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Nanoparticle approaches for hepatitis therapy and clinical translation

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Abstract

Background Hepatitis B and C viruses (HBV and HCV) remain major global health challenges, contributing significantly to liver-related morbidity and mortality worldwide. Although direct-acting antivirals (DAAs) have improved patient outcomes, key limitations persist, including suboptimal hepatic targeting, emerging drug resistance, incomplete viral eradication, and systemic side effects.

Area covered Nanotechnology offers a promising avenue for enhancing and personalizing antiviral treatments. This review explores recent advances in nanoparticle (NP)-based strategies for HBV and HCV therapy, focusing on design principles, delivery platforms, and translational applications. Lipid-based, polymeric, metallic/inorganic, and biomimetic nanocarriers are examined for drug delivery, gene editing, and vaccine development. Targeted strategies—such as galactose-mediated hepatic uptake and pH-responsive release—demonstrate the potential to improve drug localization and reduce off-target toxicity. Preclinical studies, including siRNA-loaded lipid nanoparticles, have shown significant antiviral effects in animal models. In addition, emerging AI-driven frameworks for nanoparticle design and prediction are highlighted as tools that may accelerate optimization and therapeutic personalization. Despite these advances, translation into clinical practice remains limited due to challenges such as immunogenicity, systemic instability, manufacturing scalability, and regulatory complexity.

Expert opinion To facilitate clinical translation, a clear developmental roadmap is needed that emphasizes interdisciplinary collaboration, standardized safety evaluations, and patient-centered treatment strategies. Continued innovation—including integration of nanotechnology with artificial intelligence, gene-editing approaches, and immunomodulatory platforms—holds strong potential to transform HBV and HCV management and enable safer, more effective therapeutic options beyond current antiviral approaches.

Keywords Hepatitis B and C, Nanoparticle drug delivery, CRISPR/Cas9 delivery, Hepatic targeting, Nano-vaccines

1 Introduction

Viral hepatitis is a significant global health crisis, with HBV and HCV infections resulting in over 1.5 million deaths each year from complications such as cirrhosis and hepatocellular carcinoma [1–3]. Despite notable progress in antiviral treatments, these viruses continue to resist eradication, underscoring the need for innovative therapies that align with the World Health Organization's (WHO) 2030 eradication targets [4].

The hepatitis virus family comprises five distinct viruses, ranging from hepatitis A (HAV) to E (HEV), each with its own unique transmission methods and disease progression. HBV and HCV cause most hepatitis-related deaths worldwide because these viruses result in chronic infections and cancer development in patients [5, 6].

The treatment of HBV and HCV with nucleoside analogs (e.g., tenofovir, entecavir) and direct-acting antivirals (DAAs) has led to significant improvements, but essential challenges remain. These medications face several issues, including low oral absorption rates below 30% for ribavirin, rapid renal elimination, and severe side effects that limit treatment doses, such as neuropsychiatric problems triggered by interferon [7, 8]. HBV persists through its covalently closed circular DNA (cccDNA) reservoir [9]. The lack of prophylactic vaccines for HCV, along with liver immunosuppression during infection, makes achieving eradication more difficult [10, 11].

Nanotechnology has emerged as a promising solution to overcome these challenges. Nanoparticles (NPs) smaller than 200 nm utilize the enhanced permeability and retention (EPR) effect to passively accumulate in the liver, while active targeting ligands, such as galactose, improve delivery to hepatocytes by binding to the asialoglycoprotein receptor (ASGPR) [12]. Using polyethylene glycol (PEG)-coated liposomal interferon-α reduces systemic toxicity by 40% in mouse studies compared to free interferon [13]. Delivering siRNA via lipid nanoparticles results in a more than 90% reduction in viral load in patients with chronic HBV infections [14]. These delivery systems prevent resistance by delivering antivirals alongside host-targeting agents, such as cyclosporine A, which blocks HBV capsid assembly [15], thereby providing combined solutions that extend beyond traditional therapies.

Taking all this into account, the emerging role of nanomedicine in treating HBV and HCV infections is organized around three main objectives:

1. To analyze the physicochemical parameters of nanoparticle design—including particle size, surface charge, and ligand-mediated functionalization—and their impact on hepatic biodistribution and antiviral efficacy.
2. To assess the translational challenges involved in clinical implementation, such as immunogenicity, scalability of nanoparticle synthesis, and adherence to regulatory frameworks.
3. To highlight recent technological advancements, including CRISPR/Cas9 systems for targeted HBV cccDNA disruption, AI-driven nanocarrier optimization, and the development of multi-antigenic nano-vaccines.

Importantly, this review combines findings from both preclinical models and clinical trials to provide a translational roadmap for nanomedicine-based interventions that support the WHO's 2030 goal of eliminating viral hepatitis.

2 Pathophysiology of hepatitis and therapeutic challenges

Viral hepatitis results from five major hepatotropic viral infections: HAV, HBV, HCV, HDV, and HEV [16, 17]. HAV and HEV are typically associated with acute, self-limiting infections that spread through the fecal–oral route. According to the WHO and the Global Burden of Disease study, HAV causes about 1.5 million symptomatic cases each year, while HEV infects an estimated 20 million people annually, leading to roughly 3.3 million symptomatic cases and over 40,000 deaths worldwide. The Centers for Disease Control and Prevention also report ongoing HEV outbreaks in low-resource areas with limited access to clean water [15–17]. HDV, a satellite virus that depends on HBV for replication, infects around 12 million people globally—about 5% of HBV carriers—and is linked to more severe liver disease and faster progression to cirrhosis. However, the leading causes of persistent infections and related complications are HBV and HCV [18, 19]. These viruses have evolved complex, sophisticated mechanisms to invade liver cells, along with immune evasion strategies that make treatment more difficult.

HBV enters hepatocytes via the sodium-taurocholate co-transporting polypeptide (NTCP) receptor. Once inside the cell, the virus releases its genetic material as relaxed circular DNA, which is then converted into stable, covalently closed circular DNA (cccDNA) [20–22]. The cccDNA functions as a persistent reservoir that is highly resistant to current antiviral drugs. HCV employs a combination of surface receptors—including CD81, scavenger receptor class B type I, as well as claudin-1 and occludin—to invade liver cells [19, 23]. The virus replicates within specialized cytoplasmic structures that produce numerous viral variants, known as quasispecies, which evade the immune system. HDV relies on HBV surface proteins and uses NTCP as its entry route, leading to increased liver tissue damage during co-infections [21, 22]. After entering the liver, the body initiates an immune response. Liver cell pattern recognition receptors (PPR) detect viral genetic material, triggering an immune reaction that results in the release of interferons and pro-inflammatory cytokines. This response recruits various immune cells, including Kupffer cells, dendritic cells, and natural killer cells, into the liver. Ideally, virus-specific T cells are activated, and neutralizing antibodies are produced. However, HBV and HCV have developed mechanisms to interfere with these immune processes [11, 24–26]. These viruses suppress PRR signaling pathways and reduce the expression of key molecules needed for immune cells to recognize infected cells [11, 27]. HCV produces proteins that disrupt the interferon pathways and undergo rapid mutation, which slows immune responses. These strategies lead to T-cell exhaustion and increased regulatory T-cell populations, enabling viral persistence. HBV and HCV remain in the liver for prolonged periods, causing ongoing cell destruction and subsequent liver tissue regeneration (Fig. 1) [11, 26].

The ongoing inflammation activates hepatic stellate cells, which impair the antifibrotic abilities of immune cells [28], produce collagen, and lead to the development of scar tissue that progresses into fibrosis. As fibrosis advances, it damages liver architecture and obstructs drug delivery systems, eventually resulting in cirrhosis and liver cancer [3]. The process of HBV DNA integrating into host genes contributes to cancer development, while HCV causes persistent inflammation and oxidative stress, increasing cancer risk. Platelet disease involves the release of TGF-β and neutrophil extracellular traps, linking reduced platelet counts to worsening liver health [3]. Despite recent advances in treatment, several barriers remain. The liver's structural features pose challenges

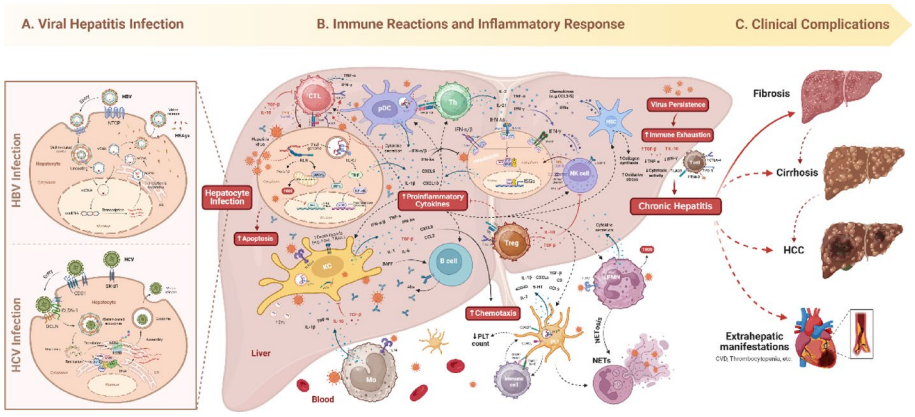

Fig. 1 Representative mechanisms of pathogenesis involved in HBV and HCV infections. Key mechanisms are discussed in the main text. Abs: antibodies; BAFF: B-cell activating factor; cccDNA: covalently closed circular DNA; CCL: CC chemokine ligand; CD: Cluster of Differentiation; CD40L: CD40 Ligand; CD62P: P-selectin; CLDN-1: Claudin-1; ER: Endoplasmic Reticulum; E1: envelope glycoprotein 1; CTL: Cytotoxic T Lymphocyte; CTLA-4: Cytotoxic T-Lymphocyte-Associated protein 4; CVD: cardiovascular diseases; CXCL: CXC chemokine ligand; C3: third complement component; DNAM-1: DNAX accessory molecule 1; EVs: extracellular vesicles; E2: envelope glycoprotein 2; FasL: Fas Ligand; GzmB: Granzyme B; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HCC: hepatocellular carcinoma; HCV: Hepatitis C virus; HSC: hepatic stellate cell; IFN: Interferon; IFNGR: IFN gamma receptor; IFNLR: Interferon lambda receptor; IL: Interleukin; IL10R2: IL10 receptor 2; IRFs: IFN Regulatory Factors; ISGs: IFN stimulated genes; JAK1: Janus kinase 1; KC: Kuppfer cell; LAG3: Lymphocyte Activation Gene 3; MAVS: Mitochondrial Antiviral-Signaling protein; MHC: Major Histocompatibility Complex; Mo: monocyte; NETs: Neutrophil Extracellular Traps; NF-kB: Nuclear factor kappa B; NK cell: Natural Killer cell; NKG2D: NK group 2 member D; NS2: Nonstructural Protein 2; NS3: Nonstructural Protein 3; NS4B: Nonstructural Protein 4B; NS5A: Nonstructural Protein 5A; NS5B: Nonstructural Protein 5B; NTCP: Sodium Taurocholate Co-Transporting Polypeptide; OCLN: Occludin; pDC: plasmacytoid dendritic cells; PD-1: Programmed cell Death protein 1; PD-L1: Programmed Death-Ligand 1; PFN: Perforin; pgRNA: pregenomic RNA; PLT: platelet; PMN: polymorphonuclear neutrophil; PSGL1: P-selectin glycoprotein ligand-1; rcDNA: relaxed circular DNA; RLR: RIG-I-like receptor; ROS: Reactive Oxygen Species; sCD40: soluble CD40; SR-B1: Scavenger Receptor class B type 1; ssDNA: single-stranded DNA; STATs: signal transducer and activator of transcription proteins; TCR: T-cell receptor; TGF: Transforming Growth Factor; Th: T helper; TIM-3: T-cell immunoglobulin and mucin domain-3; TLR: Toll-like receptor; TNF: Tumor Necrosis Factor; TRAIL: TNF-related apoptosis-inducing ligand; Treg: regulatory T cells; TRIF: TIR domain-containing adaptor inducing interferon-β; TYK2: Tyrosine Kinase 2; 5-HT: 5-hydroxytryptamine (serotonin). *Created using BioRender.com*

because its endothelial fenestrations measure 100–150 nm, restricting the passage of large drug molecules [13]. Therapeutic agents are often trapped by Kupffer cells before reaching their targets [29, 30]. The liver produces high levels of IL-10 and TGF-β, resulting in immunosuppression that reduces the effectiveness of antiviral treatments and vaccines. The viral reservoir, HBV cccDNA, remains resistant to standard antiviral drugs such as tenofovir and entecavir, requiring ongoing treatment for many patients [13, 31]. The high mutation rate of HCV causes resistance to DAAs, especially when targeting the NS5A protein [32].

In this complex scenario, nanomedicine provides innovative strategies to tackle key challenges in therapeutic delivery. Designing NPs smaller than 150 nm enables them to cross hepatic endothelial barriers while avoiding clearance by Kupffer cells. Ligand functionalization—such as attaching galactose or LDL analogs—allows targeted delivery to hepatocytes. Stimuli-responsive nanoparticles release their payloads in response to environmental triggers, such as pH or enzymes, enabling controlled, site-specific drug delivery. Co-encapsulating antiviral agents and immunomodulators within a single NP system can simultaneously suppress viral replication and reactivate T-cell responses. These strategies underscore the need for advanced delivery platforms that can overcome anatomical barriers, eliminate latent viral reservoirs, and prevent resistance.

Hepatocyte-targeted nanocarriers with immunoregulatory functions offer a promising approach to improve therapeutic effectiveness and safety.

3 Overview of nanoparticles in drug delivery

The biomedical field has adopted NPs as important tools for delivering drugs to treat complex diseases, including viral hepatitis. NPs are submicron particles ranging from 1 to 1000 nm that can be engineered to protect and deliver therapeutic agents to specific tissues or cellular locations. Moreover, NPs offer versatile properties in terms of their composition, surface functionalization, and payload capacity, making them well-suited to address the pharmacological challenges of liver-targeted therapy [33–35].

The main benefit of NPs is their ability to improve the pharmacokinetic and pharmacodynamic properties of antiviral drugs. NPs enhance drug delivery by controlling release kinetics and increasing solubility and hepatic fenestrated absorption, resulting in improved bioavailability and reduced systemic toxicity [36]. The fenestrated endothelium of hepatic sinusoids enables the passive targeting of NPs to hepatocytes, while surface ligands, such as ASGPR, scavenger receptors, or mannose receptors, facilitate active targeting [37].

Various nanoparticle types for hepatitis treatment, each offering distinct benefits, have been examined so far. Lipid-based nanoparticles (LNPs), including liposomes and solid LNPs, improve the encapsulation of hydrophobic drugs while maintaining biocompatibility. Poly (lactic-co-glycolic acid) and chitosan-based polymeric NPs provide controlled drug release and biodegradability. Metallic and inorganic NPs, such as gold, silica, and graphene oxide, also offer potential antiviral and immunomodulatory effects, along with imaging capabilities. These NPs enable tracking within biological systems for real-time monitoring of biodistribution, interact with viral particles to inhibit entry or replication, and modulate host immune responses. This dual functionality enhances both diagnostic and therapeutic strategies for managing viral hepatitis. Biomimetic carriers, including virus-like particles (VLPs) and exosomes, are promising due to their low immunogenicity and improved intracellular delivery [36, 38]. Table 1 below summarizes common nanoparticle classes studied for liver drug delivery.

Enhancing nanoparticle delivery to hepatic cells involves modifying their physicochemical properties, reducing enzymatic degradation, and minimizing off-target effects. These improvements provide a foundation for next-generation antiviral treatments that could improve hepatitis management.

Table 1 Nanoparticle Classes for Liver Drug Delivery: Composition, Advantages, and Challenges

Nanoparticle type	Composition	Advantages	Challenges
Lipid-based	Liposomes, SLNs	Biocompatibility, high drug loading	Stability, rapid clearance
Polymeric	PLGA, Chitosan, PE-Gylated NPs	Controlled release, tunable degradation	Potential immunogenicity
Metallic/Inorganic	Gold, Silica, Graphene Oxide	Imaging potential, surface functionalization	Toxicity, bioaccumulation risks
Biomimetic	Exosomes, Virus-Like Particles (VLPs)	Low immunogenicity, high targeting efficiency	Complex production, scalability

4 Types of nanoparticles used in hepatitis treatment

Although current therapies for HCV and HBV have improved over time, they still face significant limitations, including limited bioavailability, off-target effects, and inadequate delivery to infection sites, all of which reduce overall therapeutic effectiveness [39–41]. These challenges can be addressed by using NPs engineered with optimized physicochemical properties to enhance pharmacokinetics, improve targeting efficiency, and reduce side effects, making them effective carriers for antiviral therapies. Nanomedicine has emerged as a promising strategy for combating HCV and HBV by enabling targeted delivery of antivirals, diagnostic agents, and immunotherapeutics [39, 42, 43]. These agents can be either encapsulated within or attached to the surface of NPs made from various materials such as polymers, carbohydrates, lipids, metals, and metal–organic frameworks. Compared to free drugs, such nanocarriers improve therapeutic efficacy and safety by enhancing biodistribution and pharmacokinetics, thanks to their unique size and surface features. Nanoformulations offer multiple advantages: (i) protecting drugs from enzymatic degradation, (ii) reducing renal and RES-mediated clearance, (iii) targeted uptake by specific liver cell types, (iv) minimizing off-target accumulation and toxicity, and (v) overcoming drug resistance mechanisms [44].

One of the primary benefits of nanoparticle-based drug delivery is its ability to provide sustained drug release, thereby reducing the dosing frequency and minimizing related side effects. This controlled release has been achieved through methods such as PEGylation of NPs [42], regulating drug diffusion from the nanoparticle matrix [45], and using electrostatic interactions with oppositely charged carriers. Additionally, nanotechnology helps protect labile agents from enzymatic breakdown and enhances their stability in serum [46–49]. Moreover, nanoparticle formulations exhibit lower cytotoxicity than traditional drug delivery systems [45, 50]. The small size of these carriers gives them unique properties that can improve drug solubility [51], bioavailability [52, 53], chemical stability [49, 54], safety profiles [49, 55], controlled release [45, 53], cellular uptake [56–58], and targeted delivery to specific sites [49, 59, 60]. Notably, early applications of nanotechnology in HCV treatment included the use of PEGylated interferons, which significantly extended their half-life by reducing clearance [61]. Since then, extensive research has been conducted to develop nanotechnology-based approaches for the targeted delivery of anti-HCV agents, including therapeutic, diagnostic, and immunoprophylactic compounds [46, 62, 63].

The rapid advances in nanotherapeutic applications for HCV and HBV underscore their potential as key components of future treatment plans. This section provides an overview of nanomedicine-based approaches for managing hepatitis, with a focus on HCV and HBV, including vaccination, early diagnosis, and therapeutic removal of the virus. It also discusses various nanoparticle systems, stressing their composition, drug loading capabilities, targeting methods, and biocompatibility, and includes examples from both preclinical and clinical data (Fig. 2).

4.1 Lipid-based nanoparticles

Lipid-based nanoparticles (LNPs), including liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs), have been extensively researched for decades due to their high biocompatibility and ease of surface modification. They are expected to provide benefits, such as polymer-based nanoparticles. Both types share the ability

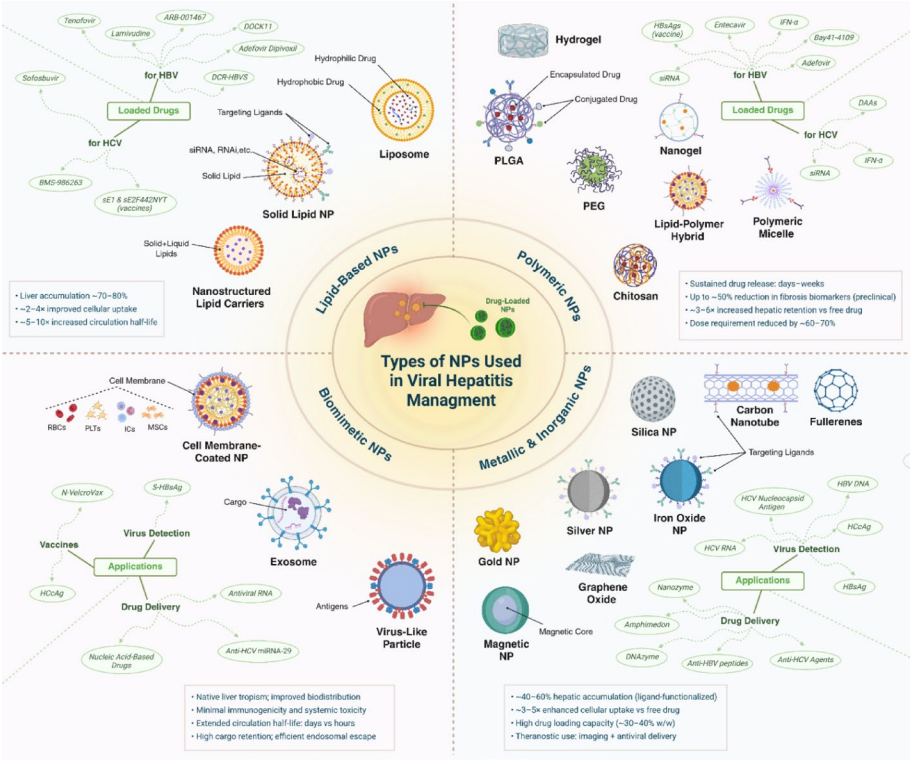

Fig. 2 Types of nanoparticle platforms and examples of their therapeutic cargoes and applications in the management of viral hepatitis. DAAs: direct-acting antivirals; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; HCcAg: HCV core antigen; HCV: Hepatitis C virus; ICs: Immune Cells; IFN: Interferon; miRNA: microRNA; MSCs: Mesenchymal Stem Cells; NP: Nanoparticle; PEG: polyethylene glycol; PLGA: poly(lactic-co-glycolic acid); PLTs: Platelets; RBCs: Red Blood Cells; RNAi: RNA interference; siRNA: small interfering RNA. *Created using BioRender.com*

to accumulate in areas with increased vascular permeability, such as sites of inflammation, infection, or tumors — a phenomenon known as the EPR effect [18]. The EPR effect of LNPs was first shown in 1985 by Morgan et al., who used indium-111-labeled liposomes for imaging deep infections and later for solid tumors [19–21]. Because they can encapsulate both hydrophobic and hydrophilic drugs in their bilayer and aqueous core, liposomes have become a promising platform for sustained drug delivery to organs with high EPR, such as the liver [22, 44, 64].

These systems can be designed to target hepatocyte-specific pathways by including ligands such as glycyrrhetinic acid or galactose moieties that bind to ASGPR receptors. For example, liposomal formulations of tenofovir and sofosbuvir have demonstrated improved hepatic uptake and prolonged plasma circulation, resulting in better antiviral activity in murine hepatitis models [33]. SLNs loaded with lamivudine also exhibited sustained drug release and increased bioavailability, leading to a significant reduction in viral load in HBV-infected hepatocyte cultures [35]. In another study, Ray et al. examined the in vivo effects of mRNA-LNP vaccines containing soluble ectodomain-1 and modified soluble ectodomain-2, which can enhance immune responses, including antibody production and T-cell activation, making them promising vaccine candidates [65].

BMS-986263, an LNP delivering siRNA targeting HSP47, was evaluated in a Phase 2 trial for treating advanced liver fibrosis in HCV-cured patients during a 12-week study. Results showed improvements in 13% (placebo), 17% (BMS-986263 45 mg), and 21% (BMS-986263 90 mg) of patients, with the BMS-986263 90 mg group showing the most

significant reduction in liver fibrosis, including improvements in ≥ 2-stage Ishak scores in five patients. The drug exhibited dose-proportional pharmacokinetics, with no accumulation, and only mild to moderate adverse events, primarily infusion-related. Baseline data suggested low levels of active fibrogenesis [66]. Another LNP-based drug, ARB-001467—a third-generation siRNA molecule encapsulated in LNPs—has completed clinical trials and showed promising results. However, in 2019, Arbutus Biopharma Corporation discontinued its development to prioritize its newer siRNA candidate, AB-729 [67]. DCR-HBVS is another LNP-formulated synthetic RNA interference (RNAi) agent currently under investigation for the treatment of chronic HBV. The drug is undergoing Phase 1 clinical trials and aims to achieve early proof-of-concept in humans [67]. A recent study showed that targeting the host factor dedicator of cytokinesis 11 (DOCK11), which is involved in the synthesis and maintenance of HBV cccDNA in vitro, using DOCK11 inhibitors loaded into LNPs effectively reduced DOCK11 expression and HBV cccDNA levels in mice without adverse effects. This suggests a promising nucleic acid-based therapeutic strategy for HBV in vivo [68].

Compared to LNPs, NLCs offer several key advantages, including higher entrapment efficiency, greater drug-loading capacity, controlled drug-release profiles, longer shelf life, effective taste masking for oral use, and increased stability in the gastrointestinal tract. Ibrahim et al. demonstrated that NLCs can encapsulate Adefovir Dipivoxil (AD), an effective antiviral agent against HBV with poor oral bioavailability and frequent dosing-related side effects. Encapsulating AD in NLCs (AD-NLCs) improved its stability, liver-targeted delivery, and intestinal absorption. Additionally, AD-NLCs significantly boosted liver uptake and the liver-to-blood ratio in a mouse model, indicating enhanced therapeutic potential and bioavailability [69].

Examples of using SLNs as a drug delivery system include employing Tristearin lipids to create solid SLNs that are later used to carry the HBV surface antigen (HBsAg). The drug is loaded into SLNs and then administered subcutaneously in animal models to evaluate its potential for HBV vaccine delivery. Results showed that SLNs loaded with Tristearin/polysorbate 80 exhibited better cellular uptake, lower toxicity, and a stronger immune response [70]. Another example involves using Dynasan 114 and soya phosphatidylcholine lipids to prepare SLNs encapsulating AD. This SLN formulation resulted in twofold and 1.5-fold increases in oral bioavailability compared with microsuspension and nanosuspension formulations, respectively. Additionally, the SLNs enhanced drug accumulation in the liver, kidneys, intestine, and stomach, highlighting their potential for improved systemic absorption and targeted organ delivery.

Despite these advantages, lipid-based systems can encounter problems such as physical instability, challenges in lipid functionalization, and rapid clearance by the reticuloendothelial system (RES), which has led to the development of PEGylated versions to enhance stealth properties and prolong circulation time [44].

4.2 Polymeric nanoparticles

The liver's essential roles in metabolism, detoxification, and immunity underscore the urgent need for effective treatment of hepatic diseases, such as HCV and HBV, and highlight the importance of developing new therapeutic and diagnostic tools. Among emerging strategies for treating liver diseases, the use of NPs as carriers for targeted drug delivery has gained significant attention due to their ability to enhance therapeutic

accuracy and reduce systemic side effects. The second type of NPs is polymeric NPs, especially those made from FDA-approved polymers such as PLGA, chitosan, and PEG, which are commonly used for controlled and sustained drug delivery, protection of bioactive compounds from degradation, and targeted delivery to specific sites in the body. Additionally, these materials offer adjustable degradation rates, precise drug-release profiles, and the capacity to co-deliver multiple therapeutic agents [71].

Chitosan-based NPs are increasingly being researched for hepatitis treatment due to their natural antiviral properties and strong mucoadhesive properties, which improve drug retention and absorption at infection sites. When encapsulating interferon-α or siRNA targeting viral genomes, these NPs showed enhanced stability, cellular uptake, and antiviral effectiveness in both HBV and HCV models [37]. PLGA NPs have also been studied for the delivery of DAAs and nucleoside analogs, enabling better targeting and reduced systemic toxicity.

Haigang et al. evaluated nanogels (Ngs) as carriers for delivering HBsAg as a prophylactic vaccine against HBV. Two types of Ngs—positively charged (Ng + +) and negatively charged (Ng-) were created using chitosan and poly-γ-glutamic acid and tested in vivo with hepatitis B mouse models (pAAV/HBV1.2 plasmid challenge). Ng (+) promoted strong humoral and cellular immunity in mice, inducing HBsAg-specific antibodies and effective viral clearance after a single dose. Ng (-) mainly stimulated cellular immunity and memory T cell responses. Ng (+) showed better stability and was more effective overall, making it a promising delivery system for long-term HBV protection and for use with weak immunogenic antigens [72].

Another study focused on developing sustained-release PLGA microspheres of Adefovir to reduce daily dosing in the treatment of chronic hepatitis B. The microspheres were evaluated for drug EE, particle size, surface structure (observed via scanning electron microscopy, or SEM), and drug-polymer interactions (assessed by Fourier transform infrared spectroscopy, or FTIR, and differential scanning calorimetry, or DSC). Key formulation factors included the drug-to-polymer ratio, polymer viscosity, and lactide content. In vitro, drug release extended up to 90 days, and in vivo tests in rats showed prolonged drug exposure, with increased Tmax, AUC, and mean residence time, and decreased Cmax, indicating effective sustained release. The formulation was stable and exhibited promise as a long-acting alternative to daily oral Adefovir [73].

The nasal mucosa has a dense network of capillaries that allows vaccines to enter the bloodstream quickly. Based on this, Subbiah et al. developed N, N, N-trimethyl chitosan NPs to deliver HBsAg through the nasal route. These NPs increased drug-loading capacity and provided sustained antigen release in vitro. Immunological tests showed that the adjuvant effect of the N, N, N-trimethyl chitosan NPs was highly stable and superior to that of standard formulations in vivo [74].

Hu et al. loaded HBsAg onto mannose-modified PLGA NPs, which released the antigen gradually and enhanced antigen presentation to lymphocytes, resulting in long-term immunity. The study demonstrated a significant increase in NP uptake by bone marrow-derived dendritic cells (BMDCs) and RAW 264.7 cells. Furthermore, subcutaneous administration of the NPs sustained humoral immunity and boosted cellular immune responses in animal models [75].

Bay41-4109 is a powerful inhibitor of HBV replication. Jiang and colleagues developed chitosan nanoparticles loaded with Bay41-4109, which significantly enhanced the

drug's bioavailability, offering an effective approach for treating HBV [76]. Recently, Hamdi et al. developed lipid-polymer hybrid nanoparticles that combine the advantages of both polymers and liposomes for the delivery of entecavir. The vitamin E-modified lipid-polymer hybrid nanoparticles demonstrated favorable physicochemical properties, confirmed through various tests. These nanoparticles specifically targeted macrophages, resulting in increased retention within these cells and reduced viral replication [77].

Despite significant advancements in the field of polymeric NPs, challenges persist, including potential immunogenicity and issues related to large-scale production, particularly in ensuring consistent batch quality.

4.3 Metallic and inorganic nanoparticles

Metal-based NPs—such as gold-NPs (AuNPs), silver, iron oxide, and graphene oxide—possess unique physical and chemical properties that make them highly versatile. They can serve not only as carriers for targeted drug delivery but also as imaging agents for diagnostics and as active antiviral agents in treatment. Their high surface area-to-volume ratio enables multifunctionality, allowing for the attachment of therapeutic, targeting, and imaging components. Additionally, some metal-based NPs may have intrinsic antiviral or anti-inflammatory effects. However, it is essential to note that most studies to date have focused on their diagnostic potential rather than their therapeutic applications.

4.3.1 Gold nanoparticles

AuNPs are promising tools for drug delivery and biosensing due to their chemical stability, non-toxicity, and biocompatibility. Their surfaces can be readily functionalized for targeted therapy, and their photophysical properties enable controlled drug release. AuNPs also serve diagnostic purposes, eliminating the need for separate imaging tools. In biosensing, they can be conjugated with antibodies to allow sensitive and specific detection of hepatitis virus antigens [43, 78, 79]. Gold NPs functionalized with antiviral peptides have shown inhibitory effects on HBV replication. Meanwhile, graphene oxide sheets have been used to co-deliver anti-HCV agents, thereby enhancing cellular uptake and mitochondrial targeting. However, concerns about the bio-persistence and potential toxicity of metallic NPs remain, requiring thorough evaluation of their pharmacokinetics and long-term effects.

Chen et al. developed an efficient method for detecting HBV genomic DNA using non-reactive PCR. This approach provided significantly better detection of low-concentration samples compared to other HBV DNA detection methods based on micro- and nanotechnology [80]. Liang et al. created an amperometric safety sensor with AuNPs on a blue-branched toluidine electrode (CHIT-TB) for detecting HBsAg. The CHIT-TB composite prevented cell leakage and preserved electrochemical activity, while the addition of AuNPs improved the sensor's electronic conductivity and overall performance [81, 82]. Sabouri et al. designed a fast, simple, and highly sensitive broad-spectrum immunoassay for detecting HBsAg. The method used a primary antibody targeting HBsAg, which was immobilized in polystyrene wells, followed by a secondary antibody conjugated to luminol-AuNPs. This approach achieved high accuracy, a low detection limit, and was cost-effective.

Ma et al. developed a novel unlabeled detection method for HCV nucleocapsid antigen using a sandwich safety sensor. The detection system employed AuNPs/ZrO2-ChI-TOS nanocomposites, which demonstrated superior biocompatibility. The resulting sensor showed high stability, increased sensitivity, and the ability to detect HCV at early stages [83]. Li et al. introduced an innovative, unlabeled, reagent-free electrochemical method for detecting HCV RNA. The approach utilized a switched hairpin DNA probe to enhance selectivity and AuNPs to amplify the electrostatic signal, thereby improving sensitivity. Key advantages of this technique include its effectiveness in complex samples and its ability to prevent false signals [84]. Moldoveanu et al. developed a novel microsensor using diamond paste modified with three types of chitosan (I, II, and III) and 10 nm AuNPs. The III/AuNPs/DP microsensor displayed increased sensitivity and a lower detection limit, making it suitable for HCV screening [85]. Wang et al. synthesized an artificial AuNPs complex designed to mimic the RNAi machinery for efficient targeted RNA cleavage. They developed a nanozyme for HCV treatment that specifically cleaves HCV RNA. This nanozyme showed resistance to proteolytic degradation, efficient internalization into human hepatoma Huh7 cells, and nontoxicity. In a xenotransplantation mouse model, it triggered detectable cellular interferon responses and demonstrated significant antiviral activity against HCV both in vitro in Huh7 cells and in vivo in mice [62].

4.3.2 Silver nanoparticles

Silver nanoparticles (AgNPs), typically measuring between 1 and 100 nm, are composed of elemental silver or silver oxide, the latter forming due to their high surface area-to-volume ratio. They have been explored as delivery carriers for both small-molecule drugs and large biomolecules and can be surface-functionalized for medical imaging applications. AgNPs also exhibit notable antibacterial activity. However, unlike gold NPs, their biomedical applications are limited by their inherent toxicity, instability, tendency to aggregate, and inconsistent behavior under different environmental conditions [86].

Liu et al. developed a platform for HBV DNA detection based on exonuclease III cleavage and silver nanocluster-dependent detection. This method was both sensitive and selective, requiring no modifications to DNA probes. It is the first report of using Exo III with silver nanocluster as a fluorescent probe for HBV DNA detection [87]. Jin et al. developed a fluorescence-enhancement strategy using AgNPs to detect HBV DNA sequences. This method demonstrated high sensitivity with a detection limit of 50 femtomolar (FM), which was 1,560 times lower than that of unenhanced fluorescence experiments [88].

Shady et al. developed an anti-HCV drug candidate using a green-synthesized AgNP compound derived from the whole extract and petroleum ether fraction of the marine sponge Amphimedon. The compound showed strong in vitro inhibitory activity against HCV NS3 helicase and protease enzymes, which are essential for viral replication. These effects resulted from direct enzymatic inhibition of the viral replication machinery, enhanced by the high surface reactivity and functional group interactions of the AgNPs. In silico analyses also supported favorable pharmacokinetic properties, further strengthening the case for the drug candidate. The study concluded that the Amphimedon extract, its petroleum ether fraction, and the synthesized nanoparticles are promising biological resources for the development of novel anti-HCV therapeutics [89]. In

parallel, Valipour et al. designed an eco-friendly, highly sensitive detection system using AgNPs combined with thiophene-functionalized graphene quantum dots (GQD-SH) to detect HCV core antigens. This hybrid nanoplatform served as an effective substrate for antibody loading, offering a cost-effective, rapid diagnostic alternative [90].

4.3.3 Magnetic nanoparticles

Magnetic nanoparticles (MNPs) are utilized in various fields, including geology, magnetic recording, and medical treatments such as magnetic hyperthermia for cancer therapy. In healthcare, MNPs aid in delivering drugs, genes, proteins, and cells directly to target areas and are also employed in imaging and biosensing applications. Their unique magnetic properties make them valuable for both diagnosis and treatment of conditions such as cancer, cardiovascular diseases, and neurological disorders.

Vaculovicova et al. developed an integrated method for isolating a specific HBV DNA fragment using MNPs, followed by immediate analysis using a capillary gel electrophoresis microchip. This approach was simple, rapid, and easy to automate [91]. Mashhadizadeh et al. created a carbon paste electrode modified with magnetic iron and AuNPs, which successfully immobilized a thiol-modified HBV DNA probe. This modified electrode allowed for the detection of low concentrations of target HBV DNA. The method was selective, reproducible, and had a low detection limit. The inclusion of MNPs and gold on the electrode surface supported efficient immobilization of the HBV single-strand DNA probe [92].

Ryoo et al. introduced a new DNA delivery system, therapeutic DNAzyme nanoparticles, to treat hepatitis C by targeting and degrading the HCV NS3 gene. The study showed that the DNAzyme-conjugated nanoparticle system effectively accumulated in the liver and specifically targeted liver cells in vivo. This nanocarrier, composed of mineral nanoparticles, was identified as a safe, effective, and functional delivery platform with significant potential to enhance DNAzyme-based therapies for hepatitis C [63]. Shawky et al. developed a new method to detect HCV RNA in serum using MNPs and unmodified cationic AuNPs. The assay successfully detected dual HCV RNA NPs in serum, yielding results comparable to those obtained with real-time PCR. It had a specificity of 96% and sensitivity of 96.5%, with a detection limit of 15 IU/ml. This method is simple, affordable, and suitable for the rapid detection of unmodified HCV RNA in serum [93].

4.3.4 Carbon nanoparticles

Carbon-based nanomaterials, including carbon nanotubes, fullerenes, and graphene oxide, are widely produced across various industries. These materials, especially GQDs, possess unique properties, including water solubility, photoluminescence, and high biocompatibility. Due to these features, GQDs are increasingly utilized in drug delivery, biological applications, sensors, photocatalysis, and adsorption [94].

Ghanbari et al. developed an electrochemical aptasensor using GQD to stabilize the aptamer on a glassy carbon electrode for the detection of the HCV core antigen. GQD, known for its unique chemical and electrochemical properties, enhanced the sensor's performance. The aptasensor demonstrated greater efficiency than other methods, offering a simple, fast, selective, and highly sensitive detection system [95]. Hu et al. developed an electrochemical sensor for detecting HBsAg, using ZnAgInS quantum dots

(QDs) as photoactive materials to generate an electric current upon excitation by a white LED. The sensor proved to be safe, sensitive, selective, and repeatable [96]. Muain et al. developed an antigen-based sensor with a reduced graphene oxide-enhanced AuNP nanocomposite to target the anti-HBV core antigen (HbcAg). This sensor effectively detected anti-HBcAg, a serological marker of prior HBV exposure, at relevant clinical concentrations [97].

Xiang et al. developed an electrochemical platform for detecting HBV-DNA using a GQD-modified glassy carbon electrode. The sensor leveraged the strong interaction between single-stranded DNA and graphene materials, achieving high sensitivity with a detection limit of 1 nM [98]. Kim et al. developed a DNAzyme-based delivery system (Dz) that utilizes a nanoscale graphene oxide complex (nGO) to inhibit NS3 gene expression and block HCV replication. This cost-effective system leverages Dz's catalytic activity to silence the HCV gene, eliminating the need for RNAi. Dz is more chemically stable than RNA and does not require modifications. The system effectively monitors HCV gene expression, selectively silences the target gene, and inhibits viral replication [99]. Ma et al. designed an electrochemical sensor with high selectivity and sensitivity for detecting HCV core antigen. The sensor used methylene graphite carbon nanocomposite electrode and peroxidase-horseradish-coated multicolored carbon nanotubes as the secondary antibody layer. The multi sHRP-DNA-CMWNT immunosorbent assay demonstrated excellent stability, reproducibility, and enhanced detection of HCV nuclear antigen in human serum [100].

4.4 Virus-like particles and biomimetic systems

VLPs and biomimetic NPs offer a particular and immunologically compatible platform for vaccine development and targeted delivery. These NPs mimic the structural features of viruses without containing infectious genetic material, enabling them to induce strong immune responses. VLPs based on HBV surface antigens have been extensively tested as both preventive and therapeutic vaccines, and recent research has explored their use for delivering nucleic acid-based drugs in chronic hepatitis models [38]. Similarly, exosomes derived from dendritic cells or hepatocytes have shown potential as carriers for antiviral RNA molecules, capitalizing on their natural tropism and ability to evade the immune system [36].

Ahmed et al. studied lentiviral vectors for integrating the full-length Core, E1, E2, and P7 genes of HCV genotype 4 into HEK293T cells, resulting in the expression of HCV structural proteins that self-assembled into VLPs. These VLPs, which resemble mature HCV virions with a diameter of 60–65 nm, were purified and characterized as potential vaccine candidates. The study highlights the successful creation of VLPs for HCV genotype 4, providing a promising platform for vaccine development, particularly in regions such as Egypt, where this genotype is prevalent [101]. Rowlands et al. introduced N-VelcroVax, an alternative VLP vaccine platform created by inserting an Affimer that recognizes a SUMO peptide tag into the N-terminus of monomeric HBV core protein (HBc). Unlike its predecessor, the tandem form, N-VelcroVax VLPs are efficiently expressed in E. coli. These VLPs successfully bind SUMO-tagged Junín virus glycoprotein, gp1, as demonstrated by structural and serological analyses. Cryo-EM confirmed binding and target-antigen capture. The findings suggest that N-VelcroVax has potential as a versatile vaccine scaffold for future applications [102].

Strengert et al. presented a method for producing recombinant S-HbsAg VLPs through transient expression in mammalian cells, followed by purification to ensure uniform antigenic presentation. The VLPs were compared to standard HbsAg samples using transmission electron microscopy and mass photometry to evaluate their assembly, composition, and antigenicity. The S-HbsAg VLPs showed improved sensitivity and specificity for detecting anti-HBs antibodies in multiplex serology, outperforming yeast- and serum-derived HbsAg. Therefore, recombinant S-HbsAg VLPs are considered the most suitable antigen for assessing HBV immunity via anti-HBs serostatus testing [103]. Drummer et al. demonstrated that a VLP vaccine incorporating the duck HBV S antigen fused to the HCV glycoprotein E2 assembles into particles that display epitopes recognized by broadly neutralizing antibodies. These VLPs induce the production of such antibodies in guinea pigs. This platform offers a promising HCV vaccine candidate suitable for large-scale, cost-effective production [104].

Ho et al. investigated the role of exosomes in the immune response to anti-HCV during HCV infection, focusing on the interaction between liver hepatocytes and macrophages. Their results showed that supernatants from Toll-like receptor-3-activated macrophages could effectively inhibit HCV replication in Huh7 cells, an effect mediated by exosomes. Moreover, blocking exosome release removed this anti-HCV effect. The study also found that Toll-like receptor-3-activated macrophages release exosomes containing members of the anti-HCV miRNA-29 family, and inhibiting miRNA-29 restored HCV replication. These findings reveal a novel antiviral mechanism in liver innate immunity and suggest the potential of exosome-based delivery systems for therapeutic applications [105].

Despite their promise, the complexity of producing and scaling up VLPs and exosome-based systems remains a barrier to their widespread clinical use. Different nanoparticle types offer unique advantages for hepatitis treatment: lipid carriers can target cells, metallic NPs offer multifunctionality, and VLPs display immunogenic properties. The choice of delivery system depends on the therapeutic goal: antiviral treatment, immune system regulation, or liver gene therapy.

4.5 Targeting strategies and mechanisms for hepatic delivery

The effective treatment of hepatitis with NPs requires a dual approach that combines passive and active targeting methods. The structure of the liver, with its porous, fenestrated blood vessels and the phagocytic activity of Kupffer cells, allows NPs to enter liver tissue through passive targeting. The process of concentrating therapeutic agents in the liver relies on physiological mechanisms, including the EPR effect and liver-specific transporters such as NTCP and OATPs, which facilitate their accumulation in the liver.

The active targeting method enhances the precision of the treatment process. Delivering treatments directly to specific liver cell groups—such as infected hepatocytes and immune cells—can be achieved by functionalizing NPs with antibodies, peptides, or sugar molecules, such as galactose. These ligands enable selective binding to receptors, such as the ASGPR, thereby improving treatment accuracy, minimizing off-target effects, and increasing overall effectiveness [36, 37].

Liu et al. found that PLGA NPs accumulated in liver tissue due to its leaky vasculature; however, adding HBV-specific PreS1 peptides to these particles enhanced hepatocyte delivery fourfold, while decreasing spleen accumulation [37]. Tang et al. used

mannose ligands to modify liposomes for targeting dendritic cells rather than Kupffer cells, thereby improving antiviral immune responses [36].

Combining passive and active targeting methods overcomes the main limitations of using passive delivery alone, as it performs better in fibrotic livers with limited vascular access and rapid clearance by Kupffer cells. To ensure efficient delivery of NPs to liver cells, it is essential to carefully control their size, keeping it below 200 nm, and maintaining a neutral or slightly negative surface charge. Additionally, the number of targeting molecules on the nanoparticle surface must be precisely controlled to improve liver accumulation and enable accurate delivery to target cells. When these factors are optimized together, nanoparticle-based treatments for hepatitis have a strong potential to enhance both safety and therapeutic effectiveness. This integrated approach offers a promising strategy for liver-targeted nanomedicine therapies.

Effective hepatitis treatment using nanoparticle systems relies on achieving high drug encapsulation efficiency and targeted delivery to hepatocytes or virus-infected liver cells, while minimizing distribution to non-target tissues. Combining passive targeting through the liver's vascular structure with active targeting via ligand-receptor interactions enables the delivery of specific compounds to liver cell populations, as noted by Liu et al. [37] and Tang et al. [36]. While both strategies aim to improve bioavailability and reduce systemic toxicity, active targeting can overcome the limitations of passive methods, such as non-specific distribution and inconsistent uptake (Fig. 3).

4.5.1 Passive targeting

The liver has a distinctive structure that facilitates more precise drug delivery. The fenestrated nature of sinusoidal endothelial cells allows NPs and macromolecules to pass from the blood space into the space of Disse because their fenestrations range from 100

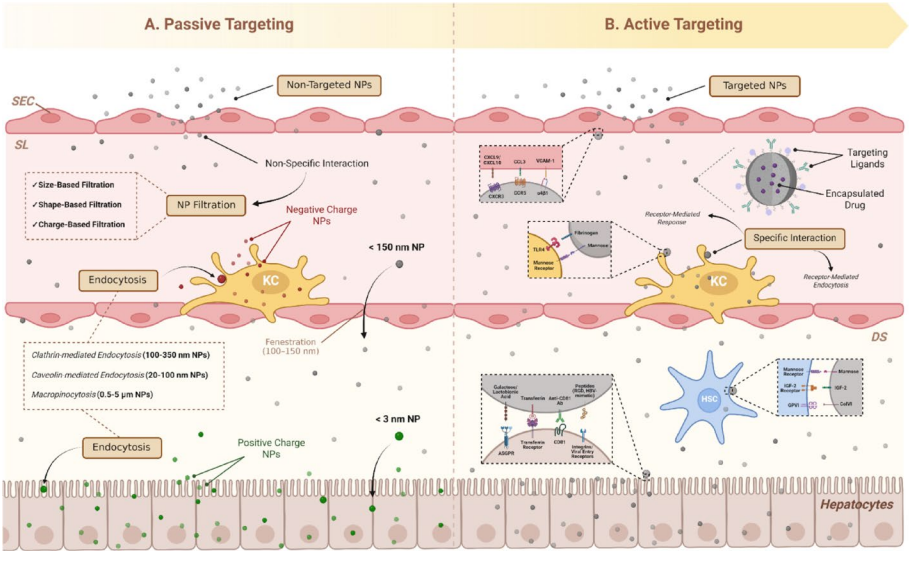

Fig. 3 Nanoparticles target liver cells via passive and active mechanisms. Ab: Antibodies; ASGPR: Asialoglycoprotein receptor; CCL: CC chemokine ligand; CCR: CC chemokine receptor; CD: Cluster of differentiation; ColVI: Collagen type VI; CXCL: CXC chemokine ligand; CXCR: CXC chemokine receptor; DS: Disse space; GPVI: Glycoprotein VI; HBV: Hepatitis B virus; HSC: Hepatic stellate cell; IGF-2: Insulin-like Growth Factor 2; KC: Kupffer cell; NP: Nanoparticle; RGD: Arginylglycylaspartic acid; SEC: Sinusoidal Endothelial Cells; SL: Sinusoidal Lumen; TLR: Toll-like receptor; VCAM-1: Vascular Cell Adhesion Molecule 1. *Created using BioRender.com*

to 150 nm. The combination of blood flow, Kupffer cell activity, and the tissue's permeability creates an optimal environment for drug accumulation.

Therapeutic agents and nanoparticles exploit the EPR effect to accumulate in liver tissue selectively. The fenestrations of dextran polymers (70 kDa) enable them to accumulate at nearly 29 times higher levels in the liver than in serum, as they can pass through these fenestrations. This principle has been used to develop formulations such as lamivudine-dextran, which delivers 50 times more drug to the liver than lamivudine alone [106–109]. The combination of methylprednisolone with dextran, linked via glycine-based connectors, enables controlled drug release in lysosomes, thereby extending therapeutic effects in liver conditions [110].

Multiple transporters specifically expressed in liver tissue facilitate the passive transport of drugs across the liver. NTCP is expressed exclusively in hepatocytes and functions to transport bile acids into cells. Researchers have modified bile acid–drug conjugate delivery to target liver tissue specifically [93, 111–115]. Hepatic drug uptake is facilitated by two transporters, OCT1 and OATPs. Large NPs (> 200 nm) and hydrophobic particles with positive surface charges are most effectively captured by Kupffer cells, which are large phagocytic cells lining the liver sinusoids. Most of these particles enter cells through endocytic processes such as clathrin-mediated endocytosis or micropinocytosis [116–128]. Cylindrical silica NPs demonstrate better liver accumulation than spherical ones, while hydrophobic surfaces promote opsonin binding, leading to Kupffer cell uptake [124–128].

Passive targeting in therapy has practical applications using lipid-based delivery systems. Liposomes (~ 100 nm) efficiently enter Kupffer cells to deliver RNAi agents, achieving 66.5% transfection efficiency in antiviral models [129–131]. PLGA NPs (160 nm) transport SYK inhibitors to the liver, reducing fibrosis and fat accumulation during hepatitis studies [132]. The healing properties of cerium oxide and gold particles, such as inorganic NPs, include anti-inflammatory effects by modulating macrophage responses during liver injury and sepsis models [133–135].

The process of passive targeting simplifies ligand development but has some limitations. The use of non-biodegradable inorganic NPs in clinical applications poses challenges because they are rapidly taken up by Kupffer cells, thereby reducing drug availability to hepatocytes. Researchers are now creating new methods that combine passive accumulation with stimulus-responsive release systems activated by liver tissue disease markers, such as pH or enzymes, to improve therapeutic effectiveness.

4.5.2 Active targeting

Active targeting enhances the delivery of therapeutic agents to specific cells by attaching ligands (such as antibodies, peptides, carbohydrates, or small molecules) to NPs, enabling receptor-specific binding to hepatocytes or immune cells. These ligands promote receptor-mediated endocytosis, intracellular drug release, and reduce off-target toxicity. Common ligand-receptor pairs and their applications are summarized in Table 2.

Moreover, carbohydrate receptors such as the ASGPR, which are highly expressed on hepatocytes, are key targets for ligand-functionalized nanoparticles. Galactose, lactose, and pullulan are commonly used to leverage ASGPR-mediated uptake, as reported by Satishchandran et al., who utilized the natural affinity of the liver-tropic AAV8 capsid

Table 2 Key Ligand-Receptor Systems in Hepatic Active Targeting

Ligand	Target receptor	Cell type
Galactose, lactobionic acid	Asialoglycoprotein receptor (ASGPR)	Hepatocytes
Mannose	Mannose receptor	Kupffer cells, DCs
Transferrin	Transferrin receptor	Hepatocytes, cancerous cells
Antibodies (e.g., anti-CD81)	CD81 (HCV co-receptor)	Hepatocytes, immune cells
RGD/HBV-mimetic peptides	Integrins, viral entry receptors	Infected hepatocytes

for hepatocyte receptors to achieve targeted gene delivery [136]. In this context, Zein nanoparticles loaded with 5-fluorouracil showed significant liver specificity in murine models, with a 31.33% improvement in targeting efficiency and a 2.79-fold increase in hepatic uptake compared to the free drug, along with sustained release and extended circulation—crucial advantages for chronic hepatitis treatments [137]. Beyond preclinical studies, active targeting is now progressing into clinical trials. For example, magnetic doxorubicin conjugates are being explored for targeting liver tumors, emphasizing the translational potential of receptor-guided delivery.

For HCV-specific strategies, PLGA NPs conjugated with the liver-targeting peptide CKNEKKNKIERNNKLKQPP delivered cyclosporine A to hepatocytes, reducing HCV replication in mice and the immunosuppressive effects of free cyclosporine [138]. Similarly, cuprous oxide NPs inhibited HCV entry by 70%, likely by masking viral envelope proteins critical for host cell interaction [139]. The combination of HCV NS3 protein and CpG adjuvant in liposomes altered the Th1 immune response, leading to a fivefold increase in IFN-γ secretion, which is beneficial for therapeutic vaccine development [140]. For gene-based approaches, the DNAzyme Dz681 delivered through MNPs demonstrated exceptional RNA silencing of HCV NS3 compared to traditional transfection methods [63, 141].

Research on HBV has used ApoA1-modified liposomes to enhance the bioavailability of baicalin, thereby reducing HBV DNA, RNA, and antigens by modulating the HNF–estrogen receptor axis [142]. Additionally, biomimetic nanoparticles guided by the PreS/2–21 peptide effectively delivered HBV-specific siRNA in preclinical models, achieving over an 80% decrease in cccDNA—successfully addressing siRNA instability and off-target effects [143].

These findings highlight promising active targeting strategies, including carbohydrate- and peptide-based hepatocyte delivery systems and immune-modulating vaccines. Future research should focus on optimizing ligands—such as using phage display libraries to identify new peptides—and advancing the clinical testing of combination therapies, including co-delivery of siRNA and TLR agonists, to accelerate translation to human applications.

5 Safety, toxicity, and regulatory considerations

Nanoparticle-based hepatitis therapies show promise but face significant safety and regulatory challenges because their physicochemical properties influence their potential to cause liver damage and affect compatibility and unintended effects [144, 145]. The clinical use of LNPs for hepatitis vaccines highlights the tradeoffs between neutral or zwitterionic lipids, which exhibit low cytotoxicity [146], and their cationic variants that can cause mitochondrial dysfunction and oxidative stress at high doses [147], as well as PEGylation, which prolongs circulation time but can lead to anti-PEG antibody

production and hypersensitivity [148, 149]. Polymeric NPs made from PLGA, and chitosan are biodegradable and considered tolerated in the liver. For instance, self-assembled FGF21 NPs comprising chitosan and heparin in CCl4 have been reported to induce an acute liver injury model, reduce serum ALT/AST, and diminish hepatic TNF-α and IL-6 levels compared with free FGF21, while not exacerbating necroinflammation, thus illustrating how optimized nanoformulation can improve therapeutic index without adding overt inflammatory or fibrotic burden [150]. However, polymeric NPs face regulatory hurdles because their degradation by-products and residual solvents may induce hepatic stress and type I IFN responses in plasmacytoid dendritic cells [151–154]. Chitosan has been shown to activate dendritic cells through cGAS-STING-driven type I IFN signaling, fostering a pro-inflammatory TH1 response and upregulation of IFN-stimulated genes, thereby establishing a pro-inflammatory milieu rather than a tolerogenic profile [154]. Consistently, repeated exposure to chitosan-coated silver NPs induced dose-dependent hepatic injury, manifested by elevated ALT/AST, oxidative stress (increased malondialdehyde, reduced glutathione), and histopathological damage affecting hepatic, renal, and splenic tissues, while only low-dose regimens avoided measurable injury [155]. The liver retention of inorganic NPs, such as gold and silica, poses specific risks because their non-biodegradable cores generate reactive oxygen species, leading to DNA damage and hepatocyte apoptosis [156–158]. For synthetic amorphous silica (E551), a 90-day oral study in Sprague–Dawley rats exposed to pyrogenic SAS (NM-203) identified a NOAEL of 5 mg/kg/day in males based on enlarged hepatic sinusoids, with a LOAEL of 2 mg/kg/day in females due to endocrine and renal changes [159]. In a complementary 28 day oral toxicokinetic and tissue distribution study comparing fumed vs precipitated food-grade SAS, repeated dosing at 2000 mg/kg/day resulted in measurable particle accumulation in the liver. Still, silica levels returned to baseline during a 90 day recovery period, and no significant histopathological liver lesions were detected, suggesting low long-term accumulation at that exposure window [160]. In the context of the immune response, silica NPs have been shown to elicit robust pro-inflammatory cytokine responses. Pyrogenic SAS (~ 14 nm) induced a stronger increase of TNF-α, IL-6, and IL-1β secretion in RAW264.7 macrophages than precipitated SAS at 10–20 μg/mL, illustrating how manufacturing route and surface state modulate innate immune activation [161]. In vivo, mesoporous silica NPs administered for 30 days promoted oxidative stress and systemic inflammation in rats, with significant elevations of serum TNF-α, IL-1β, and IL-6 and activation of TLR4/MyD88/NF-κB and JAK2/STAT3 signaling in the liver and kidney. The study documented clear fibrotic remodeling, with increased collagen deposition and upregulation of collagen I/III and profibrotic markers, reinforcing the need to monitor noninvasive fibrosis biomarkers when developing silica-based platforms [162]. The regulatory process is further complicated because various agencies classify inorganic nanomaterials as either medical devices or combination products, resulting in inconsistent toxicological testing and approval procedures [158]. The study documented clear fibrotic remodeling, with increased collagen deposition and up-regulation of collagen I/III and profibrotic markers, reinforcing the need to monitor non-invasive fibrosis biomarkers when developing silica-based platforms [162]. The regulatory process is further complicated because various agencies classify inorganic nanomaterials as either medical devices or combination products, resulting in inconsistent toxicological testing and approval procedures [158].

Biomimetic strategies, such as cell membrane coatings and VLPs, have been developed to address these issues by reducing immunogenicity and enhancing targeting [163–165]. The use of platelet membrane-coated NPs decreases complement activation while improving delivery to liver injury sites [166–169]. Exosomes utilize their natural biocompatibility to transport cargo safely [163–165]. Biomimetic systems face dual regulatory challenges because they combine biological and nanomaterial features, requiring thorough assessments of membrane proteins, vesicle purity, and viral safety, often using standardized protocols that are not yet established [166]. The future success of nanoparticle therapies for hepatitis depends on advancing biomanufacturing platforms and establishing standardized, global regulatory frameworks that balance innovation with safety.

6 Current challenges and research gaps

Although substantial progress has been made in developing nanoparticle-based drug delivery systems for the treatment of hepatitis, their translation into clinical practice is still limited. Key obstacles include unresolved scientific challenges, manufacturing and scalability issues, and broader translational hurdles related to safety, regulatory compliance, and clinical performance (Fig. 4). Nanomedicine requires comprehensive solutions to overcome these barriers and fully unlock its therapeutic potential.

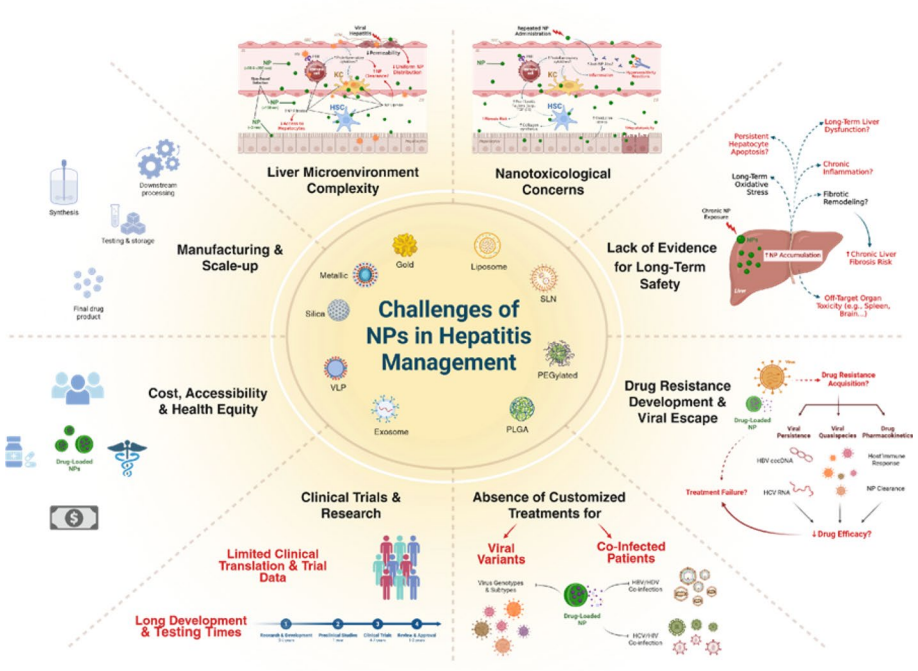

Fig. 4 Schematic representation of the significant challenges of nanoparticle-based strategies in hepatitis management. Abs: antibodies; cccDNA: covalently closed circular DNA; DS: Disse space; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HDV: Hepatitis D virus; HIV: human immunodeficiency virus; HSC: Hepatic stellate cell; HV: Hepatitis virus; ECM: Extracellular Matrix Composition; KC: Kupffer cell; NP: nanoparticle; PRR: innate pattern recognition receptors; SEC: Sinusoidal Endothelial Cells; SL: Sinusoidal Lumen; SLN: solid lipid nanoparticles; TGF: Transforming Growth Factor; VLP: virus-like particles. *Created using BioRender.com*

6.1 Standardization and scalability of nanoparticle production

Nanoparticle development faces a significant challenge in achieving reliable large-scale production. The therapeutic effects of nanoparticle treatments are significantly influenced by batch-to-batch variations in size, surface properties, and drug loading, which can alter biodistribution and effectiveness [167]. Recent reviews indicate that continuous processes, such as microfluidics and flash nanoprecipitation, outperform traditional batch methods by producing narrower size distributions and greater reproducibility, offering a clear path for scaling up both organic and inorganic systems [168–170].

Each nanoparticle type presents unique challenges in scaling up, requiring specialized strategies: (i) LNPs benefit from established liposome production methods, but scaling ionizable lipid formulations, as used in mRNA vaccines, demands precise control of mixing kinetics and solvent removal to preserve stable size and encapsulation [171]; (ii) polymeric NPs like PLGA and chitosan offer adjustable degradation profiles but face challenges related to solvent residue, with testing for residual solvent and maintaining consistent monomer ratios remaining obstacles [172, 173]; (iii) Metallic/inorganic NPs, including gold and iron oxide, require exact control of nucleation, often achieved with continuous flow reactors to prevent broad size distribution. However, equipment fouling and energy-intensive processes can limit throughput [174, 175]; (iv) Biomimetic NPs, such as cell membrane-coated NPs and exosomes, need scalable upstream cell culture and strict downstream membrane isolation under aseptic conditions that comply with Good Manufacturing Practice. This complex process currently lacks standardized protocols and reference materials [176, 177]. Complex biomimetic systems and VLP require careful attention, as even small changes in production parameters can significantly impact their bioactivity [178]. In fact, advanced analytics, such as real-time particle size monitoring and machine-learning-based processes, are increasingly used in production to detect deviations and enable automated adjustments. Still, these technologies are in the early stages of pharmaceutical manufacturing [179, 180].

6.2 Biological barriers and liver microenvironment complexity

As described above, the liver's unique microarchitecture and cellular environment pose significant challenges for efficiently delivering drugs to infected hepatocytes while minimizing unnecessary distribution to other tissues. The fenestrated sinusoidal endothelium, Kupffer cell network, and other nonparenchymal cells can filter NPs, restricting their access to hepatocytes and causing off-target clearance [29, 181, 182]. Moreover, conditions such as fibrosis, cirrhosis, and chronic inflammation further alter sinusoidal permeability and extracellular matrix composition, creating a biophysical barrier that impedes the diffusion and even distribution of NPs within the liver [183].

Beyond physical barriers, the liver microenvironment exerts dynamic immune pressures. Sinusoidal endothelial cells, Kupffer cells, pDCs, and other resident immune cells, which express high levels of pattern recognition receptors, trigger the release of proinflammatory cytokines (e.g., TNF-α, IL-1β) upon nanoparticle uptake, potentially worsening liver inflammation, altering vascular permeability, and speeding up nanoparticle clearance [182, 184, 185].

Collectively, these biophysical and immunological barriers underscore the need for multifunctional targeting strategies to ensure reliable drug delivery for effective

management of viral hepatitis. Personalized delivery systems should be developed to address the various stages of disease progression.

6.3 Immunological and toxicological concerns

Nanoparticles (NPs), recognized as foreign entities by the host immune system, can trigger undesirable immune responses, especially when administered repeatedly, necessitating careful evaluation in hepatitis-related applications. These NPs can activate innate immunity via the complement system and PRR pathways, leading to the release of pro-inflammatory cytokines (e.g., IL-1β, IL-6, TNF-α) and hypersensitivity reactions. Such immune responses can increase clearance, enhance immunogenicity, and, over time, potentially lower therapeutic effectiveness [186, 187]. The uptake of NPs by pCDs and Kupffer cells results in type I IFN production, which, although providing antiviral effects, may also cause off-target immunopathology in the inflamed liver [188]. Additionally, the long-term presence of non-biodegradable NPs, especially metallic and carbon-based nanomaterials, induces oxidative stress and disrupts mitochondrial function, thereby activating cell death pathways in hepatocytes [155]. This body of evidence underscores the significance of comprehensive immunological and toxicological evaluations in preclinical models, including standardized cytokine panels, complement activation tests, and long-term histopathological studies, to ensure the safe development of nanoparticle therapies for viral hepatitis. Approaches such as optimized surface PEGylation and the co-delivery of anti-inflammatory agents may help mitigate the immunotoxic responses associated with nanoparticles (NPs).

6.4 Long-term safety and chronic exposure

The long-term effects of NPs on the liver after repeated or prolonged use raise significant safety concerns and are largely understudied. Chronic use can cause ongoing inflammation, fibrotic remodeling, and possible organ dysfunction, indicating a need for further research in relevant models. For example, a recent study on zebrafish showed that delayed exposure to biodegradable PGA microplastics disrupted the gut-liver-brain axis. This systemic disruption was characterized by impaired barrier integrity, changes in the gut microbiota, and increased lipopolysaccharide accumulation, ultimately leading to disturbances in hepatic lipid metabolism and neurobehavioral issues, suggesting inflammation-driven systemic dysfunction [189]. Another in vivo study found that repeated intravenous injections of silica NPs in mice led to liver accumulation, activation of TGF-β1 signaling, oxidative stress, and hepatocyte death, which could contribute to the development of liver fibrosis [190]. Similarly, exposure to silver NPs has been shown to cause significant liver toxicity in rats, as evidenced by oxidative stress and elevated levels of pro-fibrotic (e.g., TGF-β1), pro-inflammatory, and apoptotic markers, all showing dose- and duration-dependent effects [191]. Additionally, metallic NPs have been shown to remain in the liver for months after exposure, indicating long-term risks of oxidative stress [192]. The accumulation of nanoparticles over time may sustain immune cell activation, leading to chronic inflammation. Therefore, comprehensive long-term toxicity studies that include biodistribution, histopathology, and cytokine analysis are crucial for determining safe dosage levels.

6.5 Resistance development and viral escape

While nanoparticle-based systems have demonstrated the ability to enhance antiviral drug potency and delivery efficiency, hepatitis viruses, particularly HBV and HCV, retain a remarkable capacity to develop resistance and evade therapeutic pressure. Despite this being a critical determinant of long-term treatment success, relatively few studies have systematically investigated nanoparticle-specific escape mechanisms or the influence of viral genetic diversity on therapeutic outcomes.

The high genotype and subtype variability of both HBV and HCV further complicates nanoparticle targeting strategies, as sequence heterogeneity can modulate drug sensitivity, influence the stability of viral replicative intermediates, and accelerate the selection of resistant variants under suboptimal therapeutic pressure. For HBV, the persistence of cccDNA within hepatocyte nuclei remains a major barrier: this reservoir may be poorly accessible to nucleoside-analogue–loaded nanoparticles [34, 193], enabling low-level viral replication to continue and promoting the emergence of resistant clones during prolonged treatment. Similarly, studies using siRNA-based nanoparticles have shown that incomplete suppression of HCV RNA can facilitate the rapid development of escape mutants, particularly in genetically diverse viral populations [194].

These challenges underscore the need for next-generation nanoparticle designs capable of achieving more complete viral suppression across genotypes, improving nuclear or subcellular targeting, and integrating strategies to minimize resistance development. Continued innovation in nanoparticle engineering and a deeper understanding of genotype-linked viral evolution will be essential for overcoming these translational barriers.

6.5.1 Absence of customized treatments for viral subtypes and co-infected patients

Hepatitis viruses exhibit significant genetic diversity, including multiple HBV genotypes and HCV subtypes, which require subtype-specific and personalized pharmacological treatments. These variants differ in their replication rates, antigenicity, and drug susceptibility. However, current nanoparticle-based therapies rarely account for this diversity, which impacts both drug response and nanoparticle-cell interactions, thereby limiting their effectiveness across different patient groups. In HBV, genotypes A and D respond differently to IFN-α, with genotype A achieving a sustained response more frequently [195]. Genotype A and B patients consistently achieve higher sustained virological response and HBsAg loss rates with pegylated IFN-α compared with genotypes C and D, even when baseline viral load and ALT are similar [196, 197]. Despite this, nanoparticle drug systems for HBV have not been designed to leverage these genotype-specific sensitivities. Most nanoparticle-based platforms for HBV—whether lipid nanoparticles (LNPs) or GalNAc-conjugated siRNA systems—have been developed and evaluated primarily in East Asian populations, where genotypes B and C predominate. However, stratified analyses by genotype are rarely performed. This raises important concerns regarding the generalizability of these findings to regions where other genotypes, such as A, D, or E, are more prevalent, including large parts of Africa and Europe [197, 198]

A similar challenge exists for HCV. Distinct HCV subtypes exhibit characteristic patterns of resistance-associated substitutions (RASs), particularly against NS5A inhibitors [198, 199]. Despite this, nanoparticle-based antiviral approaches for HCV are commonly optimized using prototype laboratory strains or a narrow range of genotypes. This may

compromise their effectiveness in geographical regions where more resistance-prone subtypes—such as 4a or 3b—predominate.

Taken together, these observations highlight a critical translational gap: without rigorous consideration of genotype and subtype diversity during nanoparticle design and evaluation, therapeutic efficacy may vary substantially across populations. Addressing these issues through broader genotype coverage, resistant-variant modeling, and geographically diverse clinical sampling will be essential for developing nanoparticle-based therapies with true global applicability.

Furthermore, co-infection significantly complicates the therapeutic landscape of viral hepatitis, introducing biological and pharmacological variables that remain insufficiently addressed in current nanomedicine research. For instance, individuals co-infected with HIV and HCV often experience accelerated liver fibrosis progression and altered pharmacokinetics of antiviral agents [200, 201]. Despite these well-documented clinical challenges, nanoparticle platforms have not yet been engineered to support dual antiviral delivery or to accommodate the profoundly modified immune and inflammatory environment characteristic of HIV/HCV co-infection.

Similarly, patients co-infected with HBV and HDV represent a substantial and clinically high-risk population. The unique virological interplay between HBV and HDV, including enhanced immune activation and hepatocellular injury, likely affects nanoparticle biodistribution, uptake pathways, and immunological interactions [202]. Yet, nanoparticle-based therapeutic strategies rarely incorporate co-infection models or evaluate the need for co-delivery systems or combination nano-therapies, which may be essential to achieve adequate viral suppression in this setting.

These gaps highlight an important direction for future research. Nanomedicine studies should incorporate stratified cohorts of co-infected patients, alongside systematic evaluation of drug–drug interactions, immune activation markers, and fibrosis progression dynamics. Such data will be critical for informing optimized dosing strategies, safety assessments, and risk–benefit profiles, thereby enabling the rational design of nanoparticle platforms tailored to these high-need, clinically complex populations.

6.6 Limited clinical translation and trial data

Despite promising preclinical results, the number of clinical trials remains limited, with only a few nanoparticle-based antivirals for hepatitis advancing to early-phase trials, and even fewer progressing to phase II/III [203]. Most NP-based hepatitis therapies remain at intermediate technology readiness levels (TRL 3–5), with only prophylactic LNP vaccines reaching late-stage clinical deployment. RNAi therapeutics for HBV provide a clear example of this trajectory. To date, ARC-520 is the only lipid nanoparticle-delivered siRNA candidate to have reached Phase II clinical evaluation in chronic HBV patients, showing significant reductions in HbsAg levels but with variable responses and safety concerns in multi-dose studies [204, 205]. ARC-520 showed moderate but variable HBsAg declines in HBV patients, with deeper responses in some HBeAg-positive individuals (up to ~ 3.5 log10 {10}10 IU/mL) but more modest declines in HBeAg-negative cohorts [204]. Other mechanistic work in patients and chimpanzees demonstrated that a substantial fraction of HBsAg can originate from integrated HBV DNA, which is not efficiently targeted by the ARC-520 siRNA triggers, helping to explain the attenuated response in many patients. Although early-phase studies in healthy volunteers suggested

an acceptable safety profile for ARC-520, the overall program was terminated after lethal toxicity of the EX1 dynamic polyconjugate delivery vehicle was observed in non-human primates, highlighting how delivery-related toxicities can block TRL progression even when on-target antiviral effects are demonstrated. In contrast, newer GalNAc-conjugated siRNA platforms such as imdusiran (AB-729) avoid intravenous LNP carriers and have demonstrated robust and durable HBsAg reductions of around 2 log10 {10}10 IU/mL in virally suppressed HBV patients, with minimal direct cytokine induction in human whole blood, supporting a favorable intrinsic safety profile. Even with these improvements, functional cure rates remain limited and often require combination approaches, emphasizing that many HBV nanomedicines currently occupy mid-range TRLs without yet crossing the threshold to late-phase pivotal trials and regulatory approval. The subsequent Phase 2b MONARCH combination study, which administered ARC-520 alongside entecavir and pegylated IFN-α, faced enrollment challenges and reported early termination of several cohorts due to insufficient HbsAg clearance and safety issues [206]. In contrast, no nanoparticle-enabled delivery systems for HCV antivirals have advanced beyond Phase I trials, highlighting gaps in robust efficacy endpoints, standardized viral load biomarkers, and genotype-specific patient stratification [207]. clearance and safety issues [206]. In contrast, no nanoparticle-enabled delivery systems for HCV antivirals have advanced beyond Phase I trials, highlighting gaps in robust efficacy endpoints, standardized viral load biomarkers, and genotype-specific patient stratification [207].

Overall, barriers to clinical translation include nanoparticle pharmacokinetics, individual variability, and limited data on long-term safety. The current regulatory framework for nanomedicine approval is unclear, as it requires detailed toxicology and efficacy data that are not typically needed for conventional drugs. Within hepatitis nanomedicine, a major unresolved question concerns RNAi-based HBV therapies. While multidose ARC-520 and GalNAc-siRNAs such as imdusiran (AB-729) achieve deep HBsAg suppression, it remains unclear whether this alone can reprogram immunity toward functional cure, particularly given that a substantial fraction of HBsAg derives from integrated HBV DNA, which is not addressed by cccDNA-centered approaches [204, 208–210]. Additionally, divergent preclinical findings regarding hepatic accumulation, oxidative stress, and fibrosis risk for inorganic and hybrid NP platforms emphasize the need for harmonized, clinically relevant, long-term toxicology evaluation. Addressing these knowledge gaps through genotype-stratified trials, the inclusion of advanced disease and co-infected cohorts, and transparent TRL mapping will be key to transitioning from promising proof-of-concept to clinically adoptable and affordable therapies [209–211].

6.7 Cost, accessibility, and health equity

High manufacturing costs, complex supply chains, and restrictive intellectual property landscapes continue to limit access to nanoparticle-based therapies in many low- and middle-income countries (LMICs), creating substantial barriers to the equitable implementation of these technologies for hepatitis treatment [212, 213]. Although point-of-care nano-diagnostic platforms have demonstrated promise for affordable HBV screening, translating similarly complex nanoparticle drug-delivery systems into resource-constrained clinical settings remains a major challenge [214].

A significant portion of these barriers stems from the exceptionally high chemistry, manufacturing, and control (CMC) requirements associated with small nucleic acid therapeutics and their nanoparticle carriers. These platforms demand rigorous standards for purity, sequence fidelity, structural confirmation, and physicochemical characterization of both the oligonucleotide cargo and the delivery vehicle [211]. The associated costs are further amplified by the need for specialized formulation infrastructure, cold-chain logistics, and advanced analytical quality-control systems, all of which are limited or unavailable in many LMICs.

Recent evaluations of small-nucleic-acid–based therapies emphasize that scaling nanoparticle platforms for population-level hepatitis control will require far more than scientific refinement alone. Sustainable implementation will depend on innovative strategies for technology transfer, regionalized manufacturing capacity, differential pricing schemes, and simplified production workflows to avoid widening global health disparities [209, 211]. Addressing these economic and infrastructural constraints is therefore essential to ensure that advances in nanomedicine translate into meaningful public health impact worldwide.

7 Artificial intelligence in nanomedicine for hepatitis therapy

Artificial intelligence (AI) is increasingly reshaping the development pipeline for nanoparticle (NP)-based therapeutics for hepatitis B and C by enabling high-resolution, data-driven optimization of NP design, biological performance, and clinical translation. Machine-learning approaches have demonstrated the capacity to analyze high-dimensional datasets linking NP physicochemical attributes—such as size, surface charge, lipid composition, ligand density, hydrophobicity, and structural topology—with their biological behavior in vivo, including hepatic tropism, immune recognition, complement activation, and endosomal escape efficiency [215–218]. These models allow rapid in silico screening of thousands of candidate formulations, identifying optimal design windows that balance efficient hepatocyte uptake with minimal off-target accumulation or immunogenicity.

Furthermore, physics-informed neural networks and multiscale computational models now simulate NP–biological interactions at molecular, cellular, tissue, and organ levels. Such tools can forecast biodistribution patterns, circulation kinetics, liver microenvironment penetration, and drug-release dynamics under different physiological states, including fibrosis, steatosis, portal hypertension, and inflammation [219]. This is particularly valuable for viral hepatitis, where liver architecture and immune microenvironments vary substantially across genotypes, disease stages, and co-infection profiles. These computational platforms significantly reduce the experimental burden associated with empirical formulation optimization, allowing researchers to prioritize only the most promising NP candidates for wet-lab evaluation [220].

AI also accelerates the development of multifunctional nanoparticle systems by supporting multi-objective optimization across parameters such as cargo encapsulation efficiency, stability, manufacturability, and therapeutic synergy. Leveraging reinforcement-learning frameworks, evolutionary algorithms, and Bayesian optimization, AI can guide the rational integration of complex payloads—including siRNAs, antisense oligonucleotides, small-molecule antivirals, immunomodulators, or even CRISPR/Cas genome-editing components—into next-generation nanoplatforms capable of

addressing HBV cccDNA persistence or HCV genotype-specific replicative kinetics [215–217].

Together, these advances demonstrate that AI is becoming a central backbone of precision nanomedicine for viral hepatitis, enabling iterative refinement of NP systems that are more effective, predictable, and scalable for clinical translation.

8 Future directions and clinical translation

Hepatitis therapy utilizing nanotechnology holds promise for enhancing current antiviral treatments and enabling innovative approaches, such as immunotherapy, gene editing, and precision medicine. Future advancements in nanoparticle-based hepatitis management are expected to advance through personalized nanomedicine and multifunctional platforms that address the current translational limitations of available therapies. Researchers are now developing NPs that combine antiviral drugs, immunomodulators, and liver-protective agents into a single delivery system. These platforms aid in fighting viral clearance and inflammation while supporting tissue repair. NPs designed to release drugs in response to pH or oxidative stress have demonstrated greater efficacy in treating chronic liver inflammation [36]. Nanotherapies combining DAAs with TNFα inhibitors and IL-10 mimetics demonstrate synergistic benefits by simultaneously regulating viral load and safeguarding liver tissue.

Furthermore, HCV and HBV treatments could be combined with gene editing technologies. Researchers are increasingly using NPs as non-viral delivery systems for CRISPR/Cas tools, which interfere with viral replication or correct host genetic vulnerabilities. Polymeric nanocarriers, along with lipid NPs, effectively deliver guide RNAs and Cas9 proteins to liver cells, achieving high delivery efficiency with minimal immune response [35]. This innovative approach enables the development of functional cures for hepatitis B and D infections. With recent advances in artificial intelligence technology, these methods could be further used to improve HCV and HBV treatments. Optimization of nanoparticle formulations has made revolutionary progress thanks to AI tools that predict drug interactions with carriers, as well as their stability and release profiles. Computational models enable the creation of personalized nanocarriers tailored to individual patient features, including liver disease status, genetic makeup, and medical conditions [37].

Regulatory agencies, including the EMA and the FDA, are working to establish standardized clinical testing protocols for nanomedicines. Future clinical studies should focus on long-term safety monitoring, hepatic distribution studies, and pharmacodynamic evaluations. Medical trials involving collaboration between clinicians, materials scientists, and pharmacologists will help define regulatory pathways and develop scalable manufacturing methods. Additionally, the use of NPs extends beyond treatment, as they are increasingly employed in vaccine production through virus-like particles and in diagnostic imaging using fluorescent or magnetic nanoparticle technology. These diagnostic methods provide rapid HCV detection tools, which, when combined with post-treatment surveillance, help reduce disease progression and transmission rates. Table 3 below outlines the future direction of NPs and their potential impact.

Table 3 Emerging Nanotechnology Strategies for Advancing Hepatitis Management

Future direction	Potential impact
Multifunctional nanoplatforms	Co-delivery of antivirals, immunomodulators, and liver-protectants
Gene editing delivery (e.g., CRISPR/Cas)	Functional cure of chronic hepatitis via genome editing
AI-driven nanoparticle design	Personalized, optimized nanocarriers for enhanced efficacy
Regulatory and clinical trial advancement	Streamlined approval and safer clinical translation
Nanoparticle-based vaccines and diagnostics	Improved hepatitis surveillance and immune protection

9 Conclusion

Nanoparticle-based drug delivery systems offer transformative potential for the management of viral hepatitis by overcoming key pharmacological and biological limitations associated with conventional antiviral therapies. Nanotechnology improves the solubility and bioavailability of therapeutic agents, enables controlled and targeted delivery to hepatocytes, and supports multifunctional therapeutic strategies tailored to HBV and HCV infections.

Advances across lipid-based carriers, biodegradable polymers, inorganic nanostructures, and biomimetic systems have expanded the therapeutic landscape, supporting the delivery of antivirals, immunomodulators, and gene-editing tools. Preclinical findings demonstrate enhanced antiviral efficacy, reduced systemic toxicity, and improved hepatic accumulation. Emerging technologies—including stimulus-responsive systems, multifunctional co-delivery platforms, and CRISPR-loaded nanocarriers—highlight the promise of personalized and curative approaches to hepatitis treatment.

Despite this progress, translation into clinical practice remains limited. Major barriers include challenges in nanoparticle manufacturing scale-up and standardization, unresolved safety and immunogenicity concerns, heterogeneous patient responses, and insufficient clinical trial evidence. Addressing these hurdles will require harmonized regulatory frameworks, robust long-term safety assessments, and innovative design strategies that account for the complexity of liver disease and patient-specific variables.

Future progress may also be accelerated by integrating nanoparticle engineering with artificial intelligence, gene therapy, and immune modulation technologies. Advancing next-generation hepatitis therapeutics will depend on interdisciplinary collaboration among virologists, nanomedicine scientists, materials engineers, and clinical hepatologists.

Overall, nanoparticle-based platforms represent a highly promising path toward developing safer, more effective, and precision-targeted therapies for HBV and HCV. With continued scientific innovation and coordinated clinical efforts, these technologies could play a central role in achieving future global hepatitis elimination goals.

Abbreviations

AD	Adefovir Dipivoxil
ASGPR	Asialoglycoprotein receptor
HBc	Core protein
cccDNA	Covalently closed circular DNA
DOCK11	Dedicator of cytokinesis 11
DAAs	Direct-acting antivirals
EPR	Enhanced permeability and retention
AuNPs	Gold-NPs
GQDs	Graphene quantum dots
HAV	Hepatitis A Virus
HBV	Hepatitis B Virus

HbsAg	Hepatitis B Virus surface antigen
HCV	Hepatitis C Virus
HDV	Hepatitis D Virus
HEV	Hepatitis E Virus
LNPs	Lipid-based nanoparticles
MNPs	Magnetic nanoparticles
Ng	NanoGel
NPs	Nanoparticles
NLCs	Nanostructured lipid carriers
PRRs	Pattern recognition receptors
PEG	Polyethylene glycol
QDs	Quantum dots
RNAi	RNA interference
AgNPs	Silver nanoparticles
siRNA	Small interfering RNA
NTCP	Sodium taurocholate Co-transporting polypeptide receptor
SLNs	Solid lipid nanoparticles
VLPs	Virus-like particles
WHO	World Health Organization

Supplementary Information

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Supplementary Material 1

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