

Nanotechnology in the Battle Against HCV: Innovations in Diagnosis and Therapy

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Abstract

Hepatitis C virus (HCV) continues to affect millions globally, posing significant public health challenges. Direct-acting antivirals (DAAs) such as sofosbuvir and daclatasvir revolutionized HCV therapy with high cure rates and fewer side effects compared to older treatments. However, ongoing research has led to the development and FDA approval of newer antiviral combinations with improved efficacy, shorter treatment durations, and broader genotype coverage. A decade ago, interferon- α and ribavirin used alone or in combination were the mainstay of therapy. The limited therapy effectiveness and severe systemic side effects were the cause mainly in treatment discontinuation. Today, newer regimens are safer and more effective, yet challenges remain, including drug resistance, high costs, and limited access in low-resource settings.

Nanotechnology has emerged as a promising approach to overcome these challenges. Nanocarrier-based drug delivery systems can enhance liver-specific targeting, improve bioavailability, reduce systemic toxicity, and potentially enable simultaneous diagnosis and therapy. This review highlights recent advances in HCV treatment strategies, with a particular focus on the role of nanotechnology in enhancing therapeutic outcomes and diagnostic capabilities for better management of HCV infection.

Keywords

HCV, nanotechnology, RNA interference, DAAs, siRNA, SVR

Introduction

Hepatitis C virus (HCV) infection remains one of the leading causes of liver-related morbidity and mortality worldwide. The severe complications; including liver cirrhosis, hepatocellular carcinoma, and systemic manifestations, resulted from the fact that HCV is a "silent" disease. It often remains asymptomatic until advanced stages, making early detection and intervention challenging.

Although direct-acting antivirals (DAAs) have significantly improved treatment outcomes, complete eradication of the virus across all patient populations remains a challenge. Issues such as drug resistance, relapse, and limited accessibility in certain regions continue to hinder global elimination efforts. Additionally, while DAAs are generally well tolerated, some patients still experience adverse effects or fail to respond optimally to therapy. Emerging therapeutic strategies aim to enhance treatment efficacy while minimizing side effects and reducing treatment duration. Among these, RNA interference (RNAi) technology has gained attention for its potential to selectively silence viral gene expression and inhibit viral replication. When combined with nanotechnology, RNAi delivery becomes more targeted and efficient, opening new avenues for both treatment and early detection of HCV.

1. Hepatitis C virus

Hepatitis C virus (HCV) is a blood borne pathogen that is subdivided into 7 genotypes [1], with over than 60 subtypes [1]. Each genotype has specific geographical distribution and the major genotype distributed in Egypt is type 4. HCV is one of the major causes of chronic liver diseases, such as, hepatitis, cirrhosis and hepatocellular carcinoma. It considered as a silent killer because symptoms may not appear for decades. HCV affects about 170 to 210 million people all over the world [2-5], where Egypt has the highest epidemic ratio in the world; 17.5% is the ratio of positive HCV antibodies in Africa according to World Health Organization survey.

HCV is a member of family Flaviviridae (hepaciviral genus) that is small in size (40-60 nm in diameter), spherical in shape. Its genome is a positive single stranded RNA (+ssRNA) that can directly be translated to the corresponding viral proteins without transcription and formation of mRNA [2, 3, 6]. The virus RNA is not integrated into the host genome and localized in the cytoplasm of the infected liver cells. It subdivided into 7 genotypes [1] and over than 60 subtypes due to its wide genetic variations related to virus nature and quiescence's.

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HCV is a blood born pathogen that is mainly transmitted by parental route from infected, unsterile medical, dental instruments and percutaneous treatment. In rare cases *via* sexual intercourse and from mother to child during labor (both less than 5%) [7]. Egypt has the highest incidence rate of HCV infections, mostly due to parenteral anti-schistosome therapy in which multiple intravenous (i.v.) injections were given to patients long time ago, before disposable syringes were available [7-9].

HCV enters hepatic cells by binding to specific receptors; low density lipoprotein receptors (LDLR), glycosaminoglycans receptors (GAGs), human scavenger class B type I receptors (SR-BI) and host cellular CD81 receptors. That followed by endosomal formation after activation of clathrin proteins through coupling with adjacent claudin CLDN1 and occludin (OCLN) to engulf the virus. Then, endocytosis and uncoating to release the viral genome into cellular host cytoplasm is the next step in the virus pathogenesis.

By further processing within the endoplasmic reticulum (ER), the viral RNA is directly translated into the corresponding precursor viral polyproteins. The precursors are then cleaved by both viral and host cellular proteases into four structural and six non-structural (NS) proteins. The structural proteins are involved in the formation of viral structures, whereas non-structural proteins play an important role in polyproteins processing and viral replication. Each viral protein has a specific role in formation of the replication complex. Then the replication complex initiates synthesis of new viral genome strands, positive and negative RNA strands. The negative RNA strand works as a template for the positive one for increasing viral replication rate and infection. The viral replication complex is also responsible for formation of new infectious units, helps in assembly, maturation and release of new viruses to attack other cells [10-12]. **Figure 1** and **Table 1** demonstrate a simplified life cycle of HCV and the specific roles of viral proteins in multiplication of the virus.

2. HCV Diagnosis and Treatment

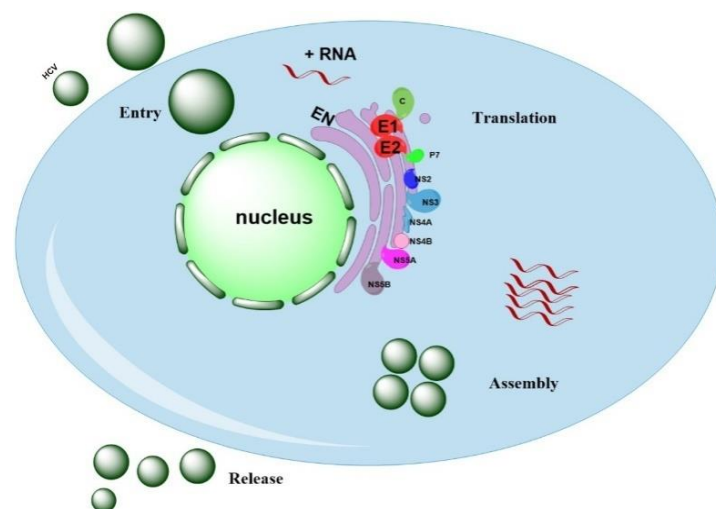
Diagnosis of HCV depends mainly on serological tests, such as antigen antibody test to detect the presence of the host antibodies in blood in response to viral infections. The serological tests are followed by qualitative polymerase chain reaction (PCR) assay to confirm the results and a quantitative one to measure the viral RNA load. In addition, liver function tests, such as measuring alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are routinely performed to monitor the liver health status (**Table 2**).

DAA are standard therapy nowadays. Decade ago, systemic administration of subcutaneous (s.c.) PEGylated interferon (INF) with oral ribavirin were considered the standard therapy [15-17]. Monitoring patients is extremely important to detect the treatment response. Liver function tests, quantitative and qualitative PCR are required to predict the appropriate dosage regimen and liver health status. Normal ALT, AST and low viral load indicate good response to treatment.

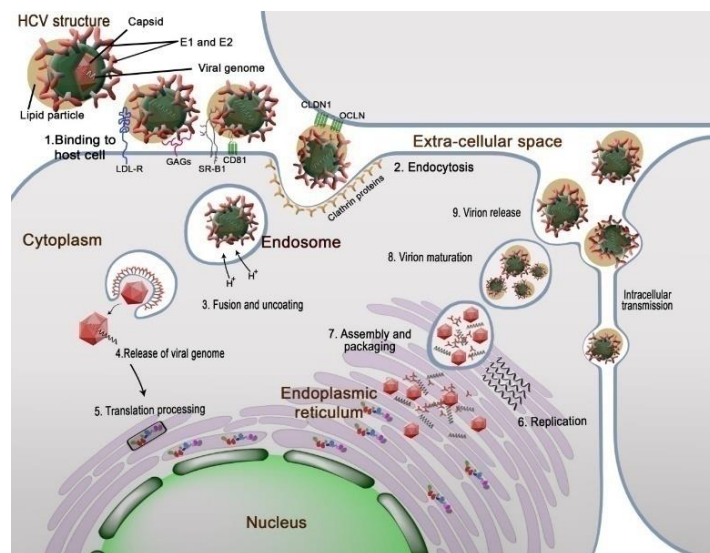
New drugs and methods are investigated to increase the sustained virological response (SVR). SVR is expressed as the percentage of patients with undetectable HCV RNA after specific period of time (depends on type of treatment and dosage regimen) after cessation of the therapy. Simeprevir is one of the protease inhibitors that has been approved by the United States Food and Drug Administration (FDA) for oral administration and was found to increase SVR to 66.7-80%, as compared to 22.7% for INF/ribavirin [18]. In addition, the direct antiviral sofosbuvir which approved by FDA in October 2013, was found to increase

SVR to 87% when given in a dose of 400 mg once daily for 12 week [19].

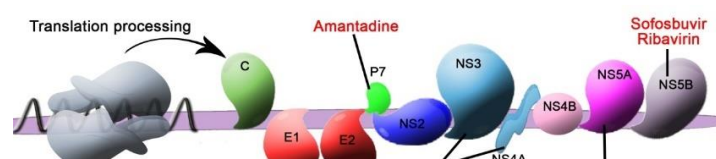
Several antiviral and host-targeted agents that were under investigation for hepatitis C virus (HCV) treatment have seen varying outcomes in clinical development. Daclatasvir and ledipasvir, both NS5A inhibitors, have progressed from clinical trials to full regulatory approval and are now widely used in combination with sofosbuvir [20-22]. Demonstrating high sustained virologic response (SVR) rates across multiple genotypes. Miravirsin, a miR-122 inhibitor, showed promising antiviral activity in early-phase trials but has not advanced further in clinical development [23]. Asunaprevir, an NS3 protease inhibitor, has been approved in certain countries, particularly in



A)



B)



C)

Figure 1. Pathogenesis of hepatitis C virus in hepatocytes; A) and B) are brief and detailed life cycle, translation, replication, and transmission of the HCV. C) Structure of viral genome and the main targets of various.

Table 1: Role of HCV proteins in viral pathogenesis and their importance as major targets for HCV therapeutics [3, 4, 7, 10, 11, 13].

Viral components		
Structural proteins	C protein	Form viral capsid which coats and protects viral genomic RNA. It is also considered as the major component that binds viral RNA during viral assembly.
	E1 and E2	Envelope glycoproteins that target host antibody response and help in viral entry into liver cells through binding to host cellular receptors.
	P7	Polypeptide localizes at plasma membrane and responsible for formation of viral ion channels, which influence membrane permeability to ions, virus entry and assembly.
Non-structural proteins	NS2	Form the viral NS2/3 protease enzyme.
	NS3	Form the viral NS2/3 protease enzyme. It also had helicase/NTPase activity that provides energy source for unwinding of double stranded RNA by hydrolyzation of nucleoside triphosphate (NTP).
	NS4A	Cofactor for viral protease enzyme.
	NS4B	Involved in concentration and optimization of replication complex, and modulate NS5A hyperphosphorylation.
	NS5A	Involved in replication complex and regulation of viral cellular pathways including cell growth, survival, cell cycle control, apoptosis (programmed cell death), and formation of new infectious HCV units. In addition, it modulates host cell interferon response. It can be phosphorylated at many sites by host cellular kinases and bind directly to NS5B to modulate its enzymatic activity.
	NS5B	Form the viral RNA-dependent RNA polymerase (RdRp) enzyme, the viral polymerase.
Viral enzymes	NS2/3 protease	Cleaves the junction between NS2 and NS3 proteins for formation of RNA replication complex.
	NS3/4A serine protease	Cleaves HCV polyproteins at NS3/NS4A, NS4A/NS4B, NS4B/NS5A, and NS5A/NS5B junctions for formation of RNA replication complex and genome translation.
	RdRp	RNA-dependent RNA polymerase (RdRp) which has characteristic right handed fingers-palm-thumb structure responsible for initiation of both positive and negative RNA strand synthesis <i>via denovo</i> mechanism and catalyze the synthesis of complementary missed RNA strands.
Ribonucleic acid	Cis acting RNA element	Contribute in binding of both cellular and viral proteins during translation and RNA replication processes. In HCV, they are mainly found in 5' and 3' untranslated region (UTRs), but, sometimes extend into some coding sequences[14].
Non-viral components		
Receptors	CD81 receptors, human scavenger class B type I receptors (SR-BI), low density lipoprotein receptors (LDLR) and glycosaminoglycans (GAGs)	CD81 is a transmembrane protein with extracellular loop that binds to HCV E2 glycoprotein with high affinity. SR-BI is a membrane protein that found in many cells and tissues, including liver cells, to facilitate uptake of cholesterol from high density lipoproteins in liver. LDLR and GAGs are considered as targets for HCV entry and adsorption. HCV E2 glycoproteins interact with GAGs and LDLRs followed by engagement of virus with SR-BI and CD81, and then interact with viral protein claudin-1 and occludin for viral entry. LDLRs and very low-density lipoproteins are essential for viral adsorption into hepatocytes.
Proteins	Cyclophilin A	Cellular proteins present in all eukaryotes and prokaryotes that interact with immunosuppressant cyclosporine A (CSA), which functions as cellular cofactors in replication of NS5B protein. It is considered as a critical component in formation of viral RNA polymerase-dependent replication complex. It also has peptidylprolyl isomerase activity which catalyzes isomerisation of peptide cis bond into trans form which facilitates protein folding. It also interacts with NS5A protein and stimulates its binding to viral RNA.
Ribonucleic acid	MicroRNAs (miRNAs)	Endogenous non-coding mRNAs present in the liver. Generally, it functions as gene expression regulator, where miR-122 is the most abundant miRNAs that interact with HCV genomes and support their translation and prevents their degradation.
Enzyme	Inosine-5'-monophosphate dehydrogenase (IMPDH)	Catalyzes the rate limiting step in <i>denovo</i> biosynthesis of guanine nucleotides, NAD (Nicotinamide adenine dinucleotide)-dependent formation of guanine monophosphate GMP pools.

combination with daclatasvir, and has shown high SVR rates in specific patient groups such as those on hemodialysis [20]. Danoprevir, another protease inhibitor, was explored in early trials but has since been discontinued due to the emergence of more effective treatments [24]. Chloroquine, although initially investigated for its antiviral properties, has not shown sufficient efficacy against HCV and is no longer pursued for this indication [25]. Similarly, nitazoxanide, an antiparasitic with some in vitro activity against HCV, has not advanced beyond early studies [26].

Alisporivir, a cyclophilin A inhibitor, targeting host factors, showed potential in early trials but its development was halted due to safety concerns [27]. While many of these drugs were originally tested in triple therapy regimens or in combination with other antivirals to enhance SVR and minimize side effects, most have been surpassed by newer, highly effective direct-acting antiviral agents that are now the standard of care.

Table 2. Outcome summary for some HCV drugs.

Drug	Mechanism\ Class	Development Status	References
Daclatasvir	NS5A inhibitor	Approved; widely used in combinations	[20-22]
Ledipasvir	NS5A inhibitor	Approved; used in fixed-dose form with sofosbuvir	[23]
Miravirsen	miR-122 inhibitor	Phase IIa completed; no further advancement	[23]
Asunaprevir	NS3 protease inhibitor	Approved in combos; high SVR in trials (incl. dialysis patients)	[20]
Danoprevir	NS3/4A protease inhibitor	Development discontinued	[24]
Chloroquine	Ant malarial agent	Abandoned for HCV; interest later shifted to SARS-CoV-2	[25].
Nitazoxanide	Antiparasitic agent	No recent clinical development for HCV	[26]
Alisporivir	Cyclophilin inhibitor	Early-stage investigated; no longer in development	[27]

Short interfering RNAs (siRNAs), microRNAs (miRNAs), and deoxyribozymes have been explored as potential therapeutic strategies for hepatitis C virus (HCV) through RNA interference (RNAi) mechanisms. RNA interference is a naturally occurring cellular process that induces post-transcriptional gene silencing by promoting the specific degradation of target messenger RNA (mRNA), thereby preventing the translation of viral proteins. Among these approaches, siRNAs have demonstrated the ability to directly target and suppress HCV RNA replication in vitro and in preclinical models. MiR-122, liver-specific microRNA essential for HCV replication, has been a key focus of research. The miR-122 inhibitor miravirsen entered clinical trials and showed dose-dependent antiviral activity, though it has not yet reached clinical approval. Deoxyribozymes, catalytic DNA molecules capable of cleaving RNA, have also been studied for their potential to selectively degrade HCV RNA, but their development remains largely experimental. While RNAi-based therapies showed early promise, challenges such as delivery efficiency, off-target effects, and long-term safety have limited their clinical advancement. Nevertheless, these approaches continue to provide valuable insights into host-virus interactions and may still hold therapeutic potential in the context of chronic HCV or future antiviral strategies [28].

3. Role of Nanotechnology in Diagnosis of HCV

Hepatitis C virus (HCV) diagnostics typically rely on blood tests that detect either viral genetic material (RNA) or viral proteins (antigens/antibodies). While several conventional detection techniques exist, including automated enzyme-linked

immunosorbent assays (ELISA) and polymerase chain reaction (PCR)-based methods, they are often limited by high cost, need for complex equipment, and dependency on trained personnel. These constraints are particularly problematic in rural or low-resource settings, where the burden of HCV is often the greatest. Consequently, nanotechnology-based diagnostic platforms are being explored as innovative alternatives due to their potential for enhanced sensitivity, speed, and miniaturization [7, 29].

One notable approach involves DNA chip technology coupled with gold nanoparticles (AuNPs) for visual gene detection. In this technique, complementary oligonucleotide probes are immobilized on a chip to capture viral RNA [30, 31], followed by hybridization with AuNP-labeled detection probes. The formation of a two-probe sandwich prevents AuNP aggregation and retains the red color, which can be intensified using silver staining to enhance visual detection [32, 33]. While this method offers specificity and a visually interpretable result, it requires

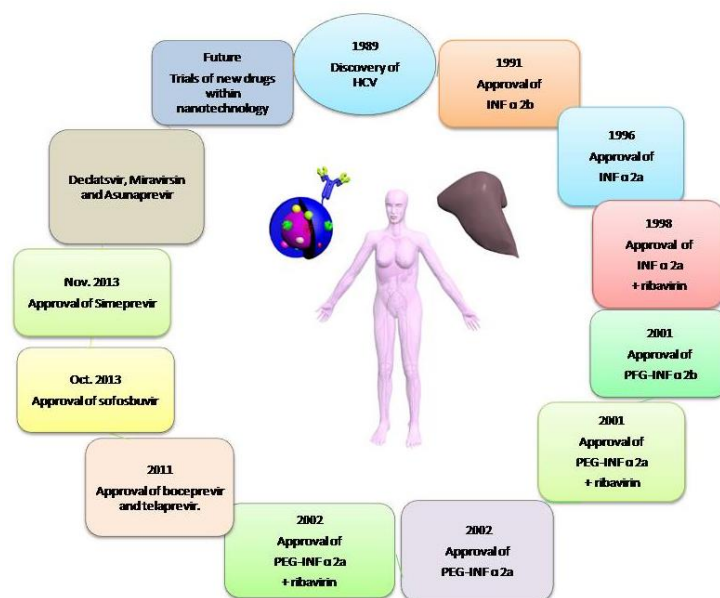


Figure 2. Brief history of development of HCV therapeutics.

multiple handling steps (e.g., serum processing, hybridization, silver enhancement), which may limit its feasibility in decentralized settings. The requirement for clean environments, chip preparation, and washing steps adds operational complexity that may hinder field application unless these steps are significantly streamlined.

Another early detection method targets the HCV core antigen (HCV-cAg), which appears in serum within 1–2 days of viral RNA. One nanotechnology-enhanced approach utilizes spectroscopic detection of Raman scattering from spatially reinforced gold nanostructures [34-36]. Coomassie Brilliant Blue G-2504 (CBBG), a common protein dye, was employed to quantify protein levels spectroscopically [36]. Although this platform enables ultra-sensitive detection, its reliance on Raman-active substrates and advanced spectroscopy equipment restricts its use to specialized laboratories. Translation to clinical settings, especially those with limited infrastructure, would require the development of compact, portable systems consider an area that still under development.

Surface plasmon resonance (SPR)-based colorimetric assays, particularly those using citrate-capped AuNPs, offer a more accessible alternative. These particles undergo a red-to-blue color change upon aggregation, which is influenced by the hybridization of viral DNA to specific probes. The assay demonstrated 92.2% sensitivity and 88.9% specificity, with a detection limit of 50 copies/reaction and a visible color change in under one minute [37]. In contrast to real-time PCR (Cobas TaqMan), which requires 2–3 hours and costs approximately \$100 per test, this AuNP-based method costs roughly \$5 and delivers faster results [38–40]. Given its low cost, speed, and visual readout, this method holds strong potential for deployment in low-resource settings, provided nanoparticle stability and assay reproducibility are optimized [39].

In areas where HCV co-infection with hepatitis B virus (HBV) is common, dual detection platforms have also been explored. One such method uses DNA-directed assembly of AuNPs to form aggregates that are visible under transmission electron microscopy (TEM) [41]. Although this approach allows for the simultaneous detection of HBV DNA and HCV RNA, its dependence on high-resolution imaging technology (i.e., TEM) limits its practicality outside research facilities. On the other hand, protein microarrays coated with viral antigens—paired

with AuNP-labeled secondary antibodies and silver staining—offer a more promising alternative [42]. These chips can detect specific IgG antibodies to HCV and HBV, producing visible black spots within 40 minutes and with a detection limit below 3 ng/mL [42]. While more practical than TEM-based methods, their widespread application still depends on cost reduction, chip shelf-life extension, and environmental stability.

Electrochemical immunosensors represent another category of promising nanotechnology-based diagnostics. One design uses a graphitized mesoporous carbon-methylene blue (GMCs-MB) nanocomposite as an electrode base, on which AuNPs are deposited to immobilize monoclonal antibodies against HCV-cAg [33, 38]. Detection is enhanced using horseradish peroxidase-DNA-conjugated carbon nanotubes (CNTs) as secondary antibodies [43]. This sandwich-type immunoassay achieves a detection limit of 0.01 pg/mL and a dynamic range from 0.25 pg/mL to 300 pg/mL [44]. Although the sensitivity is remarkable, challenges remain regarding electrode fabrication, stability, and standardization. Furthermore, electrochemical readouts require signal processors that may not yet be available in mobile, battery-powered forms suitable for rural deployment.

Table 3. Summary of the available techniques for HCV detection and diagnosis [7].

Anti-HCV antibody	Enzyme immunoassays (EIAs)	The first initial screening test that can detect the presence of HCV antibodies in the serum of patients but only after 4-6 weeks of infection. It cannot differentiate between acute, chronic or resolved infection and can yield both false positive and false negative results.
	Recombinant immunoblot assay (RIBA)	Highly specific test is used as confirmatory tests or double-check test for a positive EIA result.
HCV RNA tests	Qualitative tests	Indicate the presence of virus and results are expressed as positive or negative (for the presence of HCV-RNA).
	Quantitative tests	Measure the amount of viral RNA.
HCV genotype test		It is performed using direct sequence analysis and reverse hybridization technology. Both genotyping and subtyping tests are important tests to predict proper treatment regimen, duration and the possible barriers to drug resistance.
Biochemical indicators	Alanine aminotransferase (ALT)	Liver Function tests are used to monitor the liver health status. ALT enzyme level is high in about two third of people with chronic HCV.
	Aspartate aminotransferase (AST)	AST level increases in chronic cases, but at lesser extent than ALT, and at higher extent in cirrhosis. A high level of AST is a sign to liver damage.
	Alkaline phosphate and gamma-glutamyl transpeptidase (GGT)	Alkaline phosphate and GGT are usually normal, where elevated levels are indication to progression to cirrhosis.
	Other biochemical indicators in HCV patients	Normal levels of lactate dehydrogenase and creatine kinase. Increasing serum mitochondrial creatine kinase (MtCK) reported as risk indicator for hepatocarcinogenesis in chronic patients with HCV[45]. Low platelet and white blood cells count and high level of serum globulins indicate severe fibrosis or cirrhosis. Albumin, prothrombin, bilirubin levels are normal till late stage of hepatitis. Slightly elevated levels of iron and ferritin levels. High levels of hepatic iron deposits (HID) is associated with HCV-3 and viral-induced hepatic steatosis and in the other genotypes it may indicate late stage of liver disease or systemic iron overload[46].
Liver biopsy		It is not used for diagnosis, but to determine the severity of diseases and stage of fibrosis and liver damage.

Table 4. Nanotechnology approaches are utilized for detection of HCV infections.

Technique/Drug	Composition	Remarks	Ref.
Visual gene detection technique on a glass chip (sandwich hybridization method)	3'-mercapto oligonucleotide derivatives, 5'-amino oligonucleotide derivative, AuNPs, silver solution	Sensitive and specific method that depends on gene detection by PCR and southern blot and exclude the need for high expensive fluorescence scanner	[32]
HCV-cAG detection (enhanced roman light scattering method)	SR-Au (spatially reinforcement gold nanoparticles) nano array and CBBG dye	Linear relationship between CBBG concentration and roman intensities of CBBG-antigen complex	[47]
Visual detection of red-blue shift of AuNPs (surface plasmon resonance phenomenon)	Gold nanoparticle and DNA primer	High sensitivity reaches 92.2%, specificity of 88.9%, detection limit down to 50 copies/reaction and change in color within 1 min were observed	[39]
AuNPs aggregation detection under TEM	AuNPs and DNA oligonucleotides to both HCV and HBV	Aggregation of gold nanoparticle indicated a positive result that was observed under TEM. Using qualitative PCR technique, HBV and HCV showed amplification zones at 431 bp and 323 bp, respectively	[41]
Visual silver detection of antigen-antibody coupling in protein chip	AuNPs, HCV and HBV antigens and silver solution	Silver staining was used for enhancing visual viral detection as black spots in about 40 min with detection limit under 3 ng/mL IgG was reported	[33]
Electrochemical immunosensor with the application of nanomaterials utilizing sandwich hybridization method	Graphitized mesoporous carbon-methylene blue nanocomposite, AuNPs, monoclonal HCV core antibody and a horseradish peroxidase-DNA-coated carboxyl multi-wall carbon nanotube.	High selectivity and sensitivity with detection limit 0.01 pg/mL and the amperometry signal increased with increasing antigen concentration from 0.25 pg/mL to 300 pg/mL	[44]

4. Role of Nanotechnology in Treatment of HCV

The management of hepatitis C virus (HCV) infection has undergone a paradigm shift with the advent of direct-acting antivirals (DAAs), which now represent the cornerstone of therapy. DAAs target specific nonstructural proteins of HCV, disrupting viral replication and achieving sustained virologic response (SVR) rates exceeding 95% across diverse patient populations [48]. Pan-genotypic regimens such as sofosbuvir/velpatasvir and glecaprevir/pibrentasvir are recommended as first-line treatments [49, 50], typically administered orally over 8 to 12 weeks. Pre-treatment assessment includes evaluation of HCV RNA levels, liver fibrosis staging, and screening for coinfections (e.g., HBV, HIV), renal function, and potential drug-drug interactions [51]. Special considerations are necessary for patients with decompensated cirrhosis, advanced renal impairment, or HIV coinfection. Despite the high efficacy and tolerability of current therapies, reinfection remains a concern in high-risk populations, and no vaccine is currently available. Long-term follow-up is advised for patients with advanced fibrosis or cirrhosis, given the continued risk of hepatocellular carcinoma even after viral eradication [52].

Direct-acting antivirals (DAAs) target key nonstructural proteins essential for HCV replication, thereby disrupting the viral life cycle with high specificity and potency. HCV encodes a single polyprotein that is post-translationally cleaved into structural and nonstructural proteins, among which NS3/4A protease, NS5A, and NS5B RNA-dependent RNA polymerase are the principal therapeutic targets [53]. NS3/4A protease inhibitors (e.g., glecaprevir, grazoprevir) block the proteolytic cleavage of the HCV polyprotein, thereby preventing the formation of the viral replication complex [50, 54]. NS5A inhibitors (e.g., velpatasvir, pibrentasvir, daclatasvir) interfere with both viral RNA replication and virion assembly by targeting the multifunctional NS5A phosphoprotein, though their precise mechanism remains incompletely understood [21, 55]. NS5B inhibitors are classified into two types: nucleoside analogues (e.g., sofosbuvir), which act as chain terminators after incorporation into the nascent viral RNA by NS5B polymerase, and non-nucleoside inhibitors, which bind to allosteric sites on NS5B and induce conformational changes that impair polymerase activity. The combination of agents targeting different viral proteins enhances antiviral potency, reduces treatment duration, and minimizes the risk of resistance development [52]. Resistance-associated substitutions (RASs), particularly within NS5A, can impact treatment efficacy,

although pan-genotypic regimens have demonstrated robust activity even in the presence of certain RASs [51].

Decade ago, Interferon- α , a glycoprotein involved in antiviral activity and immune activation, was the main treatment. INF modulates immunity by its effect on the immune system. The antiviral activity is due to the ability of INF to increase the viral antigen presentation, so, facilitate its recognition by T-lymphocytes. Also, INF increases resistance of uninfected host cells to viruses and regulates cell growth by promoting apoptosis. In addition, in response to INF, production of large amount of protein kinase R that phosphorylate proteins and form inactive protein complex that affect synthesis of both host and viral proteins. RNase enzyme is also activated in response to kinase activation which causes lysis to both viral and host RNA within cells [56-59].

Two types of INF- α are used in treatment of HCV infections, INF- α -2a and α -2b. Chemical modifications to INF using different molecular weights of poly (ethylene glycol) (PEG) could improve INF pharmacokinetic profile and reduce dose frequency and side effects (Table 5). PEGylation of INF- α -2a with 40 kDa mono-methoxy-PEG (Pegasys®) resulted in slower absorption and decreased rate of systemic clearance that prolonged the half-life and improved dosing frequency of INF [56, 57, 60]. In addition, PEGylation could increase SVR to 32-39% versus 19% for non-PEGylated INF. PEGylation of INF- α 2b with 12 kDa PEG has been also utilized and it is commercially available (Pegintron®) [57].

The non-specific delivery of INF to different body tissues is the major reason for its side effects, such as, depression and hormonal problems, which eventually lead to treatment cessation. Conjugation of INF- α to hyaluronic acid (HA) was found to increase the body immune response due to the increased expression of 2',5'-oligoadenylate synthetase 1 (OAS 1, a member of synthetase family, essential proteins for immune imitation response to viral infections) enzyme in murine liver tissue by 60%, as compared to INF- α or Pegintron® [61]. Physical binding of INF- α to gold nanoparticle followed by chemical conjugation to thiolated HA was investigated to create HA-gold nanoparticles/INFs complexes [62]. Higher serum stability for 7 d after complex injections and a comparable antiviral activity to Pegintron® were noticed. Also, a higher expression of OAS 1 enzyme in liver tissues more than interferon gold nanoparticles and Pegintron® was observed.

Antiviral peptide nanocomplexes (APN) were designed for treatment of HCV/HIV co-infected patients. The virucidal C5A, an amphipathic positively charged α -helical peptide that is derived from HCV NS5A nonstructural protein was used. Electrostatic coupling between cationic C5A derivative p41 and biodegradable anionic poly(amino acid)-based block copolymer methoxy-PEG-block-poly (α , β -aspartic acid) (PEG-PLDn) [63, 64]. The self-assembled p41/PEG-PLD₂₀ APN was found to have *in vitro* antiviral activity to both HCV and HIV viruses, stable at physiological pH and form uniform nanoparticles of ca. 35 nm in diameter (compared to 900 nm of aggregated positively charged p41 in diluted aqueous solution). Partial protection of p41 peptide against proteolytic trypsin digestion and higher protease stability to APN were observed. Cellular uptake and monolayer cytotoxicity of free p41 compared to APN was studied. Lower uptake with no cytotoxicity and dose dependent accumulation in both HUV7.5 and macrophage using APN were indicated. Higher antiviral activity with significant reduction to HCV viral load after 1 h

pre-incubation of HUV7.5 cells with the APN compared to free p41 (in concentration of 10 μ M p41) were observed [64].

Short-interfering RNA (siRNA) is a double stranded RNA of approximately 21-23 bp in length with two overhanging nucleotides. The activity of siRNA depends mainly up on binding to RNA-induced silencing complex (RISC), followed by unwinding the double stranded RNA with the endonuclease enzyme [65, 66]. Micro RNAs (miRNAs) are endogenous non-coding mRNAs present in the liver. Generally, they function as gene expression regulators by promoting mRNA degradation and inhibiting protein synthesis. The most abundant miRNA is miR-122 which is a liver-specific miRNA account for about 70% of total miRNAs [67, 68], regulates HCV life cycle and helps in multiplication of the virus [23, 67, 68]. However, nucleic acid-based drugs can be easily degraded within minutes in systemic circulation. Also, they have a low cellular uptake due to their high molecular weights and anionic nature. Hence, efficient delivery of siRNA *in vivo* depends mainly on the use of viral vectors [69, 70], hydrodynamic injection [71] and non-viral vectors [72]. In particular, non-viral vectors have several advantages over other approaches, especially the ability to control their properties via modifications to the chemical structures of their building blocks.

Nanosized siRNA liposomes (siRNA nanosomes) have been prepared by using different types of lipids to create anionic, cationic and neutral liposomes, based on the type of the lipid utilized. siRNA lipoplexes have been prepared using cationic lipid (1,2 dioleoyl-3-trymethylammonium-propane (DOTAP)), cholesterol and protamine sulfate [73]. It was found that formulations which have siRNA-to-lipid ratio $\geq 1:10.56$ cleared HCV to about 85% and maintained cell viability to about 90% [73]. Also, siRNA NPs repeatedly sonicated to decrease its size up to 100 nm, and it was found that nanosomes with particle size ≥ 7.4 nm were efficiently delivered to ca. 100% of cells. Sonicated nanoparticles show higher liver deposition and gene silencing with no observed changes in zeta potential. Sonication for 30 s decreased particle size down to 122 ± 26 nm compared to 211 ± 26 nm and size decreased with higher rate with increased sonication time down to 99 ± 17 nm with 5 min sonication. By measuring effect of lyophilization on siRNA nanosomes stability and particle size it was found that particle size decreased from 642 ± 25 to 127 ± 11 nm and the zeta potential decreased from 48 ± 4 to 19 ± 2 mV by lyophilization and gradually increased with time but no evidence on effect on stability with longer time storage [74].

Other formulations were also prepared using a mixture of DOTAP, cholesterol and protamine sulfate for complexation of multiple siRNAs (thirteen), to avoid the possibility of mutant escape and drug resistance after treatment with single siRNA [75]. By further sonication, particle size reduced to 100 nm with a zeta potential of 10-15 mV. It was found that siRNA nanosomes were delivered efficiently to cells and were stable for over seven days, whereas only six of the tested siRNAs had antiviral activity. Treatment with siRNAs combination (si321, si359 that had the highest antiviral activity) decreased the viral load under the detectable level for over one month, as compared to detectable viral level with the single siRNA. By testing the drug toxicity in BALB/c mice, it was found that, a dose of 5 mg/Kg is well tolerated, and showed no evidence of liver toxicity or activation of host interferon system [75].

Lactosylated cationic liposomes composed of phosphatidylcholine (PC) and lactosylated-phosphatidylethanolamine (LA-PE) were designed for targeted

siRNA delivery [65]. The galactose terminus of lactose is considered as a ligand for the asialoglycoprotein receptors which are overexpressed on surface of hepatocytes [76]. The lactose residues strongly increased the transfection efficiency, barely activated interferon immune system, and inhibited the expression of HCV proteins in mice models. Others galactosylated cationic liposomes that are composed of different lipids were also designed and showed inhibition to Ubc13-gene by 80% after administration of 0.29 nmol/g of Ubc13 encoded siRNA that complexed with the galactosylated liposomes [77].

Other formulations based on DOTAP and cholesterol mixture, but with apolipoprotein A-I (apo A-I) as a targeting ligand were developed for siRNA delivery [78]. Apo A-I is a protein component of high-density lipoprotein which is considered as a typical serum protein that binds to receptor scavenger receptor BI (SR-BI). It was found that apo A-I increased the cellular uptake in the liver rather than other organs *in vivo*. The same lipoplexes were prepared but with modified siRNA, 2'-OMe-modification on its sense strand in two U sequences, showed higher resistance to nucleases, higher antiviral activity

maintained for 6d was noticed with the protein with 2.5 mg/kg of siRNA-core specific lipoplexes and a 50% inhibition noticed with higher concentration reach to 10 mg/kg [78].

AMO122 is a methoxy derivative to anti miR-122 oligonucleotide with phosphorothioate modifications. AMO122 were encapsulated into pH-sensitive cationic lipid as YSK05 utilizing a pH sensitive multifunctional envelope-type nanodevices (MEND) [79]. YSK05-MEND resulted in high liver selectivity, as compared to the free form of anti-miR-122 that has rapid renal excretion and lower accumulation in the liver. It shows particle size and zeta potential a rounds 71 ± 2 nm and 3.1 ± 0.5 mV respectively. No toxicity with AMO122-YSK05-MEND up to 100 nm of AMO122 compared to lipofectamine preparation LFN2K/AMO122 that shows high toxicity with more than 50 nm of AMO122.

Deoxy ribozymes or DNAzymes with nucleotide sequence complementary to viral NS3 protein in conjugation to iron nanoparticles were designed for HCV gene silencing. Aminated surface dextran coated iron oxide NPs had a zeta potential of 7.6 mV, spherical and were *ca.* 50 nm in diameter. Down regulation of NS3 protein expression *in vitro* was dose dependent manner to 91 % at higher doses. Also, there is a little evidence of cytotoxicity up to 23 μ M to magnetic nanoparticles [80].

Table 5. Nanotechnology approaches are utilized for treatment of HCV infections.

Technique/Drug	Composition	Remarks	Ref.
Interferon- α (INF/AuNPs-HA complex)	AuNPs and thiolated HA	High serum stability for 7 d within injection and a high expression of OAS 1 enzyme in liver tissue	[62]
C5A derivative (p41/PEG-PLD ₂₀ antiviral peptide nano-complexes (APN))	p41 and methoxy-PEG-block-poly (α,β -aspartic acid) ₂₀ (PEG-PLD)	Stable at physiological pH, 35 nm in diameter and higher antiviral activity with significant reduction in HCV viral load after 1 h. pre-incubation of HUV7.5 cells with the APN compared to free p41 (in concentration of 10 μ M p41)	[64]
siRNA (Cationic lipoplexes)	DOTAP, cholesterol and protamine sulfate	siRNA-to-lipid ratio $\geq 1:10.56$ can clear 85% HCV and maintain 90% cell viability. Particle size decreased down to 122 ± 26 nm and 99 ± 17 nm with 30 s and 5 min sonication, respectively	[73]
Multiple siRNA (Cationic lipoplexes)	DOTAP, cholesterol and protamine sulfate	The antiviral activity is a concentration dependent. Higher efficacy showed in 100 pmol siRNA <i>versus</i> 50 pmol. A dose of 5 mg/Kg body weight is well tolerated, show no evidence of liver toxicity or activation of host interferon system in BALB/C mice model. si321, si359 showed the highest antiviral activity.	[75]
siRNA	DOTAP, cholesterol and apo A-1	Percent of 65-75 % inhibition to the viral core protein with the unmodified siRNA lipoplexes and 95% gene silencing maintained for 6 d with the modified siRNA lipoplexes.	[78]
AMO122	YSK05-MEND	Particle size and zeta potential a rounds 71 ± 2 nm and 3.1 ± 0.5 mV respectively. No toxicity with AMO122-YSK05-MEND up to 100 nm of AMO122 compared to LFN2K/AMO122 that shows high toxicity with more than 50 nm of AMO122.	[79]
DNA oligonucleotides	RNaseA enzyme and AuNPs	NS5A gene suppression from 65 % to more than 99 % with nanozymes concentration of 0.068 nM.	[81]

Table 6. Mechanisms of action and major drawbacks of some available and old therapies for treatment of HCV [21, 50-52, 54, 55].

Name	Class	Mechanism of action	Main drawbacks	Notes
Glecaprevir, Grazoprevir	NS3/4A protease inhibitors	Block the proteolytic cleavage of the HCV polyprotein, thereby preventing the formation of the viral replication complex	Hepatic Impairment, Risk of elevated liver enzymes, Resistance	Contraindicated in moderate to severe liver dysfunction (Child-Pugh B or C) due to increased risk of hepatotoxicity. There's potential for interactions with many commonly used drugs (e.g., statins, anticonvulsants, antiretrovirals).
Velpatasvir, pibrentasvir, daclatasvir	NS5A inhibitors	Interfere with both viral RNA replication and virion assembly by targeting the multifunctional NS5A phosphoprotein, though their precise mechanism remains incompletely understood.	Low genetic barrier to resistance, potential interactions (e.g., acid-reducing agents with velpatasvir can reduce absorption).	
Sofosbuvir	NS5B inhibitors	as chain terminators after incorporation into the nascent viral RNA by NS5B polymerase, and non-nucleoside inhibitors, which bind to allosteric sites on NS5B and induce conformational changes that impair polymerase activity	Not recommended in patients with eGFR <30 mL/min/1.73 m ² or on dialysis. High cost, drug-drug interactions	Approved by FDA in October 2013. It has no action on host cell DNA polymerase and is given at a dose of 400 mg once daily.
Simeprevir	Protease inhibitor	Antiviral drug	Potentiate actions of drugs that are eliminated through the liver by CYT3A enzyme	Approved by FDA in November 2013 to be taken within triple therapy at a dose of 150 mg.
PEGylated interferon (PEG-INF)	Chemically modified interferon alpha	Antiviral action, immune activator	Blindness, depression and major blood, eye, nerve, and thyroid problems	Pegasys and Peginteron are commercially available PEG-INF α -2a and PEG-INF α -2b, with 40 and 12 kDa PEG moieties, respectively.
Ribavirin	Purine nucleoside analogue	IMPDH inhibitor, antiviral drug and Immunomodulator	Dose limiting anemia	Levovirin is the L-enantiomer of ribavirin that has higher safety, well tolerated and does not cause hemolytic anemia. Viramidine (taribavirin) is a liver targeted prodrug of ribavirin.
Boceprevir and Telaprevir	Protease inhibitor	Antiviral drugs		New generation of protease inhibitors that has been recently approved by FDA as simeprevir .
Amantadine	Ion channel blocker	Antiviral drug	CNS side effect due to dopaminergic and adrenergic actions	Monotherapy failed to reduce HCV RNA but could normalize ALT in some patients.

Other nanozymes that are composed of AuNPs loaded with DNA oligonucleotides in sequence complementary to HCV genome sequence with RNase an enzyme on its surface were designed. The nanozymes showed high stability against protease enzymes, no aggregation with about 48 ± 1.9 nm in size. *In vitro* assay showed NS5A gene suppression from 65% to > 99% with nanozymes concentration of 0.068 nM at day 1, 3, 5, 7. Also, the *in vivo* assay in mice model showed suppression in HCV RNA up to 99.6% with no toxicity or interferon induction [81].

5. Translational challenges of nanotechnology in HCV diagnosis and therapy

Despite the growing body of research showcasing nanotechnology's potential to revolutionize HCV diagnosis and treatment, the vast majority of these approaches remain

confined to **preclinical or in vitro investigations. While laboratory results are often promising demonstrating enhanced sensitivity, targeted delivery, and improved pharmacokinetics, the clinical translation of these platforms remains limited. This disconnects between laboratory innovation and real-world application is driven by several critical challenges:

5.1. Regulatory and safety barriers

Nanomaterials, particularly when used in vivo, pose unique regulatory concerns. Their novel physicochemical properties often result in unpredictable biological interactions, raising questions about biodistribution, long-term toxicity, and immunogenicity. Regulatory agencies such as the FDA and EMA have yet to establish comprehensive guidelines tailored specifically for nanomedicine, contributing to delays in approval and commercialization.

Table 7. HCV therapy regimen widely used for genotype 4 in Egypt [82-84]

Regimen	Duration	Use in Cirrhosis	Notes
Glecaprevir/ Pibrentasvir	8-12 weeks	Yes (compensated)	Not for decompensated cirrhosis
Sofosbuvir/Velpatasvir	12 weeks	Yes (compensated)	Avoid with severe renal impairment
Sofosbuvir + Daclatasvir	12-14 weeks	Yes	24 weeks + ribavirin if cirrhosis
Grazoprevir/Elbasvir	12 weeks	Yes	Rarely used; mainly for GT1/4

5.2. Manufacturing, scalability, and cost

While many nanotechnologies demonstrate high performance in small-scale studies, scaling up production under Good Manufacturing Practice (GMP) standards remains a major hurdle. Batch-to-batch variability, stability issues, and complex fabrication protocols can significantly increase development costs and limit accessibility, particularly in low-resource settings where HCV burden is the highest.

5.3. Infrastructure limitations

Some nano-based diagnostics, such as those involving Raman spectroscopy, surface plasmon resonance, or transmission electron microscopy, require highly specialized equipment and trained personnel. This limits their practical use outside of centralized laboratories and undermines their applicability in point-of-care settings, especially in underserved regions.

5.4. Limited clinical evidence

Currently, very few nanotechnology-based HCV diagnostics or therapeutics have progressed to clinical trials. Most studies are proof-of-concept or conducted in cell lines and animal models. Without robust clinical data, it is difficult to assess safety, efficacy, and cost-effectiveness in comparison to existing diagnostic tools and antiviral treatments.

5.5. Immunological and biological complexity

Nanoparticle-based therapies must navigate the complex liver microenvironment, as well as the host immune system, which may react unpredictably to foreign nanomaterials. Additionally, long-term accumulation and clearance of nanoparticles, especially non-biodegradable ones, pose potential risks that must be systematically studied.

In summary, while nanotechnology holds great promise for transforming HCV diagnosis and treatment, its clinical adoption is still in its infancy. Addressing regulatory gaps, improving nanoparticle safety profiles, reducing production costs, and generating high-quality clinical data are essential next steps. Bridging the gap between laboratory research and clinical application will require interdisciplinary collaboration among material scientists, clinicians, regulatory authorities, and public health experts.

Table 8. Comparison of classical versus nanotechnology-based strategies for HCV diagnosis and treatment.

Feature	Classical Approaches	Nanotechnology-Based Approaches
Stage of Development	Clinically approved and widely used	Mostly preclinical / in vitro
Sensitivity and Specificity	High (e.g., PCR, ELISA)	Very high (due to enhanced signal amplification and targeting)
Cost per Test	High (e.g., ~\$100 for PCR)	Potentially lower, but varies with platform
Turnaround Time	Moderate to long (hours to days)	Rapid (minutes to hours)
Equipment Requirements	Specialized laboratory instruments	May require advanced/novel equipment (e.g., Raman, SPR), but portable systems are in development
Portability	Low – lab-based	High – potential for field use and POC diagnostics
Ease of Use	Requires trained personnel	Potential for user-friendly design; some require minimal training
Clinical Readiness	Fully validated	Largely experimental
Targeting Precision (Therapy)	Low – systemic delivery	High – ligand/receptor-specific delivery
Toxicity Profile (Therapy)	Known and manageable	Unknown/under investigation
Stability and Shelf Life	Well established	Variable; often needs optimization
Regulatory Status	Approved by regulatory bodies	Lacks standardized regulatory framework

Table 9. Some definitions that are commonly utilized during therapeutic drug monitoring.

Term	Abbreviation	Definition
Sustained virological response	SVR	Percentage of patients with undetectable HCV RNA after a calculated period of time, depending on the drug and dosage regimen, after cessation of the treatment.
Rapid virological response	RVR	Percentage of patients with undetectable HCV RNA after 4 weeks of therapy.
Early virological response	EVR	Percentage of patients with declined HCV RNA $\geq 2 \log_{10}$ at week 12 of therapy.
Non responder	NR	Patients never have undetectable HCV RNA during or at the end of treatment.
Partial response	PR	Patients with $2 \log_{10}$ reduction of HCV RNA at week 12 of treatment but still detectable at week 24.
Breakthrough	BT	Patients with undetectable HCV RNA during treatment followed by a detectable result within the same treatment period.
End treatment response	ETR	Percentage of patients with undetectable HCV RNA at the end of treatment.
Relapse	RL	Return of detectable HCV RNA after EVR or at the end of treatment.

6. Conclusion

Hepatitis C virus (HCV) infection remains a significant global health concern, though major strides have been made in its treatment over the past decade. The development of direct-acting antivirals (DAAs) has revolutionized HCV therapy, offering cure rates (SVR) exceeding 95% with shorter treatment durations and improved tolerability. However, challenges such as drug resistance, limited access in low-resource settings, and the need for precise delivery to infected cells persist. Nanotechnology offers promising solutions to these limitations. One of the earliest successes was the PEGylation of interferon, which improved its

pharmacokinetics and reduced dosing frequency and side effects. More recently, nanocarrier systems are being explored to enhance the delivery, stability, and bioavailability of both approved and investigational DAAs, as well as nucleic acid-based therapeutics like siRNA and miRNA to combat resistance. Additionally, nanotechnology is transforming HCV diagnostics by enabling faster, more sensitive, and less complex detection methods that can be integrated into point-of-care platforms. As nanomedicine continues to evolve, its integration with current therapeutic and diagnostic approaches is expected to significantly enhance the precision, efficacy, and accessibility of HCV management worldwide.

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