNA has recently been developed, boosting the scalability and practicality of the siRNA nanocomplex for animal investigations on the CCl_4-induced mouse model. *In vitro* and *in vivo* results suggest that the siRNA nanocomplex has a controlled size, excellent serum stability, and high cellular absorption in activated HSCs. According to the findings, the siRNA nanocomplex effectively decreases the protein level of type I collagen and reverses liver fibrosis. As per the research, the nanocomplex efficiently distributes siRNA to the fibrotic liver and generates a powerful anti-fibrotic impact [114]. TGFβ silencing is most widely used as an anti-fibrosis therapy. This was proved in another research that aimed at curing CCl_4-induced liver fibrosis in a mouse model. There was the development of a new Cyclam-modified polyethyleneimine (PEI-Cyclam) capable of successfully delivering TGF-β siRNA. They inhibit the chemokine receptor 4 (CXCR4) for TGF-β silencing. PEI-Cyclam/siTGF polyplexes reduced inflammation, collagen deposition, apoptosis, and cell proliferation, therefore alleviating liver fibrosis [115]. Another study used vitamin A-coupled liposomes carrying siRNA HSP47 (VA-liposome siHSP47) to treat hepatic fibrosis in a dimethyl-nitrosamine-induced rat liver fibrosis model. The VA-liposome siHSP47 therapy increased hepatocyte DNA synthesis while also restoring impaired liver weight and normalizing albumin levels. Similarly, another CCl_4 rat model demonstrated the ability of the fibrotic liver to regenerate. VA-liposome siHSP47 therapy resulted in liver weight restoration and trans-differentiation of various cells [116]. As we can see, siRNA-based delivery systems for fibrosis have undergone significant development. As a result, sirnaomics has created a delivery platform based on peptides (Histidine-Lysine Co-Polymer) that are based on siRNA oligonucleotides and are currently under phase 1 clinical trial. They target COX-2, a pro-inflammatory and proliferative mediator, and TGF- β1, which regulates a variety of cellular processes, including proliferation. This drug has been found to inhibit TGF- 1 and COX-2, which are easily responsible for inducing apoptosis in human fibroblasts. Moreover, pro-fibrotic factors like α-SMA, Collagen 1 (Col1A1), and Collagen 3 (Col3A1) were downregulated in the cells after target genes were silenced [117].

MiRNAs are another form of therapy for the treatment of liver fibrosis, in addition to siRNA-based therapies. Because of their biological properties, miRNAs, which are composed of around 22 nucleotides of endogenous non-coding RNAs, have emerged as therapeutic agents [118]. RNA polymerase II transcribes miRNA genes into primary miRNAs, which are subsequently cleaved by RNase type III endonucleases to create double-stranded miRNAs. Finally, the double-stranded miRNAs are split, and one of the strands is integrated into the RISC complex, causing selective mRNA destruction. There are various kinds of miRNAs, including miR-29b, miR-150, miR-132, miR-122, miR-449, miR-335, miR-126, miR-19b, miR-101, miR-146a, miR-107, miR-449a, miR-200a, miR-214, miR-195, miR-15b, and miR-16, that have antifibrotic effects in the liver [118]. Recent research has shown that miR-130b-5p expression is highly up-regulated during HSC activation. It was also shown that there were distinct binding sites between miR-130b-5p and Sirtuin 4′s 3′ UTR (SIRT4). According to the findings, miR-130b-5p increased HSC activation by targeting SIRT4, which is involved in the AMPK/TGF-β/Smad2/3 signalling pathway. As a result, controlling miR-130b-5p might be a critical therapeutic approach for hepatic fibrosis [119]. Similarly,

several miRNAs have been discovered to be elevated during liver fibrogenesis. For an instance, miR-542–3p can cause hepatic fibrosis in mice by upregulating in CCl_4-induced hepatic fibrosis. Inactivated HSCs, miR-542–3p expression is enhanced. In contrast, antisense inhibitors can block HSC activation indicators such as α-SMA and collagen, as well as TGF-β signalling pathways, by downregulating MiR-542–3p. When these two were compared, it was shown that miR-542–3p downregulation inhibits liver fibrosis both *in vitro* and *in vivo*, indicating its potential as a new biomarker or gene therapy for hepatic fibrosis [120].

Targeting HSC lines LX-2 which are stimulated by TGF-β1 has proven beneficial in liver fibrosis. A recent study looked at the relationship between serum exosomes miR-574–5p and liver fibrosis, as well as the influence and mechanism of serum exosomes on HSC activation. The study was conducted on the HSC line LX2, and the findings show that considerably high levels of miR-574–5p were expressed in serum exosomes and were strongly linked with the expression of miR-574–5p, collagen deposition, and α-SMA expression in mice liver tissues during liver fibrosis. Also, Serum exosomes from cirrhotic patients were shown to have greater levels of miR-574–5p expression when compared to healthy participants. This demonstrates that during liver fibrosis, serum exosomes may activate HSC via the transfer of miR-574–5p to HSC [121]. Another study discovered the influence of miR-122–5p on HSC proliferation and apoptosis. LX-2 and LX-2 cells from the HSC line were employed for testing. MiR-122–5p expression was shown to be decreased in TGF-β1-stimulated LX-2 cells. Overexpression of miR-122–5p lowered collagen I and α-SMA mRNA and protein levels, hindered cell proliferation, and hastened cell death. Overexpression of miR-122–5p reduced HSC activation and fibrosis by decreasing the histone deacetylases tyrosine phosphorylation by the cellular-Abelsongene pathway [122].

Inhibition of the PI3K/Akt signalling pathway can minimize ECM deposition, limit the proliferation of HSCs, and encourage their death to achieve the goal of treatment. Recently, research on CCl_4-induced liver fibrosis in mice was undertaken to determine the effect of Idelalisib (PI3K inhibitor). TGF-β1 stimulated HSCs were employed to assess Idelalisib's antifibrosis activity. To investigate the mechanism through which Idelalisib suppressed PI3K, researchers found miR-124–3p and miR-143–3p. *In vitro* and *in vivo*, idelalisib dramatically increased miR-124–3p and miR-142–3p expression. Idelalisib also reversed the effects of the miR-124–3p inhibitor on the PI3K/Akt/FOXO3 asterisk pathway and caspase-3 [123]. During research it was found that increased levels of miR-195–3p block the expression of phosphatase and tension homologue deleted on chromosome 10 (PTEN), a negative regulator of the PI3K/Akt/mTOR signalling pathway in liver fibrosis, leading to HSC activation and proliferation and boosting the expression of profibrotic genes, such as -SMA and collagen I, in LX-2 cells, which promotes the buildup of fibrous ECM deposition in the liver, while knocking down miR-195–3p has the contrasting impact [124]. Several additional miRNAs may have a role in hepatic fibrosis, as listed in Table 4. Investigating miRNA-based molecular pathways may aid in the development of novel molecular targeted therapies for hepatic fibrosis treatment.

The translation of siRNA and miRNA-based treatments from the lab to patients is currently underway, with the FDA recently approving one siRNA. The targeted delivery of nucleic acid-based treatments such as siRNA, shRNA, and miRNA to target cells or tissues is a big difficulty. Numerous vector-based delivery methods for nucleic acid-based treatments have been developed *in vitro* and *in vivo* for a variety of disorders, including liver fibrosis.

4.2. *Vector gene delivery system*

Viral and non-viral vectors are two types of vector-based gene delivery systems. They are accurate, efficient, and have a high transmission rate.

4.2.1. *Viral vector*

The finest and most dependable carriers for gene transfer are viral vectors; these vectors have been changed in key genomic areas so that they cannot be duplicated and their immunity is improved. Viral vectors are created by modifying wild-type viruses. They are made replication incapable due to the removal of most or all of the viral genome while preserving the capacity to execute a single round of transduction to transfer the therapeutic genetic material to the target cell [140]. The appropriateness of a recombinant virus for liver directed gene therapy is determined by numerous parameters, including the infectious titre that can be established, the virus's capacity to infect nondividing cells, the effectiveness of integration into the host genome, the repeatability of administration, minimal immune responses to the virus and the transgene, and the safety of the vector system [141]. Retroviruses, lentiviruses, adenoviruses (Adv) or adeno-dependant viruses (AVVs), Simian Virus 40, and Baculoviruses are examples of viral vectors.

Retroviruses are amongst the most common viral vectors utilized in gene therapy. They ensure reliable transgene delivery into transduced cell offspring by incorporating complementary DNA into the host genome during their life cycle. They can transport the gene into target cells without causing any harm or illness. Lentiviral vectors are developed from human immunodeficiency viruses and are capable of infecting nondividing cells. The retrovirus family's lentiviruses are commonly utilized in gene delivery [140].

Interestingly, lentivirus miR-200a was employed to limit HSC activation and proliferation. The study was divided into many groups. The results indicated that the Lenti-NC group had decreased levels of α-SMA, TGF-β2, and Collagen I, whereas the Lenti-miR-200a group reduced infection development in the schistosomiasis liver fibrosis [142]. In a recent research role of (pro)renin receptor (PRR) was evaluated in hepatic fibrogenesis. To inhibit hepatic PRR expression, lentivirus-mediated PRR short hairpin RNA was adopted. Lentiviral vectors encoding PRR short hairpin RNA or complementary DNA from the α-SMA promoter were employed to knock down or overexpress myofibroblast-specific genes. The lentivirus encoded PRR contributes to liver fibrosis and HSC activation, and its inhibition reduces liver fibrosis via inactivating the ERK/TGF-β1/Smad3 pathway [143]. Lentiviruses have been extensively used in gene therapy. There have been few studies on the link between DACT2 and

Table 4
Various genes and carriers for delivery in liver fibrosis.

RNA type	Carrier	Gene	Target	Outcome	Refs.
mRNA	LNPs	HNF4A	Paraoxonase 1	Reduction in hepatic fibrosis through regulation of hepatic macrophage and HSCs	[125]
mRNA	LNPs	Human ABCB4	PFIC3	Clinical indicators such as inflammation, ductular response, and liver fibrosis were normalised.	[126]
mRNA	Adv	NDRG2	TGF-β1 and α-SMA	It can control TGF-β1 via the NF-$_k$B pathway and may be a unique therapeutic target for hypoxia-induced liver fibrosis.	[127]
mRNA	AAV	ACE2	HSC	ACE2 gene therapy lowers liver fibrosis and hyperglycaemia in diabetic NAFLD mice and has the potential to be used as a therapeutic for diabetic NAFLD humans.	[128]
miRNA	AAV serotype 8	miR-200c	SESN1	This prevented cholestatic liver fibrosis by inhibiting the IL-6/AKT feedback loop.	[129]
miRNA	Naked Gene	miR-494–3p	TRAF3	MiR-494–3p was shown to inhibit HSC proliferation and fibrosis in alcoholic hepatitis by inhibiting TRAF3.	[130]
miRNA	Naked Gene	MiR-92b-3p	CREB3L2	According to the findings, miR-92b-3p enhances HSC activation and hence the advancement of liver fibrosis through activating the JAK/STAT pathway via CREB3L2, giving a novel target for the diagnosis and therapy of liver fibrosis.	[131]
miRNA	Recombinant AAV serotype 8	miR-497	Smad7	The study found that miR-497 enhances liver fibrogenesis by targeting Smad7 to boost TGF-β/Smad signalling pathway transduction both *in vivo* and *in vitro*.	[132]
miRNA	Adv	miR-223	Gli2 and PDGFR a/b	Overexpression of miR-223 reduced Gli2 and PDGFR a/b expression in HSCs, reducing HSC activation and proliferation. This ultimately helped in ameliorating liver fibrosis	[133]
miRNA	Lentivirus	miR-129–5p	SOCS2	The findings showed that inhibiting NEAT1 might control hepatic fibrosis in ASH mice by increasing miR-129–5p and inhibiting SOCS2, hence slowing the progression of ASH.	[134]
miRNA	Lentivirus	miR-148a-3p	ERBB3 which are on HSC-T6 Cells	The study discovered that miR-148-a-3p controlled alcoholic liver fibrosis, as well as the survival and death of HSCs, via targeting ERBB3.	[135]
siRNA	AAV	shRNA	FOXA3	FOXA3 inhibition through siRNA or AAV delivery improved the fatty liver phenotype in mice. Which ultimately aided in NAFDL	[136]
shRNA	Cationic liposome	Toll-like receptors 4	Activated HSCs	TLR4 gene silencing decreased HSC activation and reduced liver fibrosis via NF-B transcriptional inactivation, pro-inflammatory cytokine production, and ROS generation.	[105]
siRNA	PA-Zn-CLD nanocomplex	siRNA of plasminogen activator inhibitor-1	KCs, ECM and HSCs	The targeting and penetration efficiency was considerably enhanced, which might lead to the development of a possible anti-fibrotic delivery system.	[137]
siRNA	mLNP	HMGB1-siRNA	Hepatic macrophages through mannose receptors	After the delivery NASH model mice's liver function recovered quickly after delivery, and hepatic steatosis restored to normal levels.	[138]
siRNA	Nanohydrogel particles equipped with ManNP	siRNA–ManNP	CD206 receptors which are expressed in M2-type macrophages	It was seen that siRNA and ManNP demonstrated high biocompatibility and vigorous absorption in fibrotic livers as measured by *in vivo* near infrared imaging, and they can be employed to alleviate liver fibrosis.	[139]

Hepatocyte nuclear factor alpha (HNF4A) N-Myc downstream-regulated gene 2 (NDRG2)Progressive familial intrahepatic cholestasis type 3 (PFIC3)sestrin 1 (SESN1)TNF receptor-associated factor 3 (TRAF3)Suppressor of cytokine signalling 2 (SOCS2)Receptor tyrosine-protein kinase erbB-3 (ERBB3)Forkhead box A3 (FOXA3)mannose residues on the surface (ManNP).

liver fibrosis. A recent study found that the DACT2 gene can prevent liver fibrosis. A lentivirus is utilized as a vector to build a lentivirus vector containing the DACT2 gene, as well as packaged DACT2 recombinant lentivirus and its control vector and delivered into CCL_4 induced fibrosis mouse model. The results demonstrated that DACT2 gene expression might suppress HSC-T6 cell activation and lower TGF-β1 expression [144].

Adv are non-enveloped, double-stranded DNA viruses that may infect both dividing and dormant cells. They can cause various types of infections in humans. The benefits of this group include relative protection (even weakened viruses cause minor respiratory infections), ease of manufacturing, simple to purify and condense even in large quantities, and the capacity to transport genes into quiet and proliferating cells. Recombinant adenoviral vectors are often created for gene transfer applications utilizing Adv5 and Adv1 along with adeno-associated virus (AAV) serotypes 2 and 5.

Recently, *in vivo* knocking down of Nestin which is an intermediate filament protein, has been linked to tissue homoeostasis during wound healing reactions and was observed in fibrotic mouse models. For the knocking down, an AAV6 carrying short-hairpin RNA targeting Nestin was employed. The result depicted that Nestin knockdown in AAV6-treated mouse fibrotic models reduced inflammatory infiltration, hepatocellular damage, and fibrosis severity [145]. In another study, the impact of IL-21 delivered by AAV on HBV was assessed. HBV-naive mice were exposed to exogenous IL-21 for a short period of time by injection of recombinant AAV expressing mouse IL-21 (AAV-IL-21). It has been observed that AAV-IL-21-injected mice eliminated HBV fast following the HBV replicon challenge [146]. Adenovirus injections are effective in animal models for targeting a certain receptor or gene. One such study employed injection of adenovirus overexpressing GATA4 through the tail vein in CCl_4-treated mice as a method to reverse the fibrosis. The investigation indicated that viral injections into the tail vein of mice efficiently reach HSCs. GATA4 inhibits Hif2 transcription via two conserved GATA sites. This aided in the regression of liver fibrosis [147].

4.2.2. *Non-viral vectors*

The majority of non-viral vectors are made up of polymeric or lipid particles that encapsulate and preserve genetic material in their interiors to allow them to enter the cell. Non-viral vectors are simpler to produce and can be scaled up industrially. They have lower immunogenicity, cytotoxicity, and mutagenesis than viral vectors, luring more researchers to investigate the promising delivery method and advance the field of gene therapy. Besides that, growing evidence supports their safety and efficacy for targeted therapy. By 2017, 33.6% of all vectors used in clinical trials were non-viral, with the diversity of NPs greatly increasing from 2012. Some non-viral vectors such as LNPs, lipid-calcium-phosphate nanoparticles (LCP NPs), lipoplexes, polymeric NPs, and inorganic NPs are employed in gene therapy for liver disease. Multiple LNPs are being studied and developed for use in cancer chemotherapy, mRNA vaccines (COVID-19 vaccines) to prevent virus infection, and gene-editing treatments for genetic diseases. The most popular nonviral vector for delivering nucleic acids is cationic LNP because it is simple to manufacture, can carry large payloads, has low immunogenicity, and can be made for multiple dosages. They interact electrostatically to bind with anionic nucleic acids, preventing nucleic acids from being degraded by nucleases while in circulation [148].

Non-viral vectors provide various benefits over viral vectors, including easy scalability, a long shelf life, low immunogenicity, theoretically infinite size of the genetic material carrier, and a superior safety profile. Non-viral vectors have limited capacity to achieve long-term transgene expression due to their poor efficacy at reaching the nucleus. In a recent research SPION decorated cationic micelle was used for miRNA delivery into rats. Vitamin A with a pH-sensitive Vitamin A-conjugated copolymer –polyethylene glycol–polyethyleneimine–poly (N-(N′,N′-diisopropylaminoe thyl)-poly(N-(N′,N′-diisopropylaminoethyl)-poly(N-(N′,N′-diisopropyla T-PBP (T-co-benzylamino) aspartamide is produced and assembled into SPION. The T-PBP micelle efficiently transports the miRNA to HSC and resulted in a synergistic antifibrosis effect via downregulating the expression of fibrosis-related genes [149]. The most basic method of non-viral vector delivery is by naked genetic material. This technique, however, is hampered by relatively poor effectiveness in cell entrance. Transposons are utilized to make irreversible changes to the cellular DNA. DNA sequences that migrate from one area of the genome to another are known as transposons. Transposon sequences are surrounded by terminal inverted repetitions that a transposase enzyme recognises and breaks, allowing them to be reinserted in new locations. As a result, transposon systems have been employed to introduce therapeutic sequences into host DNA [150]. Transposons originating from the Sleeping Beauty (SB) or piggyBac (PB) systems are currently the most promising gene therapy applications. According to the proposed investigation, the researchers created a hybrid vector system that combines recombinant AAV's high transduction efficiency with PB transposase-mediated somatic integration. The *in vivo* research on the murine model of PFIC3 has suggested that a single dosage of a hybrid vector given at birth resulted in life-long bile composition restoration, avoidance of biliary cirrhosis, and a significant reduction in carcinogenesis. This effective hybrid recombinant AAV-PB transposon vector method has the ability to mediate lifetime phenotypic correction and diminish tumorigenicity in progressive familial intrahepatic cholestasis type 3, as well as the potential for human clinical translation with additional development [151].

4.3. *Ultrasound-targeted microbubble-mediated gene therapy*

Given the benefits and drawbacks of non-viral and viral-based vectors, developing a delivery method that is reliably safe and efficacious for clinical use has been one of the most difficult tasks in gene delivery. The ultrasonic-mediated release of genes encapsulated in microbubbles (also known as ultrasound microbubbles) is one such approach that has the potential to offer regulated site-specific delivery of therapeutic drugs with minimal off-target consequences.

Ultrasound is a sound wave with frequencies ranging from 20 kHz to 20 MHz, making it inaudible to the human ear. Microbubbles are 1–10 mm polydisperse gas-filled vesicles that are mainly stabilised by a lipid or protein coating [152]. Although their shell and gas core compositions can vary greatly, all microbubbles are ideal candidates for contrast enhancement because they are resonators driven by the compressibility of their gaseous core [153]. They have been widely used as ultrasound contrast agents and in recent years have been explored as a new delivery system. This potential of microbubbles as drug delivery enhancers is based on the fact that ultrasound causes volumetric oscillations in the microbubble, which causes biological consequences.

Ultrasound is a type of pressure wave that travels through a medium. This pressure wave is distinguished by alternating rising and falling pressures that cause microbubble compression and expansion. Various studies are being conducted to improve ultrasound-mediated microbubble delivery, as well as clinical trials to introduce these delivery systems into standard clinical practice. For instance, the c-myc ASO was delivered via ultrasound-targeted microbubble destruction using a targeted ultrasound microbubble compound. In malignant mice, they were directed at hepatocellular carcinoma cells (HCC). According to the findings, ultrasonic administration of targeted microbubbles inhibited tumour development and cell proliferation the most. As a result, combining a tailored microbubble contrast agent with ultrasonic exposure might be a promising way to improve gene delivery efficiency [154]. In another study hepatocyte growth factor (HGF)-carrying lipid microbubble–cationic nanoliposomes (LMB-CNLP) were created and tested on the HSC-T6. After 24 h of transfection, cells treated with LMB-CNLP exhibited more green fluorescence than other groups, indicating that they were more effectively transfected with HGF. The scientists concluded that LMB-CNLP overcomes the common difficulties of carrying a limited quantity of genes and insufficient targeting to boost HGF transfection under ultrasound and induce the death of HSC-T6 cells, laying the groundwork for liver fibrosis gene therapy [155]. Aside from the delivery system, they also play a role in gene therapy in the treatment of liver fibrosis. Ultrasound microbubble–mediated gene therapy for hepatic fibrosis has been suggested in several animal studies. The ultrasound-targeted cationic liposome-bearing microbubble destruction gene delivery device was used to distribute artificial microRNA (miRNA) targeting connective tissue growth factor (CTGF) in a study. Using a biotin–avidin method, cationic liposomes were coupled with microbubbles. The results reveal that the plasmids were successfully delivered to the rat liver using this technique of gene delivery. The use of ultrasound-targeted cationic liposome-bearing microbubble destruction resulted in a clear decrease in fibrosis, suggesting that it might be an effective treatment technique for hepatic fibrosis [156]. By controlling homoeostasis and modification of the ECM, MMP and TIMP play an important role in hepatic fibrogenesis. A study on knockdown IGFBPrP1 was undertaken to evaluate the relationship between insulin-like growth factor binding protein related protein 1 (IGFBPrP1) and MMP/TIMP in hepatic fibrosis. Thioacetamide caused hepatic fibrosis in mice. In thioacetamide-induced liver fibrosis mice, IGFBPrP1 expression was knocked down by ultrasound-targeted microbubble destruction-mediated CMB-shRNA-IGFBPrP1 delivery. The findings showed that IGFBPrP1 knockdown lowered hepatic expression of IGFBPrP1, TGF-β1, α-SMA, and collagen I while restoring MMP2/TIMP2 and MMP9/TIMP1 balance which ultimately resulted in attenuation of hepatic fibrosis [157]. The therapeutic and delivery system applications of ultrasound guided microbubbles have expanded dramatically in recent years, with the discovery of new, more versatile agents. With an increasing interest in customized targeted therapy with fewer side effects, the use of ultrasound-mediated local delivery agents has been investigated with promising results, including but not limited to cross-linking with gene therapy.

5. Conclusion

We have reviewed and covered most of the major anti-fibrotic drugs, genes, and delivery strategies in this study. Liver fibrosis is a typical stage of CLD that has a significant impact on the human population, and innovative therapies to stop and reverse the underlying pathological conditions are desperately needed. The fact that there is no effective treatment for liver fibrosis and cirrhosis indicates the disease's complexity and the role of multiple active elements in its development. Despite these significant advances, it is undeniable that there is still a long way to go before diverse gene and delivery methods may be clinically transformed. The main impediment to creating effective antifibrotic therapy is that antifibrotic drugs cannot easily reach activated HSCs, which are key participants in liver fibrogenesis.

The underlying cause of the disease determines the status of the damaged cells. Most studies have neglected this fact, therefore developing a tailored treatment has focused on the general features of the disease rather than a complete understanding of fibrosis. Even though the majority of NPs had favourable results n animal studies, there are now just a few NP-based medications in clinical trials, indicating that further research is needed.

In addition to NPs, other types of targeted carriers have also been developed and tested in the therapy of liver fibrosis. Targeted delivery of antifibrotic drugs to activated HSCs is also crucial for effective liver fibrosis therapy. Despite significant efforts in preclinical investigations to create targeted delivery methods for liver fibrosis, the value of tailored drug delivery has not been completely understood in clinical trials. Regardless, drug targeting preparations may generate fresh leads for novel therapeutic treatments. As a result, this might open up a new avenue for treating liver fibrosis.

Gene therapy has also advanced and has several uses in the treatment of liver fibrosis. They have distinct advantages over other systems, including lengthening retention times, enhancing therapeutic outcomes, reducing side effects, safeguarding delivery molecules, and maintaining high specific targeting. Furthermore, the benefit of target-specific gene editing or siRNA technology is that these techniques can successfully inhibit important metabolic enzymes and provide distinctive targeted therapy. Gene delivery and

treatment are frequently used to target specific genes and aid in the improvement of fibrotic diseases. It is worth mentioning that viral vectors are currently used in many gene therapy techniques to transport nucleic acid cargo into cells. However, there is a lot of interest in moving toward chemical-based techniques, including polymer-based vectors, and it appears that some modifications are needed to build an appropriate and competent vector.

Considering all of this, it is obvious that all of the delivery methods have a high potential and a wide range of applications in liver fibrosis. These are simple solutions to the problem of drugs not reaching the intended place. While additional development is undoubtedly required, this approach has the potential to connect labs and patients. They are beneficial not only in the treatment of fibrosis but also in the treatment of a variety of other disorders. Keeping this in mind, additional studies will undoubtedly have an influence on the treatment of such a lethal condition.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest and have not been compensated in the development of this manuscript.

Acknowledgements

The authors are thankful to Nirma University for providing the required assistance in the preparation of this manuscript.

References

[1] Parola M, Pinzani M. Liver fibrosis: pathophysiology, pathogenetic targets and clinical issues. Mol Aspects Med 2019;65:37–55.
[2] Ezhilarasan D. Critical role of estrogen in the progression of chronic liver diseases. Hepatobiliary Pancreat Dis Int 2020;19:429–34.
[3] Novo E, Cannito S, Paternostro C, Bocca C, Miglietta A, Parola M. Cellular and molecular mechanisms in liver fibrogenesis. Arch Biochem Biophys 2014;548:20–37.
[4] Delgado ME, Cárdenas BI, Farran N, Fernandez M. Metabolic reprogramming of liver fibrosis. Cells 2021;10:3604.
[5] Kwak KA, Cho HJ, Yang JY, Park YS. Current perspectives regarding stem cell-based therapy for liver cirrhosis. Can J Gastroenterol Hepatol 2018;2018:4197857.
[6] Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol 2019;70:151–71.
[7] Marcellin P, Kutala BK. Liver diseases: a major, neglected global public health problem requiring urgent actions and large-scale screening. Liver Int 2018;38:2–6.
[8] Zhou WC. Pathogenesis of liver cirrhosis. World J Gastroenterol 2014;20:7312.
[9] Sarin SK, Kumar M, Eslam M, George J, Al Mahtab M, Akbar SMF, et al. Liver diseases in the asia-pacific region: a lancet gastroenterology & hepatology commission. Lancet Gastroenterol Hepatol 2020;5:167–228.
[10] Pinzani M. Pathophysiology of liver fibrosis. Dig Dis 2015;33:492–7.
[11] Elpek GÖ. Cellular and molecular mechanisms in the pathogenesis of liver fibrosis: an update. World J Gastroenterol 2014;20:7260–76.
[12] Luedde T, Kaplowitz N, Schwabe RF. Cell death and cell death responses in liver disease: mechanisms and clinical relevance. Gastroenterology 2014;147:765–83.
[13] Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet 2014;383:1749–61.
[14] Manning DS, Afdhal NH. Diagnosis and quantitation of fibrosis. Gastroenterology 2008;134:1670–81.
[15] Lurie Y, Webb M, Cytter-Kuint R, Shteingart S, Lederkremer GZ. Non-invasive diagnosis of liver fibrosis and cirrhosis. World J Gastroenterol 2015;21:11567–83.
[16] Lackner C, Spindelboeck W, Haybaeck J, Douschan P, Rainer F, Terracciano L, et al. Histological parameters and alcohol abstinence determine long-term prognosis in patients with alcoholic liver disease. J Hepatol 2017;66:610–18.
[17] European association for the study of the liver. EASL clinical practice guidelines for the management of patients with decompensated cirrhosis. J Hepatol 2018;69(2):406–60.
[18] Tan Z, Sun H, Xue T, Gan C, Liu H, Xie Y, et al. Liver fibrosis: therapeutic targets and advances in drug therapy. Front Cell Dev Biol 2021;9:730176.
[19] Devaraj E, Sokal E, Karthikeyan S, Najimi M. Plant derived antioxidants and antifibrotic drugs: past, present and future. J Coast Life Med 2014;2:738–45.
[20] Joudeh N, Linke D. Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. J Nanobiotechnol 2022;20:262.
[21] Ratemi E, Sultana Shaik A, Al Faraj A, Halwani R. Alternative approaches for the treatment of airway diseases: focus on nanoparticle medicine. Clin Exp Allergy 2016;46:1033–42.
[22] Hernández-Hernández AA, Aguirre-Álvarez G, Cariño-Cortés R, Mendoza-Huizar LH, Jiménez-Alvarado R. Iron oxide nanoparticles: synthesis, functionalization, and applications in diagnosis and treatment of cancer. Chem Pap 2020;74:3809–24.
[23] Salunkhe SA, Chitkara D, Mahato RI, Mittal A. Lipid based nanocarriers for effective drug delivery and treatment of diabetes associated liver fibrosis. Adv Drug Deliv Rev 2021;173:394–415.
[24] Lin Z, Monteiro-Riviere NA, Riviere JE. Pharmacokinetics of metallic nanoparticles. WIREs Nanomed Nanobiotechnol 2015;7:189–217.
[25] Cho M, Cho WS, Choi M, Kim SJ, Han BS, Kim SH, et al. The impact of size on tissue distribution and elimination by single intravenous injection of silica nanoparticles. Toxicol Lett 2009;189:177–83.
[26] Hirn S, Semmler-Behnke M, Schleh C, Wenk A, Lipka J, Schäffler M, et al. Particle size-dependent and surface charge-dependent biodistribution of gold nanoparticles after intravenous administration. Eur J Pharm Biopharm 2011;77:407–16.
[27] Disdier C, Devoy J, Cosnefroy A, Chalansonnet M, Herlin-Boime N, Brun E, et al. Tissue biodistribution of intravenously administrated titanium dioxide nanoparticles revealed blood-brain barrier clearance and brain inflammation in rat. Part Fibre Toxicol 2015;12:27.
[28] Petitclerc L, Sebastiani G, Gilbert G, Cloutier G, Tang A. Liver fibrosis: review of current imaging and mri quantification techniques. J Magn Reson Imaging 2017;45:1276–95.
[29] Kim D, Kim J, Il PY, Lee N, Hyeon T. Recent development of inorganic nanoparticles for biomedical imaging. ACS Cent Sci 2018;4:324–36.
[30] Mitrea DR, Malkey R, Florian TL, Filip A, Clichici S, Bidian C, et al. Daily oral administration of chlorogenic acid prevents

the experimental carrageenan-induced oxidative stress. J Physiol Pharmacol 2020;71(1):55–65.
[31] Kabir N, Ali H, Ateeq M, Bertino MF, Shah MR, Franzel L. Silymarin coated gold nanoparticles ameliorates CCl4-induced hepatic injury and cirrhosis through down regulation of hepatic stellate cells and attenuation of Kupffer cells. RSC Adv 2014;4:9012–20.
[32] Adhikari A, Polley N, Darbar S, Bagchi D, Pal SK. Citrate functionalized Mn_3O_4 in nanotherapy of hepatic fibrosis by oral administration. Futur Sci OA 2016;2(4):FSO146.
[33] Oró D, Yudina T, Fernández-Varo G, Casals E, Reichenbach V, Casals G, et al. Cerium oxide nanoparticles reduce steatosis, portal hypertension and display anti-inflammatory properties in rats with liver fibrosis. J Hepatol 2016;64:691–8.
[34] Kurniawan DW, Booijink R, Pater L, Wols I, Vrynas A, Storm G, et al. Fibroblast growth factor 2 conjugated superparamagnetic iron oxide nanoparticles (FGF2-SPIONs) ameliorate hepatic stellate cells activation *in vitro* and acute liver injury *in vivo*. J Control Release 2020;328:640–52.
[35] Yu Y, Duan J, Li Y, Li Y, Jing L, Yang M, et al. Silica nanoparticles induce liver fibrosis via TGF-β_1/Smad3 pathway in ICR mice. Int J Nanomed 2017;12:6045–57.
[36] Bashandy SAE, Alaamer A, Moussa SAA, Omara EA. Role of zinc oxide nanoparticles in alleviating hepatic fibrosis and nephrotoxicity induced by thioacetamide in rats. Can J Physiol Pharmacol 2018;96:337–44.
[37] Chenthamara D, Subramaniam S, Ramakrishnan SG, Krishnaswamy S, Essa MM, Lin FH, et al. Therapeutic efficacy of nanoparticles and routes of administration. Biomater Res 2019;23:20.
[38] Bartneck M, Scheyda KM, Warzecha KT, Rizzo LY, Hittatiya K, Luedde T, et al. Fluorescent cell-traceable dexamethasone-loaded liposomes for the treatment of inflammatory liver diseases. Biomaterials 2015;37:367–82.
[39] Wang J, Ding Y, Zhou W. Albumin self-modified liposomes for hepatic fibrosis therapy via SPARC-dependent pathways. Int J Pharm 2020;574:118940.
[40] Kong WH, Park K, Lee MY, Lee H, Sung DK, Hahn SK. Cationic solid lipid nanoparticles derived from apolipoprotein-free LDLs for target specific systemic treatment of liver fibrosis. Biomaterials 2013;34:542–51.
[41] Chang CC, Yang Y, Gao DY, Cheng HT, Hoang B, Chao PH, et al. Docetaxel-carboxymethylcellulose nanoparticles ameliorate CCl_4-induced hepatic fibrosis in mice. J Drug Target 2018;26:516–24.
[42] Kuznietsova H, Lynchak O, Dziubenko N, Herheliuk T, Prylutskyy Y, Rybalchenko V, et al. Water-soluble pristine C_{60} fullerene attenuates acetaminophen-induced liver injury. Bioimpacts 2019;9:227–37.
[43] Kuznietsova H, Dziubenko N, Hurmach V, Chereschuk I, Motuziuk O, Ogloblya O, et al. Water-soluble pristine C_{60} fullerenes inhibit liver fibrotic alteration and prevent liver cirrhosis in rats. Oxid Med Cell Longev 2020;2020:8061246.
[44] Mohanta D, Patnaik S, Sood S, Das N. Carbon nanotubes: evaluation of toxicity at biointerfaces. J Pharm Anal 2019;9:293–300.
[45] Hasan MT, Campbell E, Sizova O, Lyle V, Akkaraju G, Kirkpatrick DL, et al. Multi-drug/gene NASH therapy delivery and selective hyperspectral NIR imaging using chirality-sorted single-walled carbon nanotubes. Cancers 2019;11(8):1175 (Basel).
[46] Zhu C, Gong S, Ding J, Yu M, Ahmad E, Feng Y, et al. Supersaturated polymeric micelles for oral silybin delivery: the role of the Soluplus–PVPVA complex. Acta Pharm Sin B 2019;9:107–17.
[47] Li W, Zhou C, Fu Y, Chen T, Liu X, Zhang Z, et al. Targeted delivery of hyaluronic acid nanomicelles to hepatic stellate cells in hepatic fibrosis rats. Acta Pharm Sin B 2020;10:693–710.
[48] Schuppan D, Ashfaq-Khan M, Yang AT, Kim YO. Liver fibrosis: direct antifibrotic agents and targeted therapies. Matrix Biol 2018;68–69:435–51.
[49] Devaraj E, Rajeshkumar S. Nanomedicine for hepatic fibrosis. In: Nanoparticles their biomedical applications. Singapore: Springer Singapore; 2020. p. 45–64.
[50] Lee H, Jeong H, Park S, Yoo W, Choi S, Choi K, et al. Fusion protein of retinol-binding protein and albumin domain iii reduces liver fibrosis. EMBO Mol Med 2015;7:819–30.
[51] Pan TL, Wang PW, Hung CF, Aljuffali IA, Dai YS, Fang JY. The impact of retinol loading and surface charge on the hepatic delivery of lipid nanoparticles. Colloids Surf B Biointerfaces 2016;141:584–94.
[52] Zhang Z, Wang C, Zha Y, Hu W, Gao Z, Zang Y, et al. Corona-directed nucleic acid delivery into hepatic stellate cells for liver fibrosis therapy. ACS Nano 2015;9:2405–19.
[53] Borkham-Kamphorst E, Weiskirchen R. The pdgf system and its antagonists in liver fibrosis. Cytokine Growth Factor Rev 2016;28:53–61.
[54] Friedman SL. Liver fibrosis - from bench to bedside. J Hepatol 2003;38(Suppl 1):S38–53.
[55] Wang X, Wu X, Zhang A, Wang S, Hu C, Chen W, et al. Targeting the PDGF-B/PDGFR-B interface with destruxin A5 to selectively block PDGF-BB/ PDGFR-$\beta\beta$ signaling and attenuate liver fibrosis. EBioMedicine 2016;7:146–56.
[56] Chen Q, Chen L, Kong D, Shao J, Wu L, Zheng S. Dihydroartemisinin alleviates bile duct ligation-induced liver fibrosis and hepatic stellate cell activation by interfering with the PDGF-βR/ERK signaling pathway. Int Immunopharmacol 2016;34:250–8.
[57] Kocabayoglu P, Lade A, Lee YA, Dragomir AC, Sun X, Fiel MI, et al. β-PDGF receptor expressed by hepatic stellate cells regulates fibrosis in murine liver injury, but not carcinogenesis. J Hepatol 2015;63:141–7.
[58] Hayes BJ, Riehle KJ, Shimizu-Albergine M, Bauer RL, Hudkins KL, Johansson F, et al. Activation of platelet-derived growth factor receptor alpha contributes to liver fibrosis. PLoS ONE 2014;9:e92925.
[59] Kikuchi A, Pradhan-Sundd T, Singh S, Nagarajan S, Loizos N, Monga SP. Platelet-derived growth factor receptor α contributes to human hepatic stellate cell proliferation and migration. Am J Pathol 2017;187:2273–87.
[60] Maslak E, Gregorius A, Chlopicki S. Liver sinusoidal endothelial cells (LSECs) function and NAFLD; NO-based therapy targeted to the liver. Pharmacol Rep 2015;67:689–94.
[61] Poisson J, Lemoinne S, Boulanger C, Durand F, Moreau R, Valla D, et al. Liver sinusoidal endothelial cells: physiology and role in liver diseases. J Hepatol 2017;66:212–27.
[62] Friedman SL, Ratziu V, Harrison SA, Abdelmalek MF, Aithal GP, Caballeria J, et al. A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis. Hepatology 2018;67:1754–67.
[63] DeLeve LD. Liver sinusoidal endothelial cells in hepatic fibrosis. Hepatology 2015;61:1740–6.
[64] Xie G, Choi SS, Syn WK, Michelotti GA, Swiderska M, Karaca G, et al. Hedgehog signalling regulates liver sinusoidal endothelial cell capillarisation. Gut 2013;62:299–309.
[65] Géraud C, Koch PS, Zierow J, Klapproth K, Busch K, Olsavszky V, et al. GATA4-dependent organ-specific endothelial differentiation controls liver development and embryonic hematopoiesis. J Clin Invest 2017;127:1099–114.
[66] Xie G, Wang X, Wang L, Wang L, Atkinson RD, Kanel GC, et al. Role of differentiation of liver sinusoidal endothelial cells in progression and regression of hepatic fibrosis in rats. Gastroenterology 2012;142:918–27 e6.
[67] Weston CJ, Shepherd EL, Claridge LC, Rantakari P,

Curbishley SM, Tomlinson JW, et al. Vascular adhesion protein-1 promotes liver inflammation and drives hepatic fibrosis. J Clin Invest 2015;125:501–20.
[68] Kisseleva T, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression. Nat Rev Gastroenterol Hepatol 2021;18:151–66.
[69] Muhanna N, Tair LA, Doron S, Amer J, Azzeh M, Mahamid M, et al. Amelioration of hepatic fibrosis by nk cell activation. Gut 2011;60:90–8.
[70] Gur C, Doron S, Kfir-Erenfeld S, Horwitz E, Abu-tair L, Safadi R, et al. NKp46-mediated killing of human and mouse hepatic stellate cells attenuates liver fibrosis. Gut 2012;61:885–93.
[71] Wang H, Yin S. Natural killer t cells in liver injury, inflammation and cancer. Expert Rev Gastroenterol Hepatol 2015;9:1077–85.
[72] Mitra A, Satelli A, Yan J, Xueqing X, Gagea M, Hunter CA, et al. IL-30 (IL27p28) attenuates liver fibrosis through inducing NKG2D-rae1 interaction between NKT and activated hepatic stellate cells in mice. Hepatology 2014;60:2027–39.
[73] Lu DH, Guo XY, Qin SY, Luo W, Huang XL, Chen M, et al. Interleukin-22 ameliorates liver fibrogenesis by attenuating hepatic stellate cell activation and downregulating the levels of inflammatory cytokines. World J Gastroenterol 2015;21:1531–45.
[74] Rolla S, Alchera E, Imarisio C, Bardina V, Valente G, Cappello P, et al. The balance between IL-17 and IL-22 produced by liver-infiltrating T-helper cells critically controls NASH development in mice. Clin Sci 2015;130:193–203.
[75] Slevin E, Baiocchi L, Wu N, Ekser B, Sato K, Lin E, et al. Kupffer cells: inflammation pathways and cell-cell interactions in alcohol-associated liver disease. Am J Pathol 2020;190:2185–93.
[76] Sasaki R, Devhare PB, Steele R, Ray R, Ray RB. Hepatitis C virus–induced CCL5 secretion from macrophages activates hepatic stellate cells. Hepatology 2017;66:746–57.
[77] Wang HQ, Wan Z, Zhang Q, Su T, Yu D, Wang F, et al. Schisandrin B targets cannabinoid 2 receptor in Kupffer cell to ameliorate CCl_4-induced liver fibrosis by suppressing NF-κB and p38 MAPK pathway. Phytomedicine 2022;98:153960.
[78] Zhang WJ, Fang ZM, Liu WQ. NLRP3 inflammasome activation from Kupffer cells is involved in liver fibrosis of *schistosoma japonicum*-infected mice *via* NF-κB. Parasit Vectors 2019;12:29.
[79] Maeda H, Minayoshi Y, Ichimizu S, Mizuta Y, Nagasaki T, Matsusaka K, et al. Repeated administration of Kupffer cells-targeting nanoantioxidant ameliorates liver fibrosis in an experimental mouse model. Biol Pharm Bull 2020;43:93–101.
[80] Kanamori Y, Tanaka M, Itoh M, Ochi K, Ito A, Hidaka I, et al. Iron-rich Kupffer cells exhibit phenotypic changes during the development of liver fibrosis in nash. IScience 2021;24:102032.
[81] Cheng D, Chai J, Wang H, Fu L, Peng S, Ni X. Hepatic macrophages: key players in the development and progression of liver fibrosis. Liver Int 2021;41:2279–94.
[82] Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. Nat Rev Gastroenterol Hepatol 2017;14:397–411.
[83] Dewidar M, Dooley M-B. TGF-β in hepatic stellate cell activation and liver fibrogenesis—Updated 2019. Cells 2019;8:1419.
[84] Dooley S, ten Dijke P. TGF-β in progression of liver disease. Cell Tissue Res 2012;347:245–56.
[85] Wang X, Li Y, Li X, Yan L, Guan H, Han R, et al. Expression, purification, and evaluation of *in vivo* anti-fibrotic activity for soluble truncated TGF-β receptor ii as a cleavable His-SUMO fusion protein. World J Microbiol Biotechnol 2018;34:181.
[86] Huang Z, Ding M, Dong Y, Ma M, Song X, Liu Y, et al. Targeted truncated TGF-β receptor type ii delivery to fibrotic liver by PDGFβ receptor-binding peptide modification for improving the anti-fibrotic activity against hepatic fibrosis *in vitro* and *in vivo*. Int J Biol Macromol 2021;188:941–9.
[87] Wang H, Che J, Cui K, Zhuang W, Li H, Sun J, et al. Schisantherin a ameliorates liver fibrosis through TGF-β1mediated activation of TAK1/MAPK and NF-κB pathways *in vitro* and *in vivo*. Phytomedicine 2021;88:153609.
[88] Yu Q, Cheng P, Wu J, Guo C. PPARγ/NF-κB and TGF-β1/smad pathway are involved in the anti-fibrotic effects of levo-tetrahydropalmatine on liver fibrosis. J Cell Mol Med 2021;25:1645–60.
[89] Ji J, Yu Q, Dai W, Wu L, Feng J, Zheng Y, et al. Apigenin alleviates liver fibrosis by inhibiting hepatic stellate cell activation and autophagy via TGF-β1/Smad3 and p38/PPARα pathways. PPAR Res 2021;2021:6651839.
[90] Vargas-Pozada EE, Ramos-Tovar E, Acero-Hernández C, Cardoso-Lezama I, Galindo-Gómez S, Tsutsumi V, et al. Caffeine mitigates experimental nonalcoholic steatohepatitis and the progression of thioacetamide-induced liver fibrosis by blocking the mapk and TGF-β/Smad3 signaling pathways. Ann Hepatol 2022;27:100671.
[91] Chen YN, Hsu SL, Liao MY, Liu YT, Lai CH, Chen JF, et al. Ameliorative effect of curcumin-encapsulated hyaluronic acid–PLA nanoparticles on thioacetamide-induced murine hepatic fibrosis. Int J Environ Res Public Heal 2017;14:11.
[92] Fang XJ, Jiang H, Zhao XP, Jiang WM. The role of a new CD44st in increasing the invasion capability of the human breast cancer cell line MCF-7. BMC Cancer 2011;11:290.
[93] Patouraux S, Rousseau D, Bonnafous S, Lebeaupin C, Luci C, Canivet CM, et al. CD44 is a key player in non-alcoholic steatohepatitis. J Hepatol 2017;67:328–38.
[94] Uno K, Miyajima K, Toma M, Suzuki-Kemuriyama N, Nakae D. CD44 expression in the bile duct epithelium is related to hepatic fibrosis in nonalcoholic steatohepatitis rats induced by a choline-deficient, methionine-lowered, L-amino acid diet. J Toxicol Pathol 2022;35(2):149–57.
[95] Liang H, Li Z, Ren Z, Jia Q, Guo L, Li S, et al. Light-triggered no-releasing nanoparticles for treating mice with liver fibrosis. Nano Res 2020;13:2197–202.
[96] Mahdinloo S, Kiaie SH, Amiri A, Hemmati S, Valizadeh H, Zakeri-Milani P. Efficient drug and gene delivery to liver fibrosis: rationale, recent advances, and perspectives. Acta Pharm Sin B 2020;10:1279–93.
[97] Salazar-Montes AM, Hernández-Ortega LD, Lucano-Landeros MS, Armendariz-Borunda J. New gene therapy strategies for hepatic fibrosis. World J Gastroenterol 2015;21:3813–25.
[98] Saraswat P, Soni RR, Bhandari A, Nagori BP. DNA as therapeutics; an update. Indian J Pharm Sci 2009;71: 488–498.
[99] Weng SY, Wang X, Vijayan S, Tang Y, Kim YO, Padberg K, et al. IL-4 receptor alpha signaling through macrophages differentially regulates liver fibrosis progression and reversal. EBioMedicine 2018;29:92–103.
[100] Lindén D, Ahnmark A, Pingitore P, Ciociola E, Ahlstedt I, Andréasson AC, et al. Pnpla3 silencing with antisense oligonucleotides ameliorates nonalcoholic steatohepatitis and fibrosis in Pnpla3 I148m knock-in mice. Mol Metab 2019;22:49–61.
[101] Tang M, Chen Y, Li B, Sugimoto H, Yang S, Yang C, et al. Therapeutic targeting of STAT3 with small interference RNAs and antisense oligonucleotides embedded exosomes in liver fibrosis. FASEB J 2021;35:e21557.
[102] Park SY, Yun YH, Park BJ, Seo HI, Chung I. Fabrication and biological activities of plasmid DNA gene carrier nanoparticles based on biodegradable L-tyrosine polyurethane. Pharm 2022;15(1):17.

[103] Chen Y, Chen X, Wu D, Xin H, Chen D, Li D, et al. Delivery of CRISPR/Cas9 plasmids by cationic gold nanorods: impact of the aspect ratio on genome editing and treatment of hepatic fibrosis. Chem Mater 2021;33:81–91.
[104] Meng Z, O'Keeffe-Ahern J, Lyu J, Pierucci L, Zhou D, Wang W. A new developing class of gene delivery: messenger RNA-based therapeutics. Biomater Sci 2017;5:2381–92.
[105] Zhang Y, Li Y, Mu T, Tong N, Cheng P. Hepatic stellate cells specific liposomes with the Toll-like receptor 4 shRNA attenuates liver fibrosis. J Cell Mol Med 2021;25:1299–313.
[106] Higashi T, Friedman SL, Hoshida Y. Hepatic stellate cells as key target in liver fibrosis. Adv Drug Deliv Rev 2017;121:27–42.
[107] O'Keeffe Ahern J, Zhou D, Gao Y, Lyu J, Meng Z, Cutlar L, et al. Brushlike cationic polymers with low charge density for gene delivery. Biomacromolecules 2017;19:1410–15.
[108] Sahin U, Karikó K, Türeci Ö. MRNA-based therapeutics—developing a new class of drugs. Nat Rev Drug Discov 2014;13:759–80.
[109] Hu B, Li B, Li K, Liu Y, Li C, Zheng L, et al. Thermostable ionizable lipid-like nanoparticle (iland) for RNAi treatment of hyperlipidemia. Sci Adv 2022;8(7):eabm1418.
[110] Guo S, Liu B, Zhang M, Li C, Wang X, Weng Y, et al. A novel polyethyleneimine-decorated FeOOH nanoparticle for efficient siRNA delivery. Chin Chem Lett 2021;32:102–6.
[111] Guo S, Li K, Hu B, Li C, Zhang M, Hussain A, et al. Membrane-destabilizing ionizable lipid empowered imaging-guided siRNA delivery and cancer treatment. Exploration 2021;1:35–49.
[112] Omar R, Yang J, Liu H, Davies NM, Gong Y. Hepatic stellate cells in liver fibrosis and siRNA-based therapy. Rev Physiol Biochem Pharmacol 2016;172:1–37.
[113] Zhao Z, Li Y, Jain A, Chen Z, Liu H, Jin W, et al. Development of a peptide-modified siRNA nanocomplex for hepatic stellate cells. Nanomed Nanotechnol Biol Med 2018;14:51–61.
[114] Jain A, Barve A, Zhao Z, Fetse JP, Liu H, Li Y, et al. Targeted delivery of an siRNA/PNA hybrid nanocomplex reverses carbon tetrachloride-induced liver fibrosis. Adv Ther 2019;2:1900046.
[115] Ullah A, Chen G, Hussain A, Khan H, Abbas A, Zhou Z, et al. Cyclam-modified polyethyleneimine for simultaneousTGFβ siRNA delivery and CXCR4 inhibition for the treatment of CCl_4-induced liver fibrosis. Int J Nanomed 2021;16:4451–70.
[116] Sato Y, Yoneda A, Shimizu F, Nishimura M, Shimoyama R, Tashiro Y, et al. Resolution of fibrosis by siRNA HSP47 in vitamin A-coupled liposomes induces regeneration of chronically injured livers. J Gastroenterol Hepatol 2021;36:3418–28.
[117] Zhou J, Zhao Y, Simonenko V, Xu JJ, Liu K, Wang D, et al. Simultaneous silencing of TGF-β1 and COX-2 reduces human skin hypertrophic scar through activation of fibroblast apoptosis. Oncotarget 2017;8:80651–65.
[118] Kumar V, Mahato RI. Delivery and targeting of miRNAs for treating liver fibrosis. Pharm Res 2015;32:341–61.
[119] Wang H, Wang Z, Wang Y, Li X, Yang W, Wei S, et al. miRNA-130b-5p promotes hepatic stellate cell activation and the development of liver fibrosis by suppressing SIRT4 expression. J Cell Mol Med 2021;25:7381–94.
[120] Ji F, Wang K, Zhang Y, Mao XL, Huang Q, Wang J, et al. MiR-542-3p controls hepatic stellate cell activation and fibrosis via targeting BMP-7. J Cell Biochem 2019;120:4573–81.
[121] Zhou X, Liang Z, Qin S, Ruan X, Jiang H. Serum-derived miR-574-5p-containing exosomes contribute to liver fibrosis by activating hepatic stellate cells. Mol Biol Rep 2022;49:1945–54.
[122] Wu Z, Wang J, Feng J, Ying L. MicroRNA-122-5p prevents proliferation and promotes apoptosis of hepatic stellate cells by suppressing the cellular-Abelsongene/histone deacetylases 2 pathway. Hum Exp Toxicol 2022;41:096032712210846.
[123] Li X, Li H, Zhang S, Zhang R, Li J, Wei Y, et al. Protective effect of idelalisib on carbon tetrachloride-induced liver fibrosis via microRNA-124-3p/phosphatidylinositol-3-hydroxykinase signalling pathway. J Cell Mol Med 2021;25:11185–97.
[124] Wang A, Bu F, Li J, Zhang Y, Jia P, You H, et al. MicroRNA-195-3p promotes hepatic stellate cell activation and liver fibrosis by suppressing pten expression. Toxicol Lett 2022;355:88–99.
[125] Yang T, Poenisch M, Khanal R, Hu Q, Dai Z, Li R, et al. Therapeutic HNF4A mRNA attenuates liver fibrosis in a preclinical model. J Hepatol 2021;75:1420–33.
[126] Wei G, Cao J, Huang P, An P, Badlani D, Vaid KA, et al. Synthetic human ABCB4 mRNA therapy rescues severe liver disease phenotype in a BALB/c.Abcb4$^{-/-}$ mouse model of PFIC3. J Hepatol 2021;74:1416–28.
[127] Ba HZ, Liang ZH, Kim HS, Cao W. TGF-β1 can be regulated by NDRG2 via the NF-κB pathway in hypoxia-induced liver fibrosis. Ann Transl Med 2021;9:505.
[128] Rajapaksha IG, Gunarathne LS, Asadi K, Laybutt R, Andrikopoulous S, Alexander IE, et al. Angiotensin converting enzyme-2 therapy improves liver fibrosis and glycemic control in diabetic mice with fatty liver. Hepatol Commun 2022;6(5):1056–72.
[129] Song YF, Tran M, Wang L, Shin DJ, Wu JG. MiR-200c-3p targets SESN1 and represses the IL-6/AKT loop to prevent cholangiocyte activation and cholestatic liver fibrosis. Lab Investig 2022;102(5):485–93.
[130] Li H, Zhang L, Cai N, Zhang B, Sun S. MicroRNA-494-3p prevents liver fibrosis and attenuates hepatic stellate cell activation by inhibiting proliferation and inducing apoptosis through targeting TRAF3. Ann Hepatol 2021;23:100305.
[131] Huang W, Ji R, Ge S, Zhou D, Liu Z, Sun Y, et al. MicroRNA-92b-3p promotes the progression of liver fibrosis by targeting CREB3l2 through the JAK/STAT signaling pathway. Pathol Res Pract 2021;219:153367.
[132] Zhou QY, Yang HM, Liu JX, Xu N, Li J, Shen LP, et al. MicroRNA-497 induced by clonorchis sinensis enhances the TGF-β/SMAD signaling pathway to promote hepatic fibrosis by targeting SMAD7. Parasit Vectors 2021;14:472.
[133] Wang X, Seo W, Park SH, Fu Y, Hwang S, Rodrigues RM, et al. MicroRNA-223 restricts liver fibrosis by inhibiting the TAZ-IHH-GLI2 and PDGF signaling pathways via the crosstalk of multiple liver cell types. Int J Biol Sci 2021;17:1153–67.
[134] Ye J, Lin Y, Yu Y, Sun D. LncRNA NEAT1/microRNA-129-5p/SOCS2 axis regulates liver fibrosis in alcoholic steatohepatitis. J Transl Med 2020;18:445.
[135] Xiong J, Ni J, Chen C, Wang K. MiR–148a–3p regulates alcoholic liver fibrosis through targeting ERBB3. Int J Mol Med 2020;46:1003–12.
[136] Liu C, Zhou B, Meng M, Zhao W, Wang D, Yuan Y, et al. FOXA3 induction under endoplasmic reticulum stress contributes to non-alcoholic fatty liver disease. J Hepatol 2021;75:150–62.
[137] Wu P, Luo X, Sun M, Sun B, Sun M. Synergetic regulation of Kupffer cells, extracellular matrix and hepatic stellate cells with versatile CXCR4-inhibiting nanocomplex for magnified therapy in liver fibrosis. Biomaterials 2022;284:121492.
[138] Zhou JE, Sun L, Liu L, Jia Y, Han Y, Shao J, et al. Hepatic macrophage targeted siRNA lipid nanoparticles treat non-alcoholic steatohepatitis. J Control Release 2022;343:175–86.
[139] Kaps L, Leber N, Klefenz A, Choteschovsky N, Zentel R, Nuhn L, et al. *In vivo* siRNA delivery to immunosuppressive liver macrophages by α-mannosyl-functionalized cationic nanohydrogel particles. Cells 2020;9(8):1905.
[140] Lundstrom K. Viral vectors in gene therapy. Diseases 2018;6(2):42.
[141] Aravalli RN. Chapter60-Gene therapy for liver disease.

In: Liver pathophysiology. Boston: Academic Press; 2017. p. 837–51.
[142] Xu A, Zhong G, Wang J, Liu C, Liu Y, Wang W. MicroRNA 200a inhibits liver fibrosis of schistosoma. Bioengineered 2021;12:4736–46.
[143] Hsieh YC, Lee KC, Lei HJ, Lan KH, Huo TI, Lin YT, et al. Pro)renin receptor knockdown attenuates liver fibrosis through inactivation of ERK/TGF-β1/SMAD3 pathway. Cell Mol Gastroenterol Hepatol 2021;12:813–38.
[144] Huang S, Qian X, Jiang T, Xiao J, Yin M, Xiong M, et al. Mechanism of dact2 gene inhibiting the occurrence and development of liver fibrosis. Cell Mol Biol 2022;67:33–9.
[145] Chen H, Cai J, Wang J, Qiu Y, Jiang C, Wang Y, et al. Targeting nestin^{+} hepatic stellate cells ameliorates liver fibrosis by facilitating tβri degradation. J Hepatol 2021;74:1176–87.
[146] Shen Z, Gao Z, Gu C, Wu J, Wang J, Zhang J, et al. Prior transient exposure to interleukin-21 delivered by recombinant adeno-associated virus vector protects mice from hepatitis B virus persistence. Antiviral Res 2021;190:105076.
[147] Martínez MS, De Alba NA, Contreras ID, González AR. GATA4: a key factor for liver fibrosis regression. Biosaia Rev Los Másteres Biotecnol Sanit y Biotecnol Ambient Ind y Aliment 2021.
[148] Liu R, Luo C, Pang ZQ, Zhang JM, Ruan SB, Wu MY, et al. Advances of nanoparticles as drug delivery systems for disease diagnosis and treatment. Chin Chem Lett 2022;34(2):107518.
[149] Wu J, Huang J, Kuang S, Chen J, Li X, Chen B, et al. Synergistic microRNA therapy in liver fibrotic rat using MRI-visible nanocarrier targeting hepatic stellate cells. Adv Sci 2019;6:1801809.
[150] Nishihara H. Evolution of transposable elements and evolution of eukaryote genomes mediated by transposable elements. Genes Genet Syst 2019;94:231.
[151] Siew SM, Cunningham SC, Zhu E, Tay SS, Venuti E, Bolitho C, et al. Prevention of cholestatic liver disease and reduced tumorigenicity in a murine model of PFIC Type 3 using hybrid AAV-piggybac gene therapy. Hepatology 2019;70:2047–61.
[152] Kooiman K, Roovers S, Langeveld SAG, Kleven RT, Dewitte H, O'Reilly MA, et al. Ultrasound-responsive cavitation nuclei for therapy and drug delivery. Ultrasound Med Biol 2020;46:1296–325.
[153] Deprez J, Lajoinie G, Engelen Y, De Smedt SC, Lentacker I. Opening doors with ultrasound and microbubbles: beating biological barriers to promote drug delivery. Adv Drug Deliv Rev 2021;172:9–36.
[154] Jing H, Cheng W, Zhang JW, Han X, Shao H, Sun YX. Galactosylated poly-L-lysine targeted microbubbles for ultrasound mediated antisense *c-myc* gene transfection in hepatocellular carcinoma cells. Arch Med Sci 2015;11:292–300.
[155] Li WY, Wang ZX, Wang ZG, Zhao JN, Zhang SH. *In vitro* combination of lipid microbubbles and cationic nanoliposomes in enhancing HGF gene transfection. Chin J Med Imaging Technol 2012;28:1255–8.
[156] Yang D, Gao YH, Tan KB, Zuo ZX, Yang WX, Hua X, et al. Inhibition of hepatic fibrosis with artificial microRNA using ultrasound and cationic liposome-bearing microbubbles. Gene Ther 2013;20:1140–8.
[157] Ren JJ, Huang TJ, Zhang QQ, Zhang HY, Guo XH, Fan HQ, et al. Insulin-like growth factor binding protein related protein 1 knockdown attenuates hepatic fibrosis via the regulation of MMPs/TIMPs in mice. Hepatobiliary Pancreat Dis Int 2019;18:38–47.