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Review

Chitosan-based nanoscale delivery systems in hepatocellular carcinoma: Versatile bio-platform with theranostic application

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ABSTRACT

The field of nanomedicine has provided a fresh approach to cancer treatment by addressing the limitations of current therapies and offering new perspectives on enhancing patients' prognoses and chances of survival. Chitosan (CS) is isolated from chitin that has been extensively utilized for surface modification and coating of nanocarriers to improve their biocompatibility, cytotoxicity against tumor cells, and stability. HCC is a prevalent kind of liver tumor that cannot be adequately treated with surgical resection in its advanced stages. Furthermore, the development of resistance to chemotherapy and radiotherapy has caused treatment failure. The targeted delivery of drugs and genes can be mediated by nanostructures in treatment of HCC. The current review focuses on the function of CS-based nanostructures in HCC therapy and discusses the newest advances of nanoparticle-mediated treatment of HCC. Nanostructures based on CS have the capacity to escalate the pharmacokinetic profile of both natural and synthetic drugs, thus improving the effectiveness of HCC therapy. Some experiments have displayed that CS nanoparticles can be deployed to co-deliver drugs to disrupt tumorigenesis in a synergistic way. Moreover, the cationic nature of CS makes it a favorable nanocarrier for delivery of genes and plasmids. The use of CS-based nanostructures can be harnessed for phototherapy. Additionally, the incur poration of ligands including arginylglycylaspartic acid (RGD) into CS can elevate the targeted delivery of drugs to HCC cells. Interestingly, smart CS-based nanostructures, including ROS- and pH-sensitive nanoparticles, have been designed to provide cargo release at the tumor site and enhance the potential for HCC suppression.

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1. Introduction

Chitosan (CS) is a positively charged heteropolymer that occurs naturally as a polysaccharide. It is obtained from chitin, a substance found in the exoskeletons of crustaceans, fungi, yeasts, and shrimp [1]. The linear composition of CS is formed through the linkage of N-acetyl-D-glucosamine and D-glucosamine units by β(1,4)-glycosidic bonds, which are recurrent throughout the polymer's structure. The process of deacetylating chitin results in the formation of CS, which features amino groups at the C2 carbon. To qualify as CS, the sample must have a nitrogen content and degree of deacetylation of at least 7% and 60%, respectively. Commercially available CS typically has a degree of deacetylation within the range of 66-95%. Upon exposure to water, the amino groups of CS become protonated, allowing it to dissolve in acidic aqueous solutions.

As a result, CS has the ability to produce water-soluble salts such as HCl and carboxylate salts, with its amino groups being responsible for its solubility in mildly acidic environments [2]. Such function can be found during protonation status of amino groups in acid pH in chitosan-based nanoparticles [3]. Moreover, the solubility of CS varies based on its deacetylation degree. To illustrate, CS samples with 40% deacetylation can dissolve in solutions with pH values up to 9, whereas those with a deacetylation degree of 85% are soluble only up to pH 6.5 [4]. CS has a molecular weight of 5.0×10^4 to 2.0×10^6 and its viscosity is 1% in 1% AcOH [5]. Organic solvents including DMSO and *p*-toluene sulfonic acid also solubilize CS [6]. The employment of CS in the pharmaceutical industry can be summarized as drug delivery [7], gene delivery [8], tissue engineering [9], and antimicrobial infection [10,11] with a remarkable increase in use in recent years. CS has a drawback in that it exhibits poor solubility. Consequently, researchers have explored modifications to CS, including quaternization and the use of grafting agents like succinic acid or ethylene glycol, as a means of addressing this problem [12]. Fig. 1 depicts the chemical structure of CS.

In recent times, CS-based biomaterials have emerged as a promising avenue for treating a range of illnesses, with a notable surge in their use for cancer therapy. This is attributable to the unique properties of CS, including its biodegradability, low toxicity, high biocompatibility, and other attributes that make it a fitting option in the realm of cancer treatment [13,14]. A diverse range of applications for CS-based biomaterials have been explored in cancer therapy, although their predominant use has been for drug delivery via nanostructures. Specifically, CS/PEG nanostructures have been created for delivering indole-3-carbinol in the context of bladder cancer treatment, with an encapsulation efficiency of roughly 80%. These nanostructures exhibit a spherical shape and smooth surface, and a cytotoxicity analysis has indicated their capacity in bladder cancer death [15]. In addition, selenium nanostructures conjugated with CS have been developed to hinder the advancement of gastric cancer, with these structures found to boost the production of anti-inflammatory cytokines. The CS-based nanostructures also enhance immune function and suppress the proliferation of gastric tumor cells [16]. Furthermore, a drug and chemosensitizer combination can be loaded in CS-based nanostructures for cancer chemo- and phototherapy [17]. CS nanostructures can evoke a prolonged release of paclitaxel to improve the potential of bladder cancer therapy [18]. Due to the mucoadhesive function of CS nanoparticles, they are also appropriate candidates for colorectal tumor removal [19]. Curcumin-mediated tumor suppression is interesting, but its poor bioavailability is a problem [20]. Genipin-stabilized caseinate-CS nanostructures can promote the stability of curcumin and increase its cytotoxicity due to their targeted delivery [21]. Therefore, cytotoxicity of drugs can be improved using CS nanostructures [22–24]. The goal of the current manuscript is to understand the function of CS-based nanostructures in the treatment of HCC. A summary of HCC is described including its pathogenesis and existing challenges in its

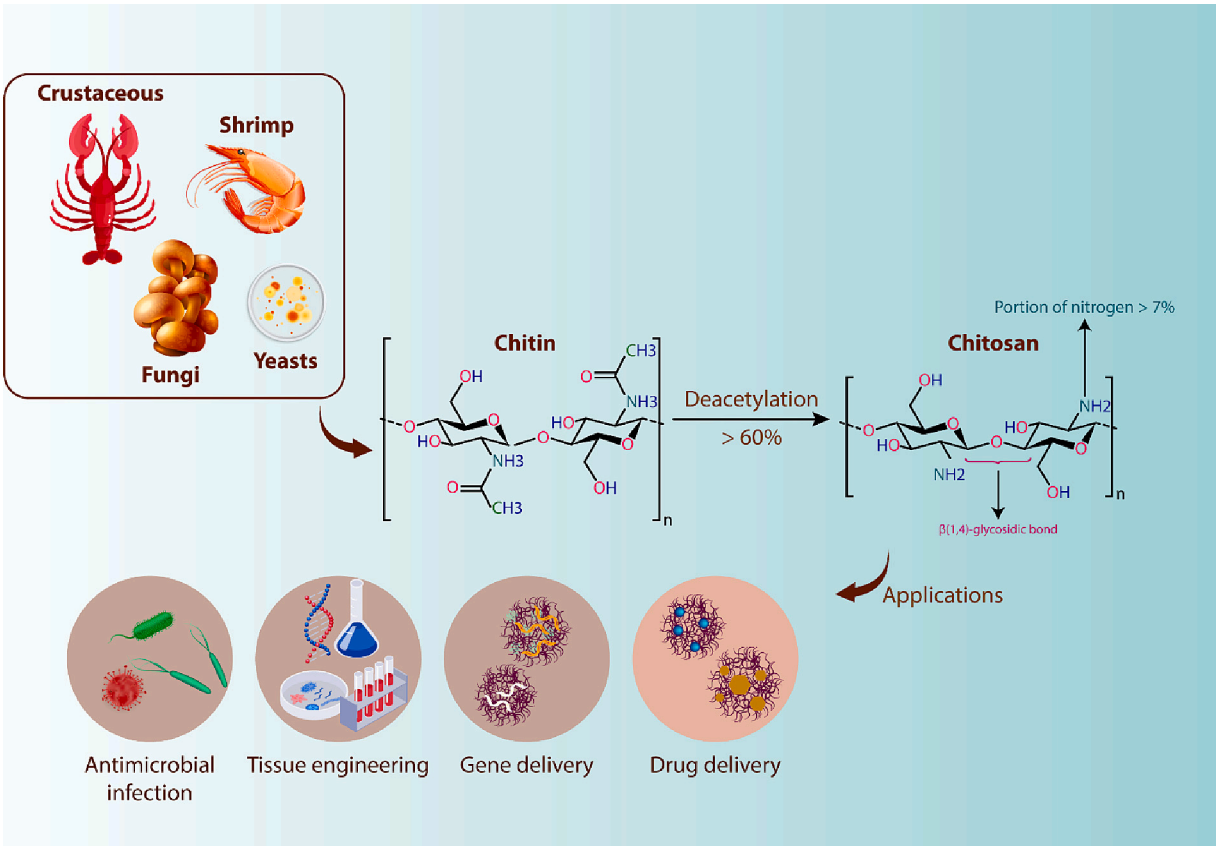

Fig. 1. The wide applications in different fields and industries.

treatment. Subsequently, the potential of CS-based biomaterials in different areas such as drug delivery, gene delivery and theranostics, is explored. Lastly, the application of stimuli-sensitive CS nanoparticles in HCC treatment is discussed.

2. Hepatocellular carcinoma: an overview

HCC poses a significant threat to patients and can greatly impact their quality of life. It is the sixth most prevalent cancer globally and ranks as the fourth leading cause of death [25]. Therefore, due to the high mortality and morbidity rates associated with HCC, greater focus should be placed on its treatment. In 2018, 841,000 new cases of HCC were diagnosed, leading to 782,000 fatalities. HCC and intrahepatic cholangiocarcinoma are the two primary types of liver cancer, with HCC accounting for 75-85% of cases [26,27]. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, obesity, diabetes, alcoholism, and aflatoxin can predispose HCC [27,28]. At present, treatment options for HCC consist of surgical resection, organ transplantation, radiotherapy, and targeted agents. However, HCC is associated with a short survival rate and high recurrence rate, and unfortunately, diagnosis often occurs at intermediate or advanced metastatic stages [29,30]. For end-stage HCC, the 1-year survival rate is 11%, and the 1-year recurrence rate after surgery is suggested to be 70% [31,32]. The effectiveness of current treatment strategies for HCC is limited due to various reasons. Surgical resection is highly effective in early stages of HCC, but the diagnosis often happens at intermediate or advanced stages when cancer cells have already spread, making this option less viable. Additionally, high recurrence rates after surgery limit its potential. Chemotherapy and radiotherapy have shown benefits in treating HCC and improving survival rates, but their frequent and extensive use can trigger alternative pathways and mechanisms that cause cancer cells to develop resistance to therapy [33–38]. There is a shortage of highly effective treatments for HCC, and researchers should aim to develop new therapies that can both suppress HCC progression and prevent therapy resistance. Additionally, these new therapies must have high biocompatibility before being introduced in clinics for patient treatment [39–41]. Recently, nano-platforms have emerged as new types of therapies for the HCC [42–44]. Due to the challenges in delivering drugs and genes to HCC and the incomplete elimination of tumor cells using previous therapeutic methods, nanoparticles have been emerged as an ideal strategy for HCC treatment. Magnetic nanostructures, in particular, have the potential to deliver chemotherapy agents and facilitate phototherapy-induced ablation of HCC cells [45]. Moreover, liposomal nanostructures can be developed for targeted imaging of HCC cells [46]. Surface functionalization of nanostructures, such as peptide modification, can be performed to increase their internalization in HCC cells [47]. Stimuli-responsive nanoparticles, such as lactosylated pH-responsive nanostructures, can be used for the synergistic delivery of curcumin and sorafenib against the HCC [48]. The next stages will cover the utilization of CS-based biomaterials in the treatment of HCC. Studies suggest that CS-based nanostructures are valuable and promising platforms for drug delivery, demonstrating high cytotoxicity against HCC cells [49–54]. Current treatment strategies for HCC have limited effectiveness for various reasons. Surgical resection is highly effective in the early stages, but the diagnosis of HCC often happens at intermediate or advanced stages when cancer cells have already spread, making this option less viable. Furthermore, high recurrence rates after surgery limit its potential. Although chemotherapy and radiotherapy have shown benefits in treating HCC and improving survival rates, their frequent and extensive use can trigger alternative pathways and mechanisms that cause cancer cells to develop resistance to treatment (Table 1). c

3. Chitosan nanoparticles and targeted drug delivery

The landscape of cancer treatment has undergone significant changes with the emergence of diverse anti-tumor agents that target multiple mechanisms within cancer cells [74–76].

There are various types of anti-cancer compounds that target different mechanisms within cancer cells. Some inhibit the replication process by preventing DNA replication, while others increase the generation of reactive oxygen species (ROS) to induce cell death. Additionally, certain compounds interfere with the cell division process. [77]. The development of unique compounds and drugs through drug discovery has significantly advanced cancer treatment. However, HCC often lacks specific symptoms in the early stages, and patients are typically diagnosed at advanced stages. Therefore, the primary focus of

Table 1
CS-based nanostructures in HCC therapy.

Nanoparticles	Remark	Ref
Chitosan composites	Reducing proliferation of different tumor cells especially HCC cells	[55]
Chitosan nanostructures	Increasing ROS levels to mediate mitochondrial damage and apoptosis induction	[56]
Chitosan-mediated synthesis of silver nanoparticles	Upregulation of caspase-3 and -9 to stimulate apoptosis	[57]
Glycyrrhizin-functionalized chitosan nanoparticles	Loading paclitaxel in nanostructures to increase its anti-tumor activity and to impair tumor progression	[58]
Labeled nanostructures	Irradiation-mediated increase in DNA damage and apoptosis induction	[59]
Micro/Nano-Lipid Bromopyruvic Chitosan Carrier	Glycolysis inhibition to suppress progression of tumor cells	[60]
Chitosan nanoparticles	Suppressing angiogenesis in decreasing tumorigenesis	[61]
Phosphorylated galactosylated chitosan	Improving survival rate of animal model High cellular internalization	[62]
Chitosan-platinum conjugated nanomaterials	A promising carrier for delivery of Cis-Platinum Apoptosis induction Better delivery and release of drug	[63]
Simvastatin-loaded chitosan nanoparticles	High internalization in tumor cells Improving bioavailability Suppressing proliferation of tumor cells Mediating ASGPR-mediated endocytosis	[64]
Norcantharidin-associated galactosylated chitosan nanoparticles	pH-sensitive release of cargo Increased internalization High cytotoxicity against tumor cells	[65]
Polymeric micelles	Internalization in tumor cells via endocytosis and suppressing progression and mediating targeted delivery of doxorubicin	[66]
Camptothecin encapsulated with N-trimethyl chitosan	Suppressing proliferation and lymph node metastasis Improving survival rate of animal model	[67]
Apocynin-loaded PLGA nanomedicine	Spherical shape with halo-like appearance High anti-tumor activity and cellular uptake Apoptosis stimulation	[68]
Mitochondria-targeted alginate/triphenylphosphonium-grafted-chitosan	70-110 nm particle size and increased mitochondrial uptake Apoptosis induction	[69]
Chitosan-Coated Iron Oxide Nanocomposite	Suppressing PI3K/Akt/mTOR and MAPK pathways to impair progression of tumors	[70]
Chitosan nanoparticles from Artemia salina	Enhancing ROS levels and mediating mitochondrial dysfunction Enhancing Bax and caspase-3 levels	[71]
Norcantharidin-conjugated carboxymethyl chitosan	Decreasing levels of ALT, AST, VEGF and MMP-9 Suppressing tumor progression	[72]
Galactosylated chitosan triptolide nanoparticles	Sustained drug release High cellular uptake through asialoglycoprotein receptor High accumulation at tumor site	[73]

treatment is on using chemotherapy agents to impede the progression, proliferation, and metastasis of tumor cells. Doxorubicin (DOX) and sorafenib are some of the most common chemotherapy agents utilized in the treatment of HCC. However, their widespread use leads to the development of chemoresistance. For instance, upregulation of WWP2 results in the development of DOX resistance in HCC [71], and therefore, nanoarchitectures have been developed for DOX delivery to impair HCC progression [72]. Likewise, sorafenib also faces the challenge of chemoresistance in HCC, as the overexpression of Ets1 and SCAP can contribute to the development of sorafenib resistance in HCC [73,74]. Due to the therapy failure caused by chemoresistance in HCC patients, natural anti-tumor compounds are being explored as potential alternatives in cancer treatment. One such compound is C0818, a curcumin derivative that disrupts the function of Hsp90, leading to increased ROS generation and anti-tumor activity against HCC [78]. Moreover, quercetin stimulates autophagy and inhibits M2 polarization of macrophages via NF-κB down-regulation in HCC therapy [79]. Despite efforts to use various strategies for HCC treatment, drug resistance remains a major challenge, and natural products may face issues related to poor bioavailability. Therefore, this section will focus on the use of CS-based nanoformulations for drug delivery to enhance HCC therapy. As previously mentioned, sorafenib is a widely used chemotherapy agent for the treatment of HCC that was initially approved by the FDA in 2007. It is commonly considered as the first-line drug for HCC therapy [80,81]. Sorafenib can suppress angiogenesis, proliferation, and metastasis of HCC cells by targeting various molecular pathways, including VEGFRF2 and PDGFR, among others [82,83]. Folate-modified CS-based nanoparticles were used in an experiment to deliver sorafenib in HCC therapy via the ionic gelation method, resulting in a high encapsulation efficiency of up to 87%, drug loading efficiencies of 18.2 and 19.9%, and a diameter of 60-80 nm (TEM). These nanoparticles can release drugs at pH 4.8, making them promising candidates for HCC therapy. Compared to sorafenib alone, the drug-loaded CS nanoparticles exhibited higher cytotoxicity on HepG2 cells and demonstrated high biocompatibility due to their low toxicity on normal cells [84].

T cell immunoglobulin mucin-3 (Tim-3) act as a novel immune checkpoint biomolecule, which can be used as a promising target for HCC treatment [85]. Researchers developed a pH-responsive drug-eluting nanoparticle (CC@SR&SF@PP) for the simultaneously delivery of Tim-3 siRNA and sorafenib to HCC. At first, sorafenib (SF) was loaded into pH-triggered positive-charged mPEG5KPAE10K (PP) nanoparticles, followed through condensing of negative-charged Tim-3 siRNA. Finally, carboxymethyl chitosan (CMCS) was adsorbed on the surface of nanoparticles. This nanoparticle increased the ability of simultaneous delivery of siRNA and sorafenib. Here, enhanced Tim-3 siRNA is able to inhibit tumor cell growth by inducing an immune response and enhancing the recruitment of cytotoxic T cells. On the other hand, The release of sorafenib at lower pH from SF@PP nanoparticles prevented tumor proliferation and angiogenesis, and finally high inhibition of tumor growth was observed in the mouse orthotopic hepatoma 22 (H22) tumor model (Fig. 2) [86].

One of the most commonly used chemotherapy agents is 5-Flourouracil (5-FU), which is a water-soluble anti-tumor compound and a fluorinated pyrimidine analog [87–89]. 5-FU is considered an antimetabolite of the pyrimidine analog that is utilized for the treatment of various tumors, including liver [90], stomach [91], colon [92], pancreas [93], and breast [94] cancers. The anti-tumor compound 5-FU has a half-life of 10-20 minutes and can be quickly absorbed into the systemic circulation. To increase its therapeutic concentration, weekly doses of 400-600 mg/m^2 are administered [95]. A study developed galactosylated CS/5-FU nanostructures that have a high affinity for accumulating in liver cancer tissue. In an animal model experiment, it was found that these nanostructures were effective in suppressing tumor progression and increasing the survival rate of the mice [96]. Hence, CS nanoparticles have shown promise as carriers for 5-FU in cancer chemotherapy. However, there is still room for improvement in increasing their therapeutic efficacy for HCC treatment through surface modification. In this regard, CeO_2 nanoparticles were synthesized from cerium chloride and rutin, and then utilized for the surface decoration of 5-FU-loaded CS nanoparticles. The study demonstrated that these nanocarriers could reduce ROS levels and decrease cytotoxicity on normal cells through their antioxidant activity, thus enhancing their biocompatibility. Additionally, they released 5-FU in a pH-sensitive manner and improved its anti-tumor activity against HCC [97].

In summary, incorporating drugs into nanostructures has been shown to increase their anti-tumor activity against HCC. One challenge in chemotherapy is the presence of side effects caused by chemotherapeutic agents. Therefore, increasing the dose of these agents is not always an option. By loading natural compounds onto nanostructures, it is possible to enhance the anti-tumor activity of chemotherapy agents while reducing their side effects. In a recent study, ginger extract (GE), a naturally occurring compound, was loaded onto CS nanoparticles. The GE-loaded CS nanoparticles induced apoptosis, decreasing HCC survival rates. Additionally, by reducing MDR1 and VEGF levels, the GE-loaded CS nanostructures suppressed HCC progression. Importantly, combining DOX with GE-loaded CS nanoparticles enhanced the cytotoxicity of DOX and reduced its side effects [98].

However, a limitation of the previous study was the lack of focus on the synergistic effect of co-delivering GE and DOX in HCC therapy, which should be addressed in future studies. Interestingly, another experiment examined the potential of CS nanoparticles for co-delivering 5-FU and aspirin in HCC therapy, with a focus on the molecular pathways and mechanisms of action of the nanostructures. The use of CS nanoparticles increased the internalization of 5-FU and aspirin, leading to apoptosis stimulation and reduced growth rates of HCC cells. Additionally, co-drug-loaded CS nanoparticles were able to decrease NF-κB levels by suppressing the synthesis of COX-2 and PGE2. [99]. An additional study has investigated the synergistic treatment of HCC through co-delivery, with promising results. DOX is a potent anti-tumor agent that can inhibit topoisomerase II activity, interfere with DNA replication, and hinder cell cycle progression and tumor cell growth [100]. Like other anti-cancer drugs, DOX resistance can occur due to the dysregulation of molecular pathways and the upregulation of drug efflux pumps [101]. The delivery of DOX by nanoparticles has garnered attention among researchers as a potential strategy for enhancing its efficacy in suppressing tumors [102,103]. The use of CS nanoparticles as a delivery system for DOX and verapamil has shown promise in reducing the expression of Bcl-2 and VEGF, as well as suppressing MDR1. These drug-loaded CS nanoparticles have demonstrated anti-tumor activity while also minimizing the side effects associated with DOX and verapamil. Notably, the application of CS nanoparticles did not result in any observed apoptosis or necrosis in liver tissue [104].

Immunotherapy has emerged as an effective therapy for HCC, and monoclonal antibodies (MAbs) are widely used for cancer therapy and imaging [105–107]. Antibodies have proven useful for diagnosing antigens and in immunotherapy. However, frequent administration can lead to HAMA. Additionally, due to their short half-life and side effects, nanoparticles are recommended for targeted delivery of antibodies. This approach can improve the efficacy of therapy while reducing side effects [108–110].

During an experiment focused on HCC treatment, N,N,N-trimethyl CS nanostructures were utilized to deliver MAbs. The nanostructures had a particle size of 59 nm and a zeta potential of 16.5 mV, and exhibited a spherical shape with a smooth surface and a mean diameter of 11.2 nm. The nanostructures showed high internalization in tumor cells, and their prolonged presence in the cells resulted in enhanced cytotoxicity against HCC [111].

Death receptor 5 (DR5), a member of the TNF-receptor family, is considered an important target for cancer therapy approaches [112]. The use of an antibody targeting DR5 leads to the oligomerization of DR5's cytoplasmic domain, which triggers an apoptotic cascade and generates death-stimulating complexes [113–115]. Hydroxyethyl CS

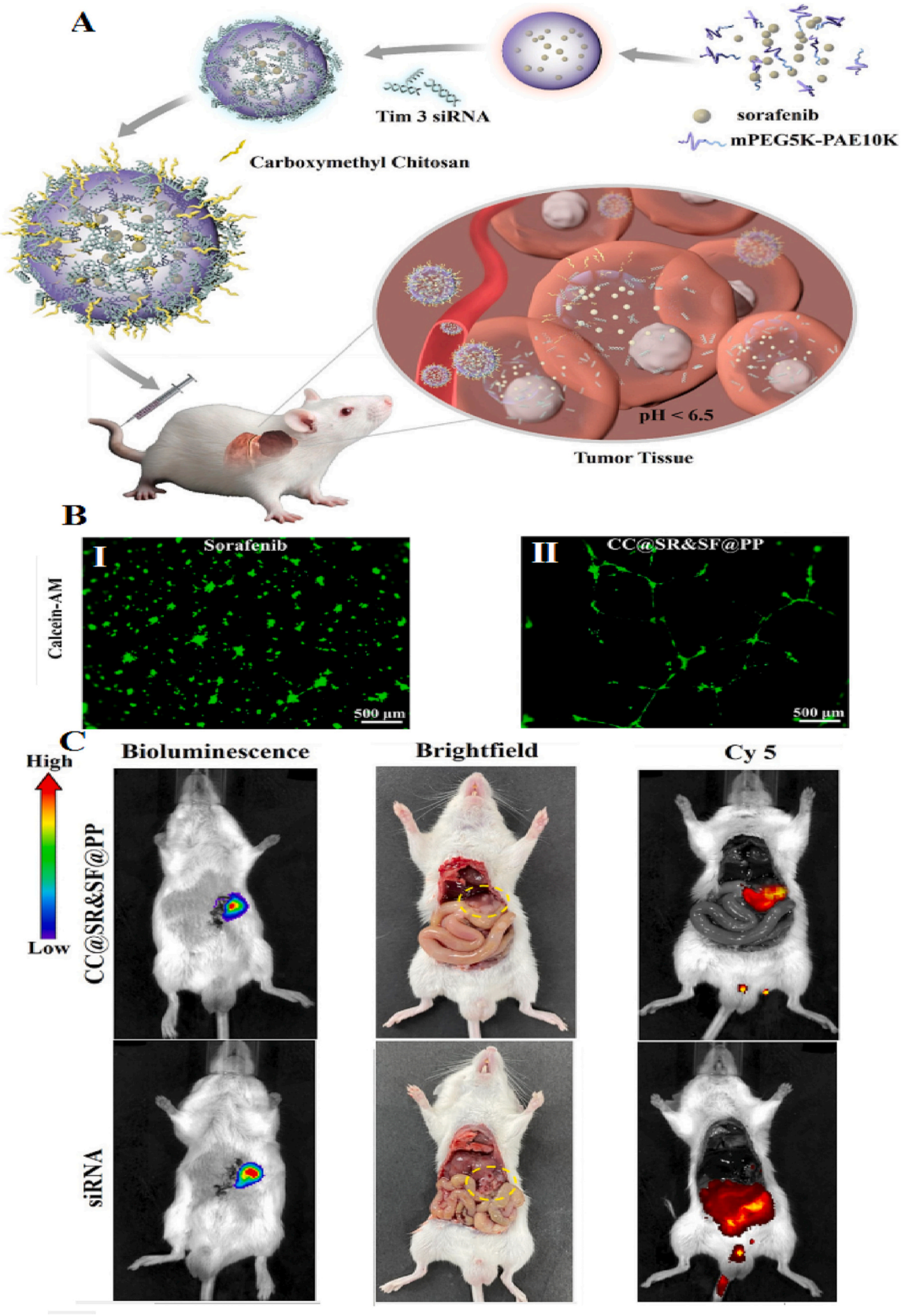

Fig. 2. (A) Schematic illustration of CC@SR&SF@PP nanoparticles for co-delivery of Tim-3 siRNA and sorafenib in HCC treatment. (B) Images of HUVECs cells induced with 5 μg/mL of sorafenib and CC@SR&SF@PP NPs. (C) *In vivo* tumor uptake assay. (a) Bioluminescence, brightfield, and Cy5 fluorescence images of the tumors recruited were attained 6 h after the mice were injected with CC@SR&SF@PP NPs (up) or free Tim-3 siRNA (down). The results showed that CC@SR&SF@PP nanoparticles have clear fluorescence on the liver and even more clusters were detected in the tumor areas of liver [86].

nanoparticles have been developed to deliver DR5-antibody for HCC treatment. These antibody-loaded CS nanoparticles displayed good stability with a zeta potential of -24.2 mV and a dispersion index of 0.203. They exhibited concentration- and time-dependent cytotoxicity and, *in vivo*, reduced tumor growth and volume in mice. Furthermore, the antibody-loaded CS nanoparticles increased levels of caspase-3, caspase-8, and BAX to induce apoptosis in tumor cells

[116]. Curcumin is also a potent anti-tumor compound in HCC therapy. Based on recent studies, curcumin can impair the progression of HCC cells by affecting various molecular pathways and mechanisms. By increasing ROS levels, curcumin mediates proptosis in HCC cells [117], and reduces VEGF levels [118]. Regardless of cancer type, curcumin has been beneficial in tumor chemotherapy [119]. However, to enhance its therapeutic index, nanoparticles and targeted delivery should be employed [120]. To enhance its potential for suppressing HCC, curcumin has been loaded onto CS/poly(butyl cyanoacrylate) nanostructures. These nanostructures have a size of 200 nm, a zeta potential of +29.11 mV, and an encapsulation efficiency of 90.04%. They have been found to induce apoptosis and decrease tumor progression *in vitro* and *in vivo* [121].

Liposomes are lipid-surrounded compartments that demonstrate promising drug delivery in cancer therapy [122,123]. Liposomes can protect drugs against enzymes, the external environment, and immune recognition, thereby improving the biodistribution of drugs and enabling targeted delivery [124]. However, free form liposomes and other nanoparticles are not very effective in drug delivery [125]. Polyphenols or SCFAs can be delivered using liposomes, which can increase drug plasma levels and enable targeted delivery to the liver when administered orally [126,127]. Liposomes have potential for delivering butyric acid in HCC therapy, and their efficacy in cancer therapy can be enhanced through surface modification with chitosan (CS). CS-coated drug-loaded liposomes significantly reduced the viability of HepG2 cells after 72 hours of incubation at concentrations of 7.5, 2.5, and 1.6 mM, displaying greater toxicity compared to free butyric acid and free liposomes. Moreover, in HCC therapy, these nanoparticles are effectively internalized by tumor cells and can reduce the levels of IL-8, IL-6, TNF-α and TGF-β by 64, 58, 85 and 73.8%, respectively (Fig. 3) [128].

4. Chitosan nanoparticles and gene delivery

Gene therapy has emerged as a new kind of treatment strategy for cancer. Although gene therapy can be used for the treatment of different diseases and pathological events, its use in cancer therapy has been a hot topic due to key underlying reasons. The primary reason for the appeal of gene therapy in cancer treatment is due to the fact that cancer is one of the deadliest malignancies worldwide. Additionally, chemotherapy, which is a conventional treatment method, has encountered significant challenges in treating HCC due to drug resistance. Therefore, gene therapy can be utilized to enhance conventional therapies and sensitize HCC cells to therapy. Despite the initial promise of gene therapy in HCC treatment, it was soon discovered that this approach also faced its own set of challenges. One such challenge was the lack of targetability towards tumor cells and the potential for enzymatic degradation. Consequently, there arose a need to encapsulate gene therapy agents in order to improve targeted delivery and provide protection against degradation [129,130]. The aim of the current section is to evaluate the function of CS-based nanostructures for gene delivery in HCC therapy.

Apoptosis is a crucial molecular pathway in HCC therapy, as cancer cells tend to enhance their progression when they acquire resistance to apoptosis. Therefore, the delivery of genes involved in apoptosis has

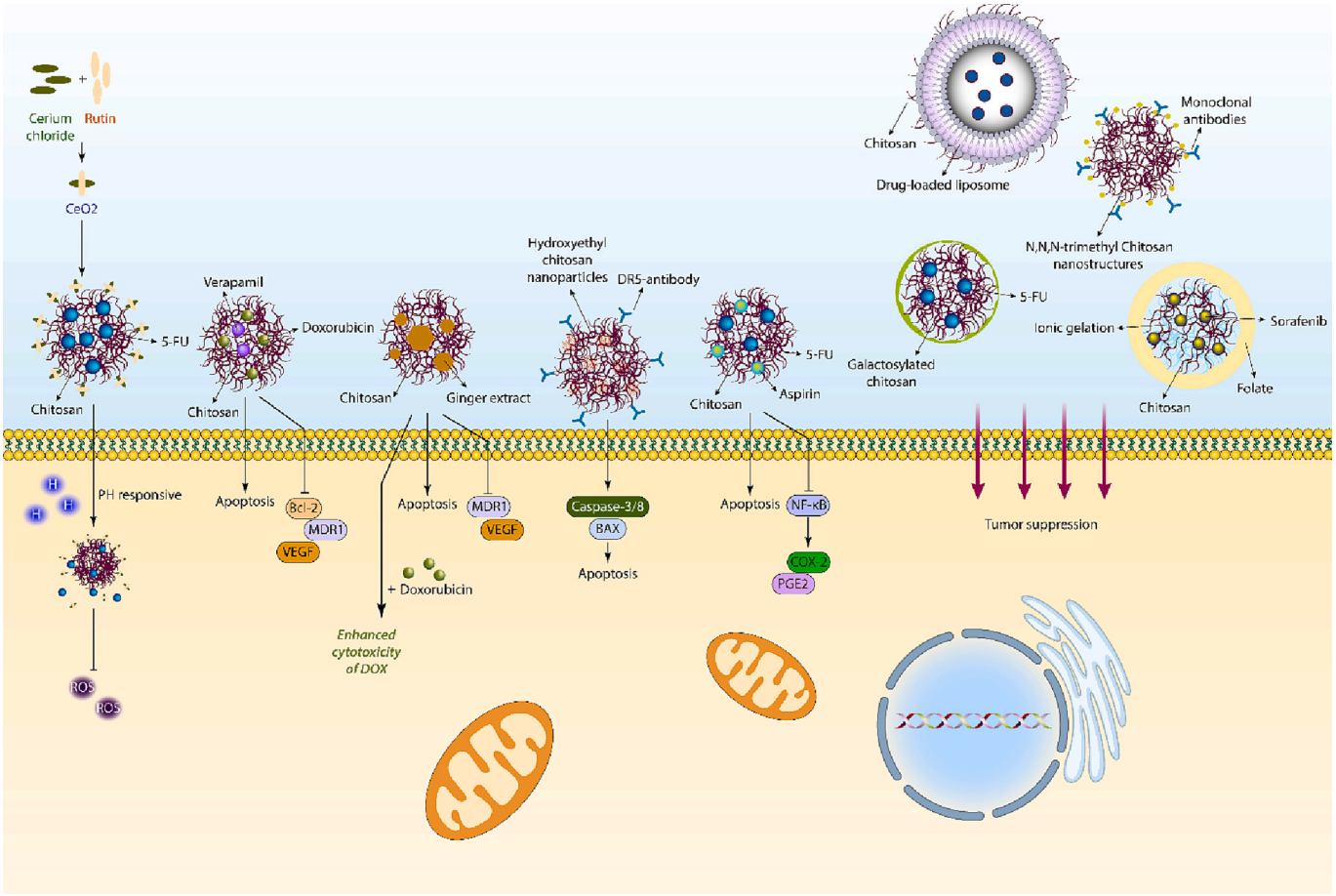

Fig. 3. CS-based nanostructures for delivery of drugs in HCC therapy.

gained significant attention. To this end, urocanic acid-modified CS-based nanostructures have been designed to deliver the p53 gene in a targeted manner. These nanostructures are capable of internalizing in HepG2 cells, inducing apoptosis, and reducing the proliferation rate [131]. Since p53 gene-loaded CS-based nanoparticles have shown promise in HCC suppression, they have been combined with doxorubicin chemotherapy for effective cancer therapy. A combination of doxorubicin chemotherapy and p53 gene-loaded CS-based nanostructures can increase the expression levels of p53 and caspase-3, triggering apoptosis in HCC cells [132].

It is noteworthy that CS can be modified to enhance its ability to deliver genes for HCC therapy. Studies have shown that the conjugation of TAT peptide and LHRH hormone to low molecular weight CS can result in the creation of a delivery system with high DNA condensation power. This system has a particle size of 70-85 nm, a zeta potential of +30 mV, and is a stable nanoscale delivery system for HCC treatment [133].

RNA interference (RNAi) has emerged as a new kind of therapy for cancer, and short hairpin RNA (shRNA) and small interfering RNA (siRNA) are two key members of the RNAi family. The use of shRNA can effectively decrease the expression of a specific gene. In the case of HCC, VEGF is known to act as an oncogenic factor, and its down-regulation through UDCA can be effective in slowing down HCC progression [134]. Moreover, lncRNA PAARH promotes HOTTIP expression to induce VEGF signaling during angiogenesis induction in HCC [135]. Therefore, targeting VEGF is of importance in the treatment of HCC [136]. For the treatment of HCC, VEGF-shRNA has been conjugated to low molecular weight CS. VEGF-shRNA/CS complex can reduce VEGF expression in HCC cells and liver tumor tissues. Upon intravenous injection into tumor-bearing mice, CS increased and sustained shRNA accumulation in tumor tissues. Moreover, the VEGF-shRNA/CS conjugate suppressed angiogenesis and reduced cancer proliferation in HCC [137]. Similar to shRNA, siRNA has been extensively employed in cancer therapy. Delivery of siRNA using nanostructures is an important strategy to improve its potential in suppressing HCC, especially in the context of immunotherapy [138–140]. Iron oxide nanostructures have been developed for the delivery of siRNA in HCC therapy. The nanoarchitectures consist of an iron oxide core coated with CS-PEG polymers, which are then functionalized with an antibody against the GPC3 receptor. These nanoarchitectures have been utilized for targeted delivery of siRNA to effectively inhibit the progression of HCC in an animal model [141].

MicroRNAs (miRNAs) are a type of factor that play a role in regulating the progression of HCC. With a length of 19-22 nucleotides, miRNAs are capable of regulating the expression of numerous genes. By modulating various signaling networks, miRNAs act as key regulators of the tumor microenvironment components, and they have a significant impact on tumor progression [142–144].

Galactosylated CS-5-FU nanostructures have been utilized for the delivery of miR-122 in HCC therapy. These nanostructures enhance the blood and salt stability of miR-122 and promote apoptosis while inhibiting the growth rate of cancer cells. Additionally, miR-122 delivery through CS nanostructures results in the down-regulation of ADAM17 and Bcl-2, ultimately reducing HCC viability [145]. This approach can induce HepG2 cell apoptosis, prevented HCC cell proliferation, and higher antitumor efficiency *in vivo*, and therefore addressed the compromise between miRNA delivery and toxicities of the system (Fig. 4). Based on these studies, the use of CS-based nanostructures in the treatment of HCC is highly suggested for the following reasons. The first reason is that genes have a short blood circulation time, and their efficacy is jeopardized due to enzyme degradation. Therefore, their conjugation to CS or encapsulation can lead to enhanced blood circulation time. Furthermore, the use of CS-based nanoparticles facilitates their accumulation at the site of the tumor. To improve the accumulation efficacy of gene-loaded CS-based nanostructures at the tumor site, ligands and antibodies are often added to the surface of these nanostructures, allowing them to bind to specific receptors present on the surface of tumor cells. More importantly, an *in vivo* experiments in animal models have also shown the potential of gene-loaded CS nanostructures in HCC therapy.

These studies highlight the benefits of using CS-based delivery systems for gene therapy in HCC. The keynote is that these nanostructures can be utilized for drug and gene delivery in HCC synergistic therapy. CS-based nanoparticles have been used for co-delivery of VEGF and sorafenib in HCC therapy, due to the oncogenic role of VEGF signaling pathway in HCC. CS-based nanostructures are utilized to enhance antitumor activity by co-delivering sorafenib and VEGF down-regulation. This approach leads to apoptosis induction, with CS-based nanoparticles playing a crucial role in the process [146]. The progression of HCC cells is influenced by the regulation of midkine, as reported in [147]. Moreover, IGF-1R can induce STAT3 signaling, leading to the enhancement of midkine expression and a feedback loop with STAT3, which further promotes HCC progression [148]. A hybrid nanostructure based on CS has been developed for the delivery of midkine-siRNA, based on the aminoalkylation modification of CS. These nanoparticles can condense siRNA with a weight ratio greater than 3. The particle sizes of the demonstrated nanostructures were between 100-200 nm, and they had a spherical shape with a zeta potential of +3 mV. There was no observed aggregation. Additionally, the CS nanostructures loaded with siRNA decreased midkine expression and hindered the growth of HCC cells [149]. In addition to the co-delivery of drugs and genes, CS-based nanoparticles have been utilized for the co-delivery of genes in HCC therapy. Nutlin-3a functions as a tumor-suppressor and acts as an inhibitor of MDM2. By doing so, it can induce apoptosis and decrease the growth rate of osteosarcoma cells [150]. Moreover, nutlin-3a can induce cytoskeleton rearrangement, impairing the progression and metastasis of cancer cells [151]. Moreover, nutlin-3a's capacity to induce apoptosis is associated with upregulation p53 expression in tumor cells [152]. To enhance the inhibitory effects on HCC progression, an experiment utilized core-shell nanoparticles to co-deliver p53 and nutlin-3a genes in HCC therapy. The core was loaded with nutlin-3a, while the shell was comprised of cyclodextrin-g-CS/p53. Compared to pure CS, the nanostructures exhibited a 5-fold increase in cellular uptake in HCC cells. They can interact with and disrupt the cell membrane. By delivering p53 and nutlin-3a, they hindered the interaction of MDM2-p53 ions, thereby reducing the progression of HCC cells [153].

RASSF1A is another important factor in HCC that enhances the number of apoptotic cell deaths in HCC and reduces the growth rate of cancer cells. Moreover, RASSF1A promotes the sensitivity of HCC cells to cisplatin and 5-FU chemotherapy [154]. During an experiment, nanostructures were created by combining carboxymethyl CS and Fe3O4. The amino groups of CS served as cross-linked groups and were conjugated to galactose ligands through an ammoniation reduction method. The nanoparticles had a particle size of 40.1 nm and a zeta potential of +6.5 mV. They exhibited a strong ability to condense DNA, and their biocompatibility was high. Researchers found that the nanoparticles preferentially accumulated in tumor tissue and promoted caspase-3 levels in cancer cells, leading to apoptosis when an external magnetic field was applied [155].

PAK1 plays a crucial role in promoting HCC as an oncogenic factor, and the upregulation of PAK1 expression is promoted by LINC00460, which down-regulates miR-485-5p, thereby accelerating the progression of HCC cells [158]. Moreover, myricetin can reduce the expression level of PAK1 in HCC cells, triggering apoptosis [159]. Down-regulation of PAK1 by IPA-3 impairs the progression and proliferation of HCC [160]. CS-based nanostructures have been designed to deliver PAK1-siRNA for HCC treatment, and these nanostructures have been modified with lactobionic acid (LA) and glycyrrhetinic acid (GA) to improve internalization in HCC cells. The nanostructures enhance the internalization of PAK1-siRNA in HCC cells, resulting in inhibition of tumor cell growth and metastasis, as well as simultaneous induction of apoptosis. The ability of siRNA-loaded CS-based nanostructures to induce apoptosis in

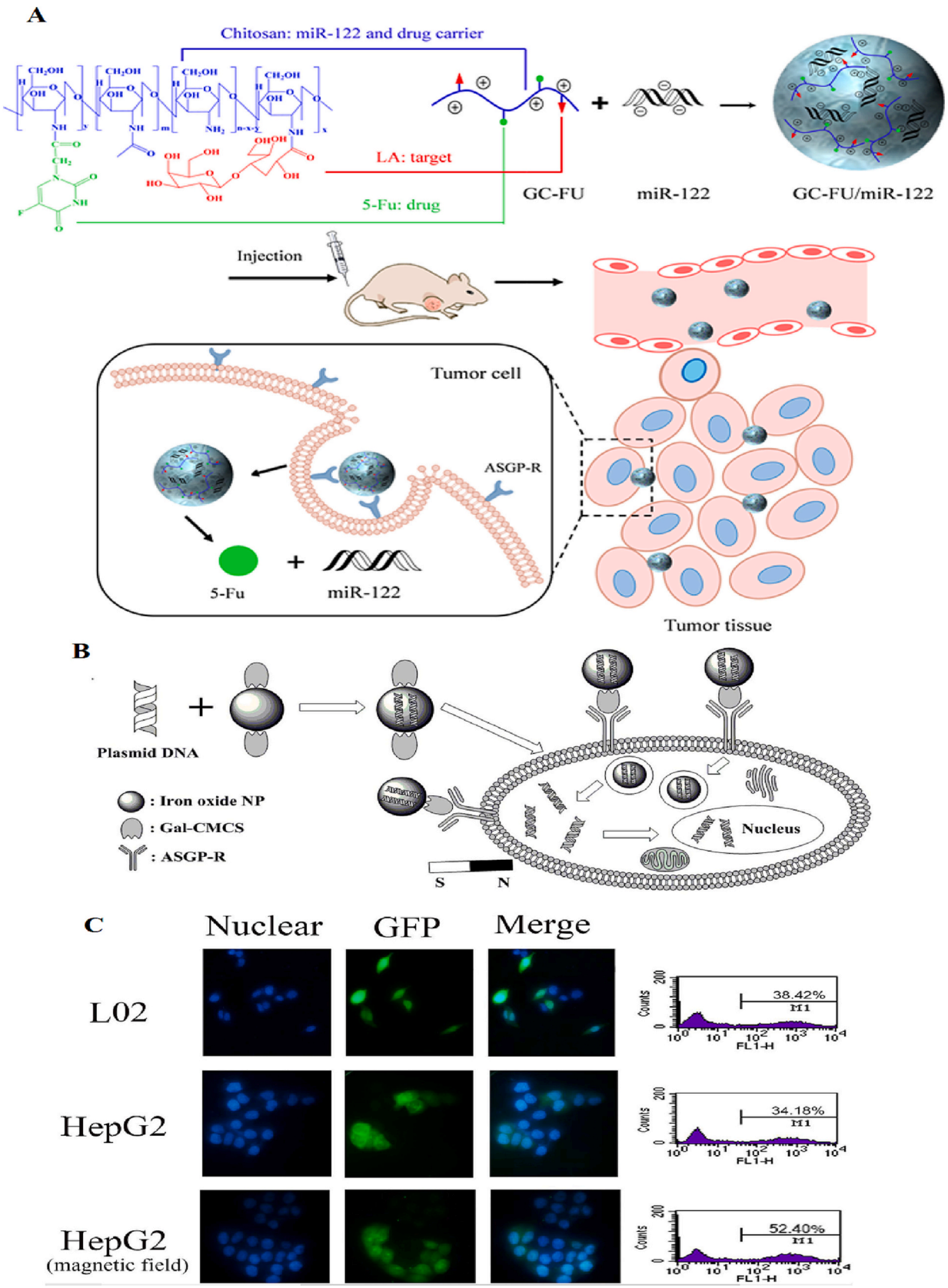

Fig. 4. (A) Schematic illustration of the synthesis process of GC-FU/miR-122 and Hepatoma-Targeted codelivery of miR-122 and 5-Fu toward Synergistic Therapy for Hepatocellular Carcinoma [156]. (B) Targeted transfection of Gal-CMCS-Fe3O4-NPs inside the nucleus of the cell. (C) Transfection efficiency of Gal-CMCSFe3O4-NPs/pcDNA6.2mir-EGFP in various cell lines. 72 hours after transfection, strong green fluorescence was detected in L02 and HepG2 cell lines [157].

HCC is attributed to the down-regulation of PAK1, which inhibits the MEK/ERK axis [161]. Similar to PAK1, targeting PLK1 is of importance in HCC therapy [162]. STK39 binds to PLK1 to increase ERK expression, enhancing HCC progression [163]. High expression levels of FBXO45 lead to upregulation of PLK1, accelerating HCC progression [164]. The galactosylated CS-graft-poly(ethylene glycol) (GCP) nanoparticles can encapsulate PLK1-siRNA and suppress proliferation rate while inducing G2/M arrest. Moreover, siRNA-loaded GCP nanostructures stimulate apoptosis by upregulating p53, p21, and Bax while reducing Bcl-2 expression [165]. CS-based nanostructures can mediate the release of siRNA into cytosol by facilitating escape from lysosomes and endosomes [166]. Moreover, the potential of CS-based nanostructures in gene delivery for HCC therapy has been improved by the development of smart nanocarriers that are pH- and redox-sensitive (Fig. 5 and Table 2) [167,168].

5. Chitosan nanoparticles and phototherapy

Phototherapy has recently emerged as a promising cancer treatment strategy, with two primary methods being photodynamic therapy (PDT) and photothermal therapy (PTT). Both methods lead to the induction of cell death in tumors. PDT relies on the metabolism of cancer cells, taking advantage of this process. A photosensitizer is used, and upon accumulation in the tumor tissue, exposure to light results in the generation of ROS, which subsequently triggers cell death and inhibits neovascularization [174–176]. PTT uses a laser to generate heat in tumor tissue, which mediates the ablation of cancer cells and slows their progression [177–180].

Recently, PTT has been extensively used for the treatment of HCC. As an example, Cu2-xS NCs were encapsulated using PLGA, which was then subjected to near-infrared II exposure, leading to photothermal therapy (PTT). This approach proved to be beneficial in achieving a synergistic photo- and chemo-therapy for HCC treatment [181]. UIO-66/Bi2S3 nanocomposites are developed for the delivery of doxorubicin and provide PTT for enhancing the potential of chemotherapy [182]. Furthermore, the potential of nanoparticle-mediated PTT in HCC suppression in an animal model has been confirmed to be free of toxic effects on surrounding liver tissues and other major organs [183]. Researchers have exploited CS-based nanostructures for the purpose of PTT in the treatment of HCC. Gold nanoparticles can be employed for PTT in HCC therapy. An experiment has focused on the chemo- and phototherapy of HCC using 5-FU-loaded gold nanoparticles. Despite its efficacy, 5-FU has a negative charge, making it challenging to interact with gold nanoparticles. To overcome this challenge, gold nanoparticles have been coated with a positively charged CS layer to facilitate their interaction with negatively charged 5-FU. These CS-coated gold nanostructures exhibited loading efficiencies of 72% for 5-FU, and upon laser irradiation, they caused PTT in conjunction with chemotherapy, thereby effectively inhibiting the progression of HCC cells [184]. Interestingly, graphene quantum dots (GQDs) can be utilized as photosensitizers to mediate PTT for the treatment of HCC.

In a recent study, GQD/magnetic chitosan nanoparticles were developed as a nano-scale delivery system for HCC treatment. These nanoparticles effectively prevented the release of doxorubicin in the bloodstream and, by mediating both chemotherapy and photothermal therapy (PTT), significantly improved the survival time of mice while inhibiting HCC progression [185]. However, there are no experiments using CS-based nanostructures for the purpose of PDT in HCC therapy.

In the future, CS nanoparticles and phototherapy are likely to gain more attention in the field of biomedicine. With the increasing need for

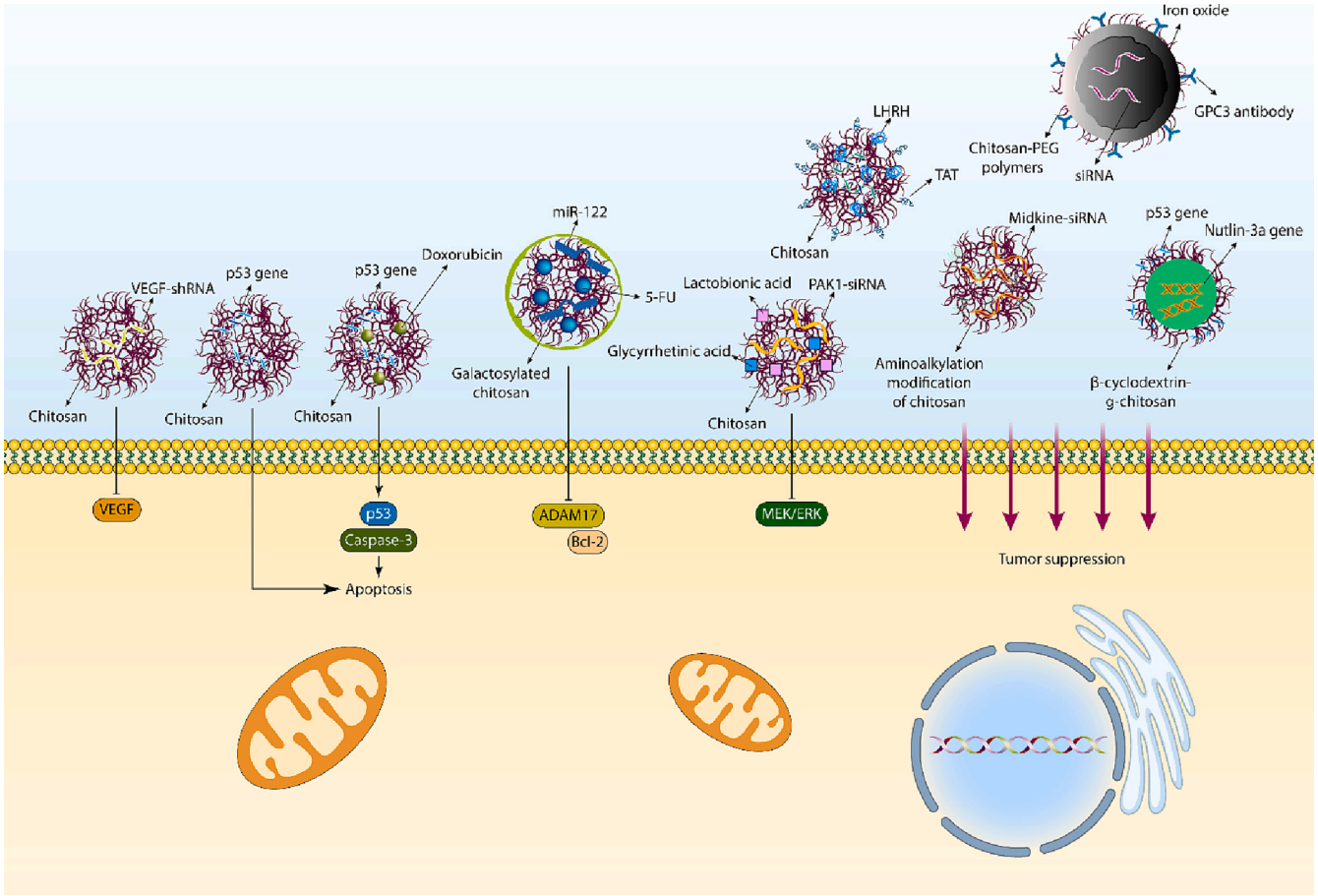

Fig. 5. CS-based nanostructures for gene delivery in HCC therapy.

Table 2
The CS-based nanoscale delivery systems for genes in HCC therapy.

Nanocarrier	Gene	Remark	Ref
Urocanic acid-modified CS-based nanostructures	p53 gene	Apoptosis induction and reducing proliferation of HCC cells	[131]
CS nanostructures	p53 gene	A combination of doxorubicin and p53 gene-loaded nanostructures promote caspase-3 and p53 levels in apoptosis induction and decreasing tumor progression	[132]
CS complex	VEGF-shRNA	VEGF-shRNA/CS complex suppresses angiogenesis and inhibits tumor proliferation	[137]
CS-PEG-coated iron oxide nanoparticles	siRNA	Targeted delivery of siRNA in animal models to suppress tumor growth	[141]
Galatosylated-CS—5-flourouracil	miR-122	Down-regulating ADAM17 and Bcl-2 levels Apoptosis induction Decreasing tumor progression	[145]
Folate-modified CS nanoparticles	IP-10 gene	Reducing tumor growth Enhancing survival rate of mice Decreasing number of MDSC cells	[169]
Folate-modified CS nanoparticles	IP-10 gene	Binding to folate receptors on the surface of cancer cells Enhancing IP-10 expression Improving activity of CTLs Enhancing IFN secretion Suppressing tumor progression Increasing animal survival time	[170]
TAT-LHRH conjugated CS/DNA nanostructures	-	Reducing renal clearance of DNA Improving circulation time Showing anti-cancer potential in animal models	[171]
CS-stabilized gold-folate-poly(lactic-co-glycolide) nanostructures	Plasmid DNA	Spherical shape of nanostructures with particle size of 199.4 nm Zeta potential of 35.7 mV High biocompatibility High anti-cancer activity	[172]
Folate-conjugated CS nanoparticles	IP-10 gene	Increasing IP-10 expression and exerting anti-cancer immune response Reducing Treg cells in spleen Enhancing MDSCs	[173]
CS-based nanostructures	PAK1-siRNA	Down-regulation of PAK1 to inhibit MEK/ERK axis Apoptosis induction Increasing cellular uptake of siRNA	[161]
CS-graft-poly(ethylene glycol) nanostructures	PLK1-siRNA	Apoptosis induction Impairing tumorigenesis	[165]

targeted therapies and personalized medicine, the use of CS nanoparticles in drug delivery and gene therapy is expected to rise. Researchers are exploring ways to improve the stability, efficacy, and bioavailability of CS nanoparticles for better clinical outcomes. In the case of phototherapy, the development of new phototherapeutic agents and light sources is likely to expand its applications beyond current use in skin disorders and cancer therapy. Additionally, the integration of CS nanoparticles with phototherapy has the potential to enable site-specific delivery and activation of therapeutics, minimizing off-target effects and improving treatment outcomes. Overall, the future of CS nanoparticles and phototherapy looks promising, with the potential to revolutionize the treatment of various medical conditions.

6. Surface functionalized nanostructures

CS has been found to be a useful tool in modifying nanomaterials, leading to improvements in their properties, including drug and gene delivery, enhanced biocompatibility, and targeted delivery capabilities. Although the benefits of using CS to modify nanomaterials for cancer therapy are promising, there is still potential to further enhance their properties. Since CS can create functional groups on the surface of nanoparticles, ligands can be attached to improve their targeting ability. The current section aims to provide an overview of how CS-based nanostructures can be functionalized for HCC therapy. Nanomaterials can be functionalized using antibodies, sugars, folate, and peptides to increase targeted delivery and reduce side effects. Tumor cells undergo angiogenesis to acquire nutrients and enhance their ability to migrate to other sites [186]. The upregulation of αvβ3 and αvβ5 integrins on the surface of cancer cells results in binding with RGD peptides [187]. CS-based polymeric micelles have been modified with RGD for HCC therapy, as they can encapsulate DOX with an efficiency of 90%. They mediate the sustained release of the drug and increase the cytotoxicity of DOX against HCC cells. Furthermore, nanoparticles with RGD-modified surfaces have high cellular uptake rates [188]. As it was mentioned, the benefit of CS is to provide a place for conjugation of ligands. For instance, graphene oxide (GO) can be synthesized for drug delivery in HCC therapy. Connecting cRGD to GO nanocomposites is challenging, and a potential solution is to first conjugate cRGD to CS. Next, the cRGD-CS conjugate can be used to modify the surface of GO nanocomposites, followed by loading DOX via π–π stacking interactions. These composites can release drugs in the acidic tumor microenvironment, and their modification with cRGD-CS enables them to selectively target hepatoma cells by upregulating integrins [189].

Celastrol (Cela) is a naturally occurring compound derived from Tripterygium wilfordii Hook, and it has shown promising anti-tumor activity against liver cancer [190]. Poor solubility, bioavailability, and systemic toxicity are among the drawbacks of Cela [191–194]. Polymeric micelles are considered the best drug delivery systems for Cela, and due to their low critical micelle concentration (CMC), they demonstrate high stability in aqueous solutions [195,196]. On the other hand, GA is a popular material for providing hepatoma-targeting capacity. In places where HCC occurs, there are high numbers of GA receptors [197,198]. The levels of GA receptors on the surface of HCC cells are higher than those of normal hepatocytes [199]. In a recent experiment, CS-based polymeric micelles were developed for Cela delivery in HCC therapy. These nanostructures have been modified with GA, and they show high release in the tumor microenvironment and low release in blood conditions. They can increase the bioavailability and therapeutic index of Cela and release the drug in a ROS-responsive manner. Although the cytotoxicity of CS-based polymeric micelles containing Cela is high against HepG2 cells, they demonstrate no significant toxicity against L-02 cells at low concentrations. Therefore, they are promising carriers for suppressing tumor progression [200]. Advances in the field of biology have shown that cancer cells exhibit overexpression of certain receptors on their surface compared to normal and healthy cells. For example, we recently demonstrated the upregulation of folate receptors on the surface of cervical cancer cells. Functionalizing nanocarriers with folic acid enhances their ability to target cancer cells [201]. As discussed in this section, receptors such as folate and CD44 have been identified on the surface of cancer cells. By functionalizing chitosan nanomaterials with ligands, these nanocarriers can specifically target tumor cells that overexpress those receptors, thereby enhancing their targeting ability (Fig. 6).

7. Stimuli-responsive chitosan nanoparticles

In recent years, there has been an increase in the use of stimuli-responsive nanostructures for cancer treatment. Out of the different types of stimuli-responsive nanoplatforms, pH is the internal stimulus that is most utilized. The pH levels in various diseases, such as cancer and inflammation, undergo alterations. Changes in pH are also observed in organelles such as lysosomes during physiological processes [202,203]. The feature has been utilized by researchers to create

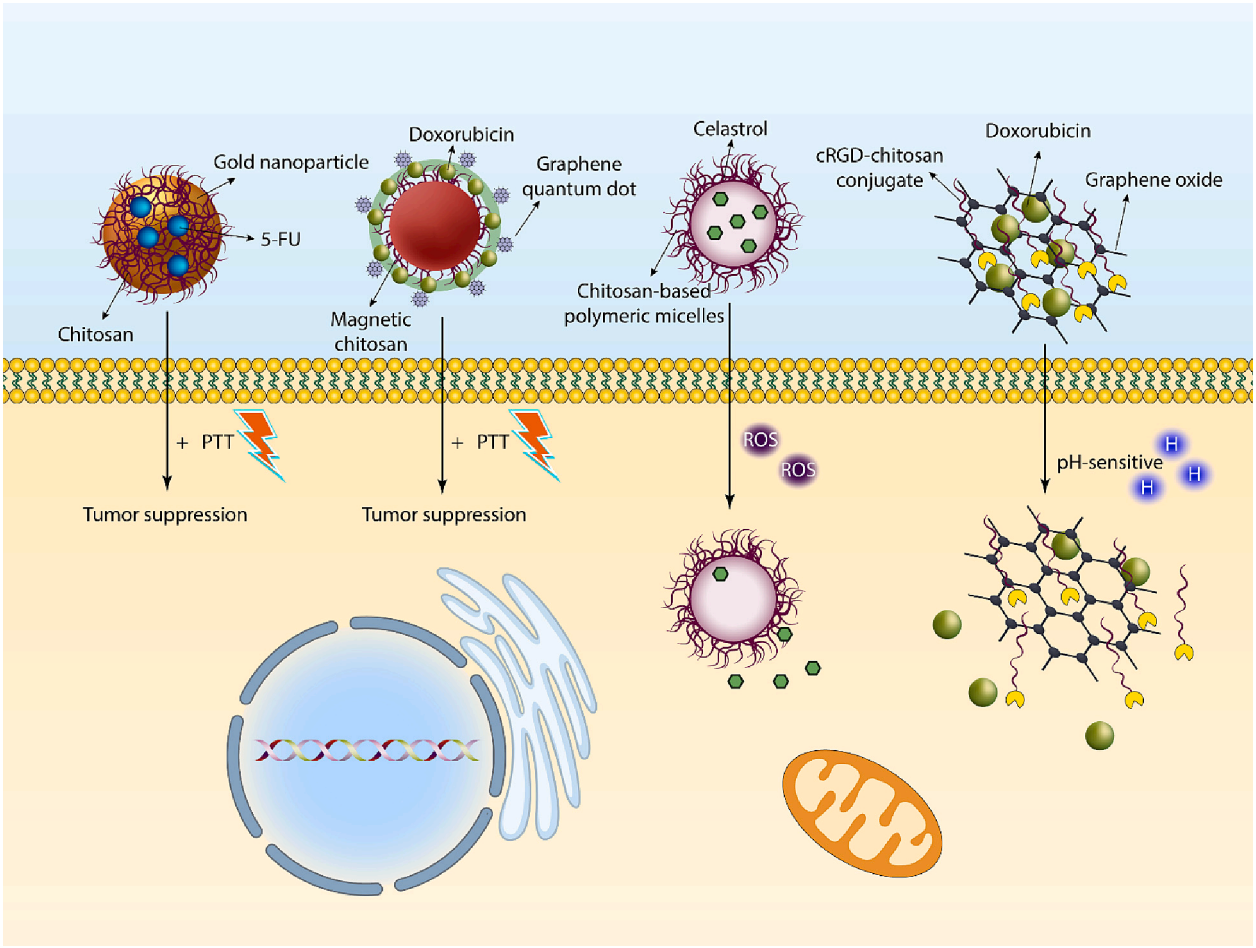

Fig. 6. The application of CS-based nanostructures for phototherapy and their surface functionalization.

intelligent nanostructures for cancer therapy. These nanostructures are sensitive to changes in pH levels and can undergo modifications in their properties or structure as a result. Specifically, they are designed to respond to pH fluctuations in their environment. Normal tissues typically have a pH range of 7.2-7.4, whereas tumor tissues have a pH range of 6-7 [204–206]. In acidic pathological eventsenvironments, there is a pH gradient around the cell membrane, and considering pH 6 of the cell membrane, this pH increases with increasing distance from the cell membrane [207]. Since the pH level of tumor tissue is lower than that of normal tissue, researchers have utilized this feature for developing pH-sensitive nanoparticles in HCC cancer therapy. In an experiment, pH-sensitive self-healing hydrogels have been designed with the purpose of suppressing HCC.

A pH-sensitive self-healing hydrogel platform was created using a combination of N-carboxymethyl CS and PEGDA. The presence of Schiff base contributed to the pH sensitivity of the platform. This hydrogel platform was then utilized for the administration of doxorubicin in the treatment of HCC.

The hydrogels that were synthesized showed excellent biocompatibility and could facilitate extended drug release. Additionally, the hydrogels that were based on CS and loaded with doxorubicin were effective in impeding HCC advancement by inducing cell death and triggering apoptosis [208]. In a more recent effort, self-healing hydrogels were prepared from CS and 4armPEGDA. Schiff base bonds were created between the amino group of carboxy CS and the aromatic aldehyde of 4armPEGDA to make them pH-sensitive. The synthesized hydrogels were biocompatible and biodegradable, and could mediate the sustained release of doxorubicin in mildly acidic pH of tumor microenvironment. MTT assay revealed low viability of HepG2 cells after exposure to pH-sensitive doxorubicin-loaded CS-based hydrogels [54].

The treatment of HCC typically involves the administration of chemotherapy agents, with sorafenib being one of the most frequently prescribed drugs. Unfortunately, the development of sorafenib resistance in HCC has posed a significant challenge for physicians. Several studies have suggested that certain factors contribute to this resistance, such as the triggering of the PI3K/Akt signaling pathway via the S-palmitoylation of PCSK9

[209], or upregulation of MCM2 to trigger the Hippo signaling [210]. On the other hand, some solutions, such as using anti-tumor compounds (CT-707 and orlistat), have been suggested for reversing sorafenib resistance in HCC [211,212]. One of the important strategies is the development of pH-sensitive nanoparticles for the targeted delivery of sorafenib. Lactobionic acid-modified pH-responsive CS-conjugated mesoporous silica nanostructures were prepared for the co-delivery of sorafenib and ursolic acid in HCC treatment. Firstly, the mesoporous silica nanostructures were equipped with COOH groups on their surfaces. Next, CS-LA was attached to these functional groups. Subsequently, sorafenib and ursolic acid were loaded onto the nanostructures for simultaneous delivery. Upon binding to the LA receptors located on the cancer cell surface, and with the stability of pH 7.4, the nanostructures internalized into tumor cells and released the drugs. The released drugs were effective in suppressing the proliferation and invasion of HCC cells, inducing apoptosis, and inhibiting angiogenesis. Besides, he nanocomplex meaningfully reduced the tumor in HCC H22 tumor-bearing mice model and prevented the lung metastasis in the H22 lung metastasis models. As a results, co-delivery of UA and SO through MSN-CS-LA nanocarriers can be developed a hopeful approach for HCC

combinational therapy, particularly for the HCC metastasis chemoprevention (Fig. 7) [213]. Moreover, mesoporous magnetic nanocomposites (Fe_3O_4@$mSiO_2$) were prepared such vthat Fe_3O_4 nanoparticles formed the core and $mSiO_2$ provided the shell for the delivery of doxorubicin in HCC therapy. All of these nanoparticles were wrapped by CS toprevent premature drug release. At pH 4, nanostructures released 86.1% of doxorubicin, suggesting their pH-sensitive function. Blank nanostructures were not toxic, while drug-loaded nanostructures suppressed the viability of HCC cells [214].

Due to the importance of using stimuli-responsive nanostructures, research has focused on developing multifunctional nanocarriers. In an experiment, magnetic- and pH-responsive hydrogels were developed for the delivery of methotrexate in cancer therapy. For developing platforms, first, Fe_3O_4-N_2 nanostructures were prepared using hydrothermal reactions. Next, methotrexate-loaded magnetic CS-based hydrogels were developed. Their encapsulation efficiency and drug loading were suggested to be 93.8% and 6.28%, respectively. Moreover, CS-based hydrogels were pH-sensitive and released methotrexate at pH 4, with a drug release rate of 90.6% at 48 hours. They showed good biocompatibility and reduced viability in HepG2 cells, revealing their high anticancer activity [215]. If both redox- and pH-stimuli are used for the development of nanostructures, they can exert more beneficial anticancer activity for the purpose of drug delivery. Carboxymethyl groups are also used in the synthesis of nanostructures with hydrophilic shells and hydrophobic cores. Nanostructures are modified with CD147 ligand to be responsive at variouspH- and redox-environments.

The nanoparticles exhibited an enhanced permeability and retention (EPR) effect, and upon modification with CD147 antibodies, they were efficiently internalized into HCC cells, thereby effectively inhibiting tumor progression. Additionally, drug release was responsive to both ROS and pH levels [216].

As a result, targeted therapy utilizing stimuli-responsive nanostructures is a promising approach for treating HCC. To fully understand the efficacy of nanostructures in HCC treatment, further research is

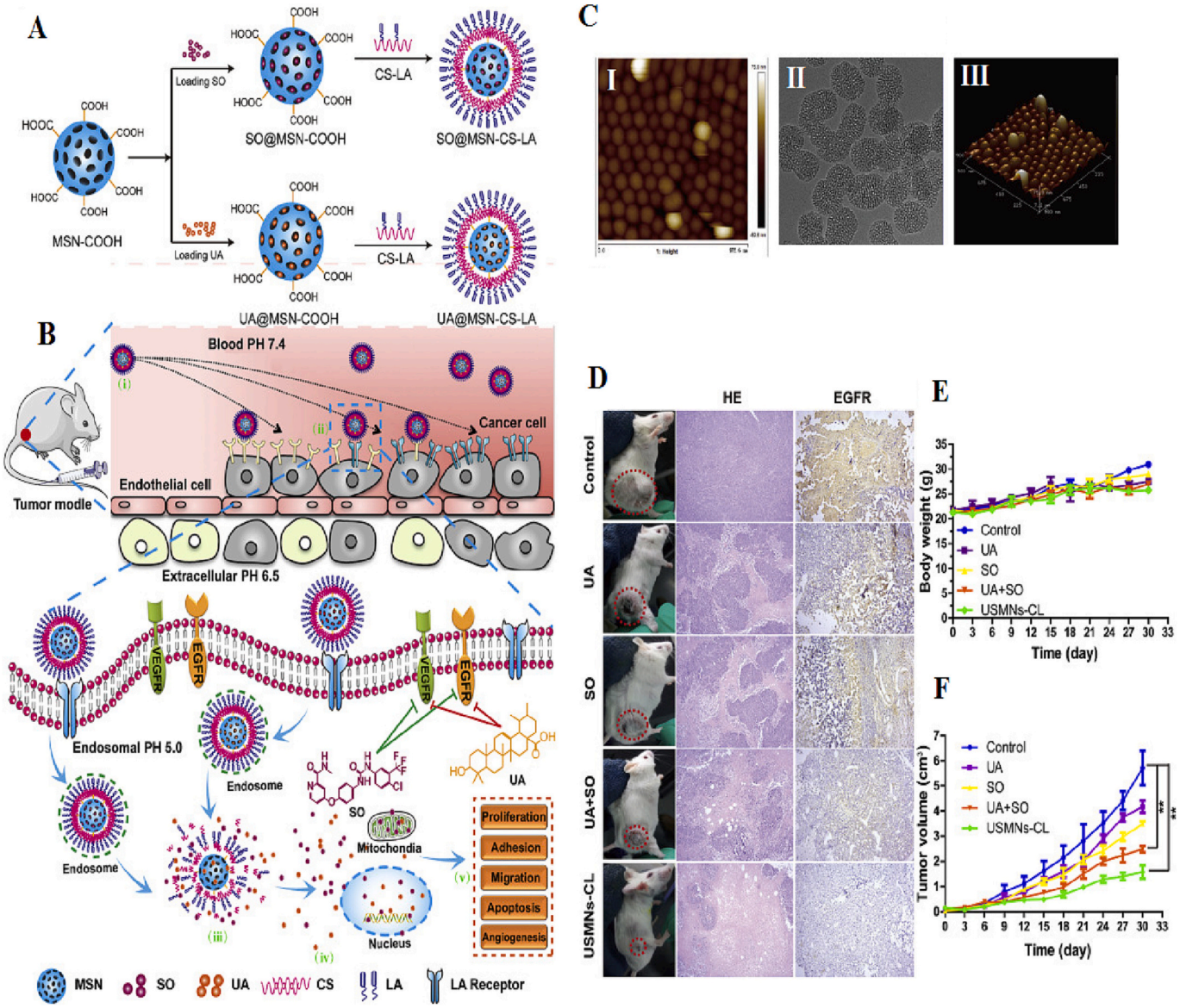

Fig. 7. (A) The synthesis process of USMNs-CL and their delivery activity. (B) Schematic presenting the co-delivery of UA and SO through MSN-CS-LA *in vitro*. Uptake of USMNs-CL complex by cells was done through endocytosis mediated by ASGPR receptor. Both UA and SO inhibited proliferation and increased cell apoptosis. (C) AFM image of MSN nanoparticles; (B) TEM image of MSN nanoparticles; (C) AFM image of MSN nanoparticles. This nanoparticle showed an average diameter of about 80 nm with uniform size diameter distribution. (D) Hematoxylin–eosin staining shows inhibitory effects on tumor growth. (E) Body weight change throughout treatment phases. (F) Tumor volume in lung metastasis model. **P < 0.01 [213].

needed. Additionally, ion- and solvent-responsive nanoparticles based on chitosan CS could be a promising avenue for HCC therapy and should be explored in future studies (Fig. 8 and Table 3).

8. Conclusion and remarks

The current manuscript provides a new insight into the treatment of HCC and highlights current difficulties in the therapy of this malignant disease. Surgery is no longer an effective therapy for HCC due to the migration of tumor cells at advanced stages.

As a result, the use of chemotherapy and radiotherapy is commonly favored for treating HCC. However, their effectiveness is being questioned due to the emergence of resistance, and they may also cause adverse reactions in patients undergoing cancer treatment.

As a result, novel therapeutic approaches for addressing HCC are being explored, with the use of nanoparticles being one such avenue. However, given that only biocompatible and safe nanostructures can be employed in clinical trials for patient treatment, the selection of appropriate nanostructures must be undertaken with great care. This review is primarily centered on CS-based nanostructures due to their high biocompatibility and safety, making them a promising candidate for future cancer treatment applications. The foremost obstacle in the treatment of HCC is the absence of drug targeting specificity, with a significant risk of drug resistance resulting from the aggressive nature of HCC cells. As a result, the use of CS-based nanostructures can facilitate targeted drug delivery and enhance the anti-tumor effects of drugs. The studies' notable strengths include their examination of both synthetic and natural compounds for drug delivery in HCC therapy. However, a potential limitation is the insufficient emphasis on the co-delivery of natural and synthetic agents, which could potentially mitigate drug resistance and increase cytotoxicity in HCC therapy. Gene therapy has emerged as a promising approach for tumor treatment, including HCC,

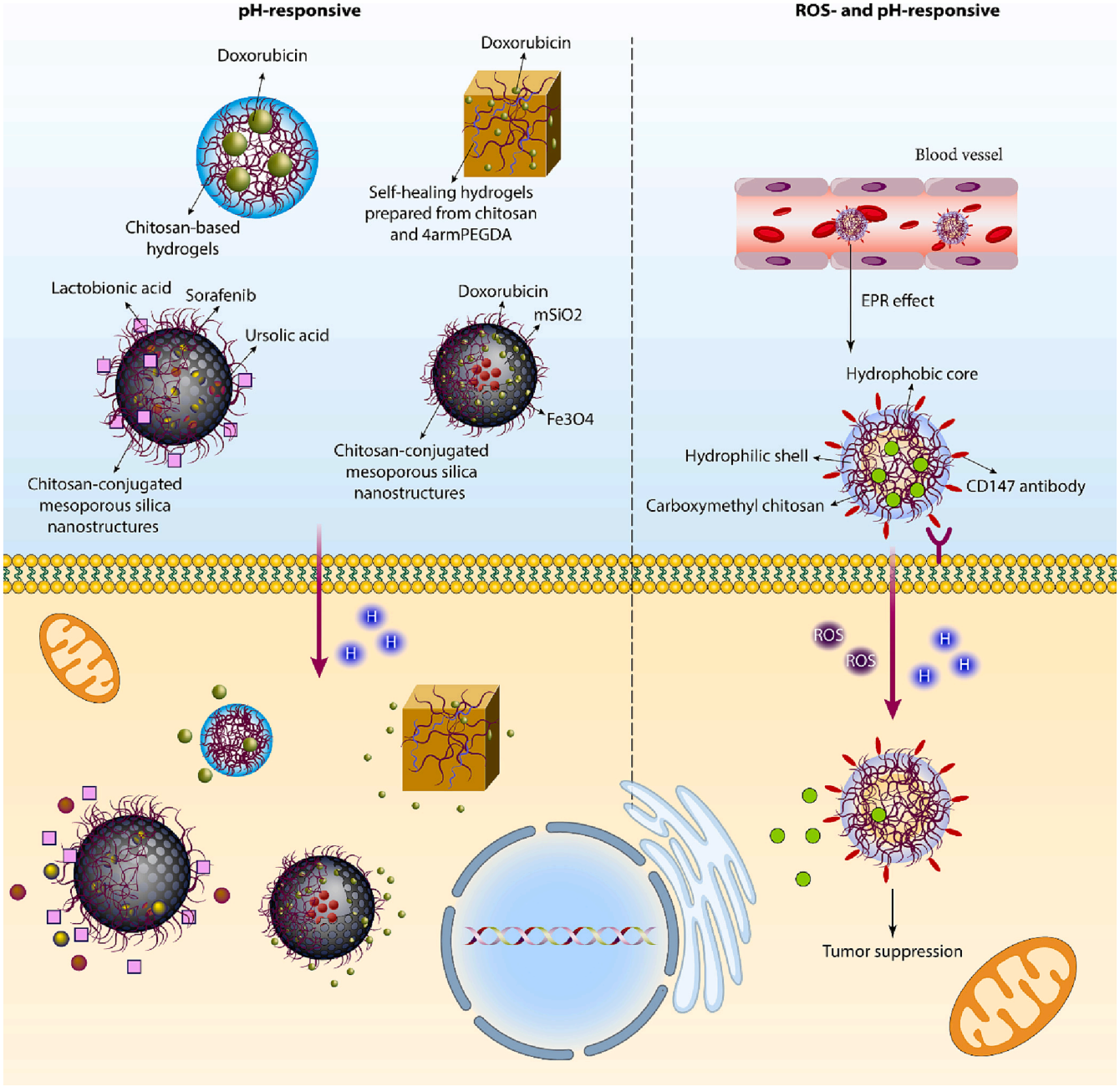

Fig. 8. Stimuli-responsive CS-based nanostructures in HCC therapy.

Table 3
The development of stimuli-responsive nanocarriers in HCC treatment.

(Nano)carrier	Responsiveness	Remark	Ref
CS/PEGDA self-healing hydrogels	pH-responsive	Sustained delivery of doxorubicin Apoptosis induction Reducing tumor progression	[208]
CS/PEGDA hydrogels	pH-responsive	Biodegradable and biocompatible hydrogels Reducing survival rate of HepG2 cells Sustained delivery of doxorubicin	[54]
CS/LA-modified mesoporous nanostructures	pH-responsive	Co-delivery of sorafenib and ursolic acid in cancer suppression pH-sensitive release of drugs Internalization in tumor cells by binding to LA receptor	[213]
Mesoporous magnetic nanoparticles wrapped with chitosan	pH-sensitive	pH-sensitive release of doxorubicin Suppressing viability of tumor cells	[214]
Magnetic CS hydrogels	pH- and magnetic-responsive	Sustained release of methotrexate and release in response to pH Anti-cancer activity High biocompatibility	[215]
CD147-modified CS nanostructures	ROS- and pH-responsive	Targeted delivery of DOX Redox and pH release of cargo Suppression of tumor cells Internalization acceleration due to modification with CD147 antibody	[216]

but its efficacy is limited due to enzymatic degradation and off-target effects. CS-based nanostructures with positive charges can interact with negatively charged genes and enhance their delivery, thereby suppressing HCC progression. However, a limitation of current studies is the absence of experiments investigating the use of CS-based nanostructures for augmenting HCC immunotherapy, particularly in terms of polarizing macrophages from M2 to M1. Future research should address this gap. CS-based nanostructures can be used for phototherapy (PDT and PTT) to induce cell death in HCC and reduce the survival rate of tumor cells. Furthermore, modification of these nanostructures with ligands like RGD and GA has been explored to enhance their targeting ability. In addition, smart nanocarriers such as pH- and ROS-responsive CS nanostructures can potentially improve the efficacy of nanocarriers in HCC therapy. It is worth noting that future studies should also consider using CS-based nanostructures for boosting HCC immunotherapy by altering the polarization of macrophages from M2 to M1.

Due to their high biocompatibility, CS-based nanostructures hold great promise for clinical applications in the future. They have demonstrated significant potential in the treatment of HCC, particularly in the form of CS-based nanoscale delivery systems. These adaptable biomaterials provide a distinct blend of properties, including biocompatibility, biodegradability, and low toxicity, which make them suitable for drug delivery and imaging purposes. In this article, we have provided an overview of the current advances in chitosan-based nanoscale delivery systems for HCC and examined their potential in theranostics. CS-based nanoscale delivery systems offer a significant advantage in enhancing drug solubility and bioavailability. This is due to the hydrophilic properties of chitosan, which enables the encapsulation of hydrophobic drugs, ultimately improving their solubility and pharmacokinetics. Furthermore, due to their small size, CS-based nanoparticles can efficiently enter cancer cells, leading to an improved drug delivery system. Many studies have shown the effectiveness of chitosan-based nanoparticles in delivering various chemotherapy drugs, including doxorubicin, paclitaxel, and cisplatin. Along with drug delivery, CS-based nanoscale delivery systems have also been utilized for imaging purposes. CS-based nanoparticles can be used to improve cancer detection by incorporating imaging agents such as MRI contrast agents and fluorescent dyes. This approach can enhance sensitivity and specificity, allowing for targeted imaging of tumor growth and metastasis. Furthermore, chitosan-based nanoscale delivery systems offer theranostic capabilities, combining both imaging and drug delivery in a single platform. This approach has the potential to provide personalized treatment strategies for cancer patients. Another potential application of CS-based nanoscale delivery systems to HCC is gene therapy. Gene therapy involves the delivery of genetic material to cells, which can then be used to correct or replace defective genes. Studies have demonstrated that CS-based nanoparticles can effectively deliver genetic material, including siRNA and plasmids, to cancer cells. This approach can be used to target specific genes that are overexpressed in cancer cells, resulting in selective gene silencing and subsequent cancer cell death. Despite the numerous advantages of CS-based nanoscale delivery systems, there are still several challenges that need to be addressed before they can be translated to clinical applications. One of the key challenges is the stability and reproducibility of these systems. The manufacturing process of CS-based nanoparticles can be complex and requires careful optimization to ensure consistent particle size and drug loading efficiency. In addition, the stability of CS-based nanoparticles under physiological conditions can be influenced by various factors, including temperature, pH, and ionic strength, which may result in particle aggregation and decreased effectiveness. Another challenge is the potential toxicity of CS-based nanoparticles. Despite the generally accepted biocompatibility and biodegradability of chitosan, there are concerns regarding the potential toxicity of nanoparticles with prolonged exposure or at high concentrations. Therefore, extensive *in vitro* and *in vivo* toxicity studies are needed to evaluate the safety of CS-based nanoparticles before they can be used in clinical applications. The CS-based nanoscale delivery systems offer a versatile bio-platform for drug delivery and imaging applications in HCC. CS-based nanoscale delivery systems have demonstrated significant potential in preclinical studies, and their ability to facilitate personalized theranostic applications has garnered considerable interest in the field of cancer treatment. Despite their promising results, there are still a number of challenges that must be overcome before these systems can be implemented in clinical settings. Issues such as manufacturing reproducibility, stability, and toxicity must be carefully addressed through further research in order to optimize these systems and evaluate their safety and efficacy in clinical trials.

The development of chitosan-based nanoscale delivery systems for HCC involves the use of mathematical equations and models to optimize their design and predict their behavior in biological systems. One example is the use of diffusion equations to model the release of drugs from nanoparticles. The release of drugs from nanoparticles is a complex process, influenced by various factors including the size and surface properties of the nanoparticles, the nature of the drug, and the physiological conditions of the target tissue. Diffusion equations can be used to model the release of drugs from nanoparticles by considering the diffusion of the drug through the nanoparticle matrix and the surrounding tissue. Another example is the use of numerical simulations to study the interaction of nanoparticles with biological systems. Numerical simulations are powerful tools that can predict the behavior of nanoparticles in different physiological conditions and provide insight into their toxicity and biodistribution. For example, computational models can be used to simulate the interaction of nanoparticles with cell membranes, providing information on the mechanisms of nanoparticle uptake and toxicity. Numerical simulations can also be used to study the behavior of nanoparticles *in vivo*, predicting their biodistribution and pharmacokinetics. Furthermore, mathematical models can be used to optimize the design of imaging agents for theranostic applications. For

example, mathematical models can be used to predict the quantum yield and photostability of fluorescent dyes used for imaging. These models can be used to optimize the design of imaging agents by predicting the optimal dye concentration and excitation wavelength. Additionally, models based on the principles of optics and electromagnetism can be used to optimize the design of MRI contrast agents, predicting their relativity and magnetization.

The studies on CS-based nanostructures have demonstrated their potential in HCC therapy and drug and gene delivery, indicating the possibility of future clinical trials. However, the clinical application of these nanocarriers is not limited to their efficacy in suppressing tumorigenesis *in vitro* and *in vivo* and their ability to deliver drugs and genes. While these potentials are crucial for clinical use in patient treatment, safety and long-term toxicity are also important factors to consider. Studies evaluating the potential of chitosan-based nanostructures in HCC treatment have demonstrated not only their ability to reduce tumor cell viability but also their lack of toxicity towards normal cells, as confirmed by MTT assay. Additionally, other studies have provided further evidence of the biocompatibility of CS-based nanostructures, which supports their potential for future application in HCC therapy. A randomized clinical study has demonstrated the potential of CS-based nanostructures as promising candidates for the treatment of facile skin sebum. Furthermore, the study showed that these nanostructures have a satisfactory safety profile, with only mild and self-limiting scaling and acneiform eruption observed [217]. Following intravenous administration, chitosan-based nanostructures were found to accumulate mainly in the lung and liver without inducing leukocytosis or hemolysis. Although a slight retardation in weight gain was observed shortly after administration, it was soon recovered. Moreover, a 2-week follow-up study in rats demonstrated the high safety of CS-based nanostructures, as they did not cause pain or distress [218]. A randomized clinical trial has confirmed the high safety and minimal side effects of utilizing CS-based nanostructures for wound dressing [219]. Chitosan and hyaluronic acid-coated nanohydrogels are suggested to be a promising option for MRI, with high biocompatibility and hemocompatibility [220]. Therefore, chitosan nanoparticles demonstrate a high level of safety and have the potential for clinical application in the future. No experiments have been conducted on the clinical application of chitosan nanostructures for the treatment of HCC patients. However, our search on clinicaltrials.gov has revealed that chitosan is being investigated for clinical use, and there are several ongoing efforts in this regard. For instance, chitosan is being studied for the modulation of AGE in prostate cancer patients (ref: NCT03712371), although one of the limitations of current clinical trials is the low number of participants (ref: NCT02591017). Additionally, chitosan has potential applications as a pH-sensitive biomaterial in predicting the prognosis of cancer patients (Ref, NCT04218188), and a phase III clinical trial involving 170 participants has evaluated its safety in breast cancer patients (Ref, NCT02967146). Despite the promising results of CS-based nanomaterials in preclinical studies, further research is necessary to investigate their efficacy and safety in clinical settings, particularly for HCC therapy. Notably, a phase IIb clinical trial has shown that a holmium-166/chitosan complex (Milican) can be used as a viable treatment option for HCC, with promising long-term outcomes and a high level of safety. This finding represents a significant step towards the clinical translation of CS-based nanomaterials for cancer therapy [221].

The current review article focused on the role of CS-based nanostructures for HCC therapy. It is worth mentioning that chitosan can also be employed for development of hydrogels [208] and microspheres [132]. However, the focus of the present paper is on the potential of chitosan-based nanoplatforms for the delivery of therapeutics and their promising role in HCC therapy. [222]. A substantial body of research has demonstrated the potential of chitosan-coated nanoparticles, which can be further modified with ligands, for targeted cancer therapy, including the delivery of drugs and genes in the treatment of HCC [149,169,170,223]. Chitosan modification of nanoparticles not only improves their stability and biocompatibility but also enhances their selectivity towards tumor cells when functionalized with ligands. Stimuli-responsive chitosan-based nanomaterials have also been explored for HCC therapy. Despite these significant advances and benefits, there are still limitations and drawbacks that need to be addressed in future studies. For instance, when nanostructures are coated with chitosan, their interactions with proteins and other agents in serum may be altered, leading to changes or suppression of the protein corona.

Future studies should focus on conducting *in vitro* and *in vivo* experiments to demonstrate how the modification with chitosan can affect the behavior of nanostructures in HCC therapy. Another significant limitation that needs to be addressed is the lack of specific focus on clinical trials, which is the ultimate goal of pre-clinical studies.

Abbreviations:

CS	Chitosan
HCC	Hepatocellular carcinoma
PEG	Polyethylene glycol
HBV	Hepatitis B virus
HCV	Hepatitis C virus
Hsp90	Heat shoch protein 90
ROS	Reactive oxygen species
NF-kB	Nuclear factor-kappaB
FDA	Food and Drug Administration
5-FU	5-flourouracil
GE	Ginger extract
VEGF	Vascular endothelial growth factor
DOX	Doxorubicin
COX-2	Cyclooxygenease-2
PGE2	Prostaglandin E2
MDR1	Multidrug resistance protein 1
MAbs	Monoclonal antibodies
DR5	Death receptor 5
RNAi	RNA interference
shRNA	Short hairpin RNA
siRNA	Small interfering RNA
miRNA	microRNA
STAT3	Signal transducer and activator of transcription 3
MDM2	Murine double minute 2
LA	Lactobionic acid
GA	Glycyrrhetinic acid
ERK	Extracellular signal-regulated kinase
PLK1	Polo-like kinase 1
GCP	Ggalactosylated CS-graft-poly(ethylene glycol)
PDT	Photodynamic therapy
PTT	Photothermal therapy
NC	Nanocrystal
GQDs	Graphene quantum dots
GO	Graphene oxide
Cela	Celastrol
CMC	Critical Micelle Concentration
Akt	Protein kinase-B
PI3K	Phosphatidylinositol-3 kinase
EPR	Enhanced Permeability and Retention
RGD	Arginylglycylaspartic acid

CRediT authorship contribution statement

All the authors participated in writing first draft, its edition during revision time, collecting manuscripts and data, language edition, drawing figures, filling tables and the final manuscript was confirmed by all authors. K.K, S.M, Po.M.T, M.M.N, A.R, S.M, E.H, F.Y, F.S and M.A.Z participated in writing first draft. N.N, N.R and Y.N.E contributed to English edition of paper and obtaining and extracting figures of other papers, checking references and improving quality of paper. S.S and M.R

drew figures and edited paper. P.R, K.H and W.Y participated in conceptualization, collecting papers, editing manuscript, writing some introductory words and supervising paper.

Declaration of competing interest

The authors declare no conflict of interest.

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