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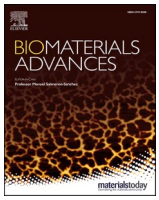
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Review



Chitosan and hyaluronic acid-based nanocarriers for advanced cancer therapy and intervention

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ABSTRACT

Cancer has become a major public health issue leading to one of the foremost causes of morbidity and death in the world. Despite the current advances in diagnosis using modern technologies and treatment via surgery or chemo- and radio-therapies, severe side effects or after-effects limit the application of these treatment modalities. Novel drug delivery systems have shown the potential to deliver chemotherapeutics directly to cancer cells, thus minimizing unnecessary exposure to healthy cells. Concurrently, to circumvent difficulties associated with conventional deliveries of cancer therapeutics, natural polysaccharides have gained attention for the fabrication of such deliveries owing to biocompatibility, low toxicity, and biodegradability. It has been exhibited that natural polysaccharides can deliver high therapeutic concentrations of the entrapped drug to the target cells by sustained and targeted release. Considering the immense potential of natural polymers, the present work focuses on naturally generated biopolymer carriers based on chitosan and hyaluronic acid. This review delineated on the role of chitosan and its derivation from renewable resources as a biocompatible, biodegradable, non-immunogenic material with notable antitumor activity as a drug delivery carrier in oncotherapy. Moreover, hyaluronic acid, itself by its structure or when linked with other molecules contributes to developing promising pharmaceutical delivery systems to setback the restrictions related to conventional cancer treatment.

1. Introduction

Cancer is considered to be the major burden of disease that results in leading causes of mortality in developed, developing, and poorly developed countries worldwide [1]. Overall, it affects one in four people over their lifetime. The most frequently recorded cancers include prostate, breast, lung, colon, rectum, urinary, uterine, and skin cancer [2–4]. In particular, cancer is the condition of uncontrollable cell development that can occur anywhere in the body due to a mutation of the genetic material [5]. These genetic mutations either occur as part of aging or due to exposure to mutants. In addition, cancer might show epigenetic abnormalities (i.e., gene expression changes that are phenotypic but do not impact the DNA sequence) such as covalent modifications of chromatin, physical modifications in nucleosomal positioning, or focal increase in DNA methylation [6]. These alterations in gene expression might lead to progressive uncontrolled growth of the cells, leading to the formation of tumors. Since genetic alterations in cancer cells are heritable, cells bearing these alterations are liable to Darwinian selection (survival of the fittest). It favors the genetically distinct subclones with more aggressive characteristics, thus shaping the evolution of cancer. Therefore, genetic and epigenetic changes collaborate to cause the development of cancer as these are mitotically heritable [7,8]. This uncontrolled cellular growth is categorized into benign, which remains localized and can be removed by local surgery, and malignant, which can invade and destroy adjoining structures and spread to different parts of the body, leading to death [9–11].

The risks associated with emerging cancer come from interactions between heritable variants and three different types of exogenous

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agents: 1) physical mutagens such as ionizing X-rays and non-ionizing ultraviolet rays, 2) chemical mutagens such as components in tobacco smoke, heavy metals (e.g., arsenic), aflatoxin, alcohol, and asbestos, and 3) biological factors such as infections by bacteria (*Helicobacter pylori*), parasites and viruses (human papillomavirus). As documented, the incidence of cancer varies with age; elderlies are more susceptible because of a build-up of risk for specific mutagens and less effective cellular repair mechanisms. In addition, other comorbid conditions including obesity or reproductive factors can also lead to carcinogenesis [12,13]. Various stimulators for developing cancer have been summarized in Fig. 1. Largely, these cancerous conditions can broadly be treated with chemotherapy, surgical resection, and radiotherapy [14]. While compared to conventional treatment strategy, targeted therapy is the most advanced treatment strategy against cancer conditions with low toxicity and high curative potential [15]. It spares the exposure of the therapeutics to the normal cells, thereby their functions are not hampered. The first small, targeted molecule that has been brought to the clinical use of cancer is imatinib, a tyrosine kinase inhibitor [16]. Further, several other approaches have been brought as targeted therapy using monoclonal therapy, gene therapy, ablation therapy, other small molecules, etc.

Alternatively, advancement in drug delivery systems has also brought several novel strategies with increased solubility of the poorly water-soluble chemotherapeutic agents and increased stability of the formulations when compared with the conventional preparations. Furthermore, these preparations could prolong the stay of the formulation within the circulation along with the release of entrapped therapeutic agent(s) at a specific site of cancer because of targeting ligands on the carrier surface and the high vascularity of the cancerous microenvironment [17]. Thus, the incorporation of the drug into advanced polymeric or lipid-based formulations, complexation, dispersion of the therapeutics, or modification of the crystal habits of the molecules showed potential implications in several preclinical and clinical outcomes [18]. To fabricate, such formulations require the incorporation of judiciously selected excipients possessing low accumulation and toxicity to the human body. Mostly, the excipients used to develop such formulations are generally recognized as safe categories of pharmaceutical components. However, considering the immense role of these excipients in the development of a suitable formulation to deliver chemotherapeutic agents, chitosan, and hyaluronic acid (HA) are widely used for their biodegradable and biocompatible characteristics with the flexibility to structural modifications to target activation and release at any specific stimuli. Several formulations using those two natural polymers have shown the potential of sustained release of the entrapped medications at the cancer site for superior control of cancer cell proliferation [19–22]. With these concepts, the current article has summarized recent progress in the delivery systems using these two natural polymers with special emphasis on targeting tumor growth for improved efficacy. A detailed account of these two natural polymers with superior preclinical findings, and clinical and regulatory aspects for future researchers has been summarized.

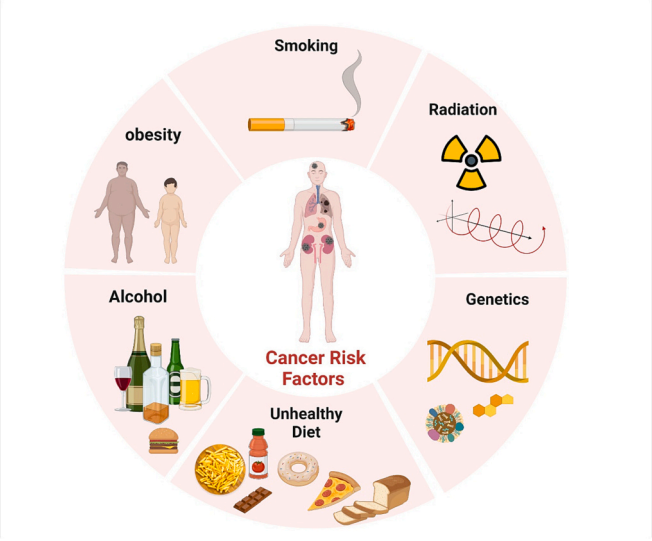


Fig. 1. Presentation of different types of cancer risk factors. (Created with Biorender.com)

2. Selection of literature

PubMed, Springer, Google Scholar, and Science Direct were explored to find significant outcomes on the scientific paper based on the English language. Two reviewers independently screened the title and abstract with consideration of keywords such as "chitosan-based drug delivery", "anticancer agent", "cancer therapy", "HA in cancer therapy," and more with a combination of "OR" and "AND". Additionally, cross reference of articles was used for further collection, later incongruities were fixed by discussion. The prerequisites for selection were papers with in-vitro data, together with cell line studies and/or animal studies, while the criteria for exclusion were review-based articles, letters to editors, conference papers, case studies, and articles in other languages. The search was restricted to the last ten years.

3. Challenges with cancer treatment

As discussed earlier, cancer is a life-threatening disease in the world and is treated by traditional therapy strategies such as chemotherapy, surgery, radiotherapy, and immunotherapy. However, these treatment strategies are inevitably associated with inadequacies, for instance, lack of target specificity, and low inhibition efficiency which may lead to cancer reoccurrence after treatment. This is evident by the literature that various pathophysiological process was affected by a surgical procedure that provoked the proliferation of cancer cell and the recurrence of the tumor. Moreover, surgical manipulation could enhance cancerous cell dissemination in blood circulation which persuades immunosuppression and surges the cancer cell colonization in target organs [23,24]. In existing scenarios, chemotherapy is abundantly used for treatment, however, it leads to detrimental side effects, high treatment costs, and drug resistance. Moreover, both chemo and radiotherapy have a lack of target specificity, however, recent research is focused on the development of targeted formulation to provide better treatment outcomes and overall survival for patients [25,26].

Besides the traditional cancer treatment options, immunotherapy gains attention to conquer the cancer. Its effectiveness relates to the regulation of the immune system instead of concentration on cancer cells. However, some challenges are also encountered with immunotherapy such as off-target effects, immune-related side effects, etc. [27,28]. Further to improve anticancer therapy outcomes, chemo-immunotherapy was discovered to provide a synergistic effect. It incorporates the two treatment mechanisms which reduce the dose with enhanced therapeutic effect; however, lack of target specificity, off-target interaction with the immune system, and toxic effect on healthy tissue is the major setback [26,29]. Recent literature demonstrated that the immunosuppressive tumor microenvironment could be remodeled by the use of a nanocarrier. In another approach, nanocarriers were developed to elicit the immune response by eliminating the immune calls along with tumor modulation to get an impressive antitumor effect [30].

In the exploration of new treatment strategies for cancer, nanocarriers emerge as a promising tool to overcome the major setback of conventional therapy. Extensive research has been done to investigate the targetability, sustained delivery, and antitumor effect of nano chemotherapeutics fabricated with natural and/or synthetic polymers [25]. This article has drawn attention to chitosan and HA-based nanocarriers

due to their stability, safety, easy accessibility, and modification friendly [24,27].

4. Structure, physiochemical properties, and anticancer potential of chitosan and hyaluronic acid as nanocarrier

4.1. Chitosan

Chitosan is the natural origin of cationic polysaccharides, belonging to the aminoglycopuran family [31,32]. Chitin is collected from crustacean shells like shrimp, crabs, crayfish, etc., and is removed by partial deacetylation [33]. It is a linear copolymer polysaccharide composed of β-(1-4)- linked- D-glucosamine and N- acetyl-D-glucosamine. Chitosan has a similar structure to cellulose, except the C-2 hydroxyl group of cellulose has been changed to an acetamide group, which is then converted into primary amino groups. This polymer is semi-crystalline and exhibits polymorphism [34]. They are flakes with no color or smell and easily soluble in an acidic aqueous solution. The solubility of chitosan is poor at pH 7.4 (physiological pH) due to its weak base (pKa 6.2–7) property. The variables utilized for de-acetylation will determine the level of de-acetylation and average molecular weight (Mw). Chitosan has a MW that ranges from 10 to 1000 kDa. The degree of de-acetylation is typically between 70 and 95 %; however, for optimum chitosan solubility, at least 85 % deacetylation is needed [35].

Chitosan is extensively used in pharmaceutical, biomedical, and cosmetic applications. It is a biodegradable (as it is metabolized by certain human enzymes, like lysozyme), biocompatible, positively charged biopolymer [37]. Chitosan, in reaction with polyanionic compounds including citrate, sulfate, and tripolyphosphate, readily produces biodegradable nanoparticles. Chitosan is different from other biodegradable polysaccharides because its primary amino groups have a cationic character, which is responsible for its distinctive characteristics like sustained drug release, mucoadhesion, transfection enhancement, efflux pump inhibition, and permeation enhancement properties.

Chitosan has been demonstrated to cause cell death and trigger caspase-3. It has also been proven to activate caspase-8, which initiates the extracellular cellular death cascade [38,39]. Necrosis has also been proposed as the pathway through which chitosan nanoparticle exposure causes cancerous cells to die. Additionally, it was further observed that proliferating cell nuclear antigens (PCNA), p27/Kip, and p21/Cip suppress the malignant cell cycles [40]. Additionally observed is inhibition of the cancer cycle in cells associated with proliferating cell nuclear antigen (PCNA), p21/Cip, and p27/Kip [41]. In a study, efforts were made to investigate the effect of low-molecular-weight chitosan individually as well as with cisplatin on oral SCC Ca9-22, and non-cancerous keratinocyte HaCaT cell lines. The method of MTT assay and laser scanning cytometry were used to evaluate the survival of cells and cell cycle characteristics, correspondingly. The TUNEL test and electron microscopy were used to investigate apoptosis, and then caspase function was measured. While cisplatin caused death in both kinds of cells, LMWC had cell-damaging effects on Ca9-22 yet did not affect HaCaT cells. The G1/S stoppage of the cell cycle, a rise in TUNEL-positive cells, a premature death shape of the cell, and moderate spikes in caspase activity were the results of exposing Ca9-22 cells to low molecular weight chitosan. The cancer-fighting properties of low molecular weight chitosan were exhibited by triggering the processes of cell death and arresting the cell cycle. Short-term treatment with low molecular weight chitosan was not as harmful to HaCaT cells in comparison to Ca9-22 cells [42].

Furthermore, Abedian et al. also compared the 2 % w/v solutions of low molecular weight and high molecular weight chitosan on three different cancer cell line types viz. HeLa, Saos-2, and MCF-7, based on cellular toxicity and induction of cell death by MTT assay. Human fibroblast cells were taken as control cell lines. Following treatment with varying concentrations of chitosan, the cell's survival was assessed using the MTT test at 24, 48, and 72 h. The distinction between necrosis as opposed to apoptosis was also identified using the Annexin V labeling technique and the method of flow cytometry analysis. Three malignant cell lines were successfully inhibited from proliferating by both forms of chitosan; however, fibroblast cells appeared to be more responsive to chitosan. Researchers observed a considerable reduction in the survival of all three cell lines, up to 90 %, but not influenced by concentration variations in the cellular toxicity qualities of both high molecular and low molecular weight chitosan. According to flow cytometry studies, MCF-7 had more visible necrosis, whereas Saos-2 and HeLa had pronounced death by apoptosis patterns. Additionally, fibroblasts were shown to have better vitality with both forms of chitosan when treated like normal cells [43].

This polymer is widely used to enhance retention time and bioavailability by designing bio-adhesive drug delivery systems [44]. High molecular-weight chitosan has higher viscosity and mucoadhesion properties than low molecular-weight chitosan. The viscosity of chitosan is also subjected to the level of deacetylation, pH, and temperature of the solution. The functional groups and molecular weight of chitosan play a key role in preventing bacterial and fungal growth [45,46]. Its application for designing controlled delivery systems is limited due to its rapid water absorption and higher swelling rate, directing the rapid release of the drug. To overcome this, various chemically altered chitosan derivatives have been designed. Chitosan is pH-sensitive; it precipitates at pH above 6.5 and mucoadhesiveness loss and penetration properties increase in the intestine, thus reducing its application to drugs having an absorption window in the upper GI tract [47]. It can transport therapeutics by enhancing transcellular and paracellular transportation modes and loosening epithelial cells' tight junctions. Its exact medication delivery mechanism to targeted areas is not fully understood. Enhanced EPR and pH-dependent release make chitosan suitable for cancer cell treatment [46,48,49]. In an investigation, chitosan was found to have an anti-cancer effect due to its degree of deacetylation and molecular weight [50]. Chitosan or its functionalized derivatives, such as polypyrrole-chitosan, sulfated chitosan, sulfated benzaldehyde chitosan, carboxymethyl chitosan, chitosan oligosaccharides, and chitosan-thymine conjugate showed promise anticancer efficacy against a variety of cancerous cells [51,52]. Combining chitosan derivatives with nanoparticles or other active compounds can further alter their anticancer properties. 3-amino-2-phenyl-4(3H)-quinazolinone was conjugated on a PPC-silver chloride nanocomposite to increase chitosan's bioavailability to tumor cells. This formulation demonstrated the prolonged release of chitosan to cancer cells while safeguarding non-cancerous cells [53]. Fig. 2 highlights the applications of chitosan and its derivatives in the treatment of cancer.

Through the reduction of new blood vessel growth, immunostimulatory, decreasing the free radicals induced damage, cell death, and enzymatic regulation, chitosan or its analogs specifically permeate cancer cells and demonstrate anti-proliferative effects [53]. Low molecular weight chitosan promotes cytotoxicity and stops cancer cell growth at the G1/S phase [42]. Low molecular weight chitosan with a molecular weight of 21 and 46 kDa are water soluble and triggers macrophages by inducing the intestinal intraepithelial lymphocytes to produce cytokines including interferons, and interleukins (12 and 18) [54]. Additionally, via regulating NF-kB mediated signaling pathways, and cell-cycle associated genes (Cdc25A, p21/Cip, and p27/Kip), chitosan increases the expression of transcriptional growth factor β and triggers cell death [55]. Chitosan-thymine-conjugates selectively target cancerous cells by exhibiting antiproliferative effects against HepG2 liver cancer cells while showing no inhibitory effects against NIH3T3 normal mice fibroblasts [56]. Likewise, polypyrrole-chitosan exhibited anticancer action against MCF-7 breast cancer cells and Ehrlich ascites carcinoma (EAC) cells [57].

Carboxymethyl chitosan inhibited the growth of new blood vessels by reducing vascular endothelial growth factor (VEGF) expression while enhancing the immune system response by activating interferon-γ and tumor necrosis factor-α levels [58]. Additionally, sulfated chitosan and

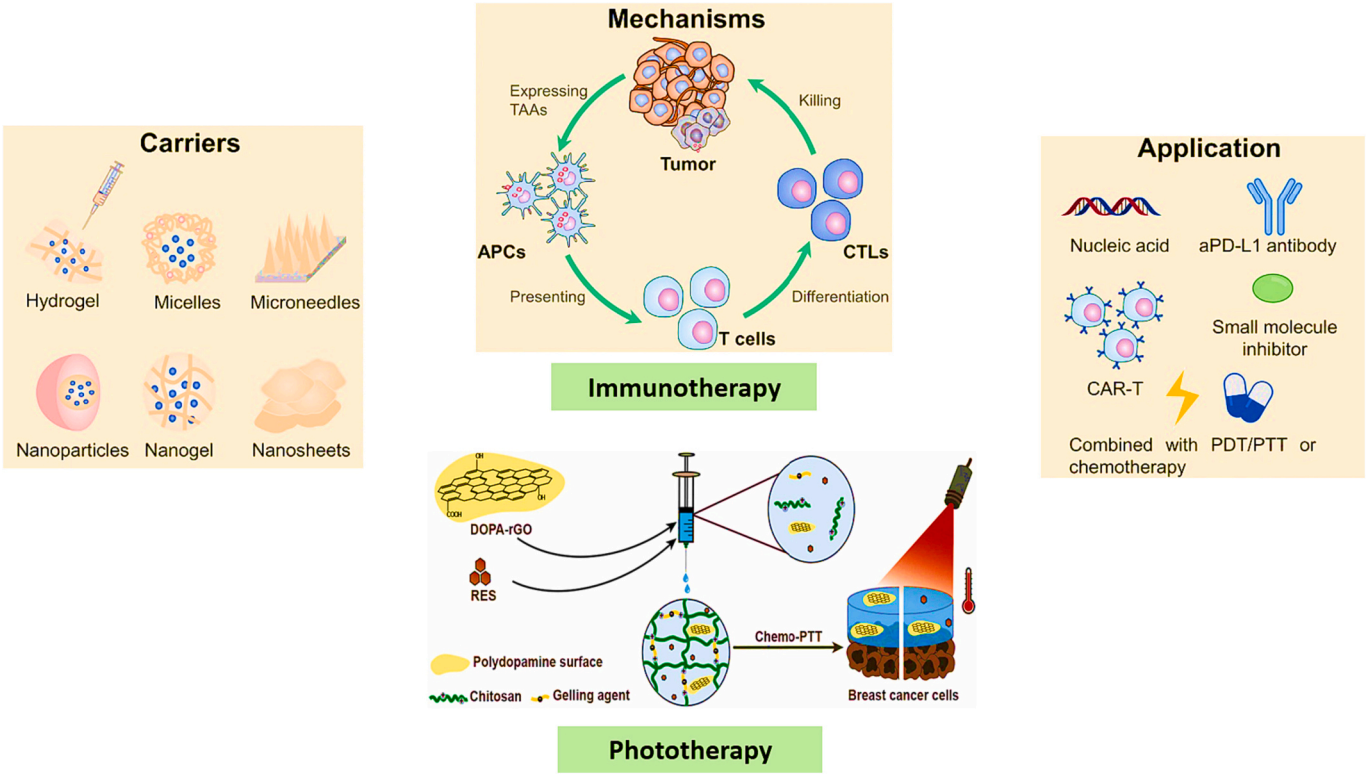


Fig. 2. Chitosan and its derivatives illustrating the mechanism and application in cancer therapy. (TAAs: Tumor-associated antigen; APC: Antigen-presenting cells; CTLs: Cytotoxic T lymphocyte; CAR-T: Chimeric Antigen Receptor T-cell; PDT/PTT: Photodynamic/Photothermal therapy).
Adapted with the permission from [27,36].

sulfated benzaldehyde chitosan showed chemotherapeutic action toward MCF-7 cells by inducing apoptotic cell death and preventing the activation of extracellular signal-regulated kinases by FGF-2. These two derivatives had significantly more apoptotic activity than chitosan [59]. Additionally, chitosan oligosaccharide demonstrates anticancer efficacy against colorectal, hepatocellular, and human cervical carcinoma cells, irrespective of their charge [60].

The zist of the structural contribution of chitosan is important as a drug delivery carrier, it has a protonable amino group in structure which imparts higher solubility in an acidic tumor environment with a pKa value of 6.5. However, hydrogen bond disruptors play an important role in changing solubility with physical and chemical characteristics. The degree of deacetylation (DD) governs the chitosan backbone's free amino groups, which determine the DNA/siRNA binding efficiency and positive charge density. It also helps to enhance transfection efficiency by escaping from an endolysosomal compartment [19,61]. A study found that low MW chitosan exhibits higher cytotoxic effects on oral squamous cell carcinoma compared with high MW chitosan. This may be attributed to the higher positive charge of the amino group which has a higher interaction with the negatively charged cancer cell membrane or by amino group medicated endocytosis [42,62].

Chitosan has received approval from the European Union (EU) and the US Food and Drug Administration (US-FDA) for use as an ingredient in foods, fat-absorbing substances, and wound dressings [48]. Due to the presence of functional amino groups on its surface, the molecule chitosan can interact via electrostatic attraction with molecules that are negatively charged like cells, nanoparticles, lipids, medicines, and macromolecules [63]. In bulk form, chitosan is safe and demonstrates low toxicity, but in the form of nanocarriers, reveals different biological characteristics viz., high surface area, and polydispersity. Therefore, safety standards for chitosan nanoformulations must be established [64].

Current investigations on the toxicity of chitosan provide perplexing data and a range of interpretations that are not entirely supported by the findings. Chitosan and its derivatives have been studied for their potential toxicity, and it has been found that irrespective of their molecular mass, chitosan becomes more toxic as the degree of acetylation increases. Additional findings demonstrate that cytotoxicity is inversely related to molecular mass [65]. In this regard, Frigaard et al have brilliantly reviewed and compiled the reported cytotoxicity of chitosan-based and derivatized-chitosan-based nanoparticles in their review. The investigators have exhaustively reviewed different kinds of chitosan nanocarriers, either as in core or in coating. They concluded, despite the difficulties in evaluating the outcomes of various tests and methodologies that investigators faced, most chitosan nanoparticles showed minimal or limited cellular toxicity irrespective of the particle composition, derivatives, cellular toxicity assay, cell lines, and animals employed in both in the laboratory and preclinical studies [66].

4.2. *Hyaluronic acid*

The glycosaminoglycan compound HA, commonly known as hyaluronan, was found in the vitreous humor of the bovine eye. It is a linear polyanionic polymer comprised of consecutive units of N-Acetyl-D-glucosamine and glucuronic acid. It is synthesized by an integral membrane protein known as hyaluronan synthases and begins to migrate in the extracellular environment and lengthen its polymeric chains [67]. The only glycosaminoglycan that neither interacts with the core protein nor undergoes post-synthesis is HA and exists in both organs and tissues [68–71].

The hyaluronidases family of enzymes regulates the fragmentation of hyaluronan. In somatic cell mass, HA is metabolized by hyaluronidase-2 (Hyal-2) and hyaluronidase-1 (Hyal-1). Hyal-2, an enzyme that is phagocytosed and transported to lysosomes where hyaluronan performs additional degradation, first fragments hyaluronan up to molecular weight 20 kDa [72]. Varying HA fragments with diverse molecular sizes

exhibit unique or occasionally conflicting capabilities. For instance, HA fragments with greater molecular weights exhibit anti-inflammatory and immunosuppressive properties, whereas HA fragments with lower molecular weights are pro-inflammatory molecules and serve as messengers for cells to learn about various stress conditions [73,74].

Research has proved that HA has a vital role in regulating various biological activities like infiltrating cancer and metastasis, embryogenesis, angiogenesis, granulation tissue formation, and repair [75,76]. Given that RHAMM (HA-mediated mobility receptor) and CD44-HA (cluster differentiation) receptors are upregulated in a variety of cancer types, the HA-drug complex has been employed to deliver anticancer medicines because of their adaptability. These conjugates offer various advantages such as enhanced solubility, prolonged release, and improved targeting properties. Many HA-coupled delivery systems have been studied, and they demonstrate the tumor-specific release of anticancer agents, resulting in their increased antitumor activity. Folate, epidermal growth factors, and transferrin are a few examples of upregulated receptors that are found on the exterior of malignant cells and are what allow tumors and normal cells to be distinguished from one another. Another important surface receptor is CD44 also known as H-CAM. It is a transmembrane glycoprotein and is stem-like in structure, associated with metastasis, cancer progression, development of malignancies, and lymph node infiltration. Additionally, it is a key biomarker for the early diagnosis of numerous cancers, including ovarian, hepatocellular, and basal-type breast carcinoma. The interaction between HA and CD44 can activate several signaling pathways, leading to the growth, adhesion, and metastasis of cancer cells [77]. This HA's unique ability to bind with CD44 receptors has been used to develop tumor-specific release of anticancer drugs using HA-coupled nanocarriers, in addition, it aids in strengthening the chemo-resistance against anticancer medications [78].

To adequately elucidate the role that HA size plays in both physiological equilibrium and cancer, consideration must be given to the various molecular weight (MW) forms of HA viz. oligomeric HA, Low MW-HA, High MW-HA, and Medium MW-HA. HMW HA has contrary outcomes, namely the suppression of mitogenic processes and inflammation, whereas low molecular weight HA increases multiplication and inflammatory processes. HMW-HA has been scientifically demonstrated to have antiangiogenic properties that prevent the formation of vascular smooth muscle cells by keeping them in the stage of G1 [79]. HMW-HA inhibits proliferation by strengthening cell-to-cell interaction and reducing ECM penetration. HMW-HA may aid in preventing metastasis by producing a more substantial ECM. The application of HMW-HA enhanced the single-layer cancer lymphatic endothelium cell integrity, preventing the growth of cancerous cells [80]. Furthermore, the particular inhibition of Rac GTP-loading and Rac-dependent communication to cyclin D1 is caused by HMW-HA attachment to CD44 which in turn inhibits the proliferation of cells [81]. By suppressing the synthesis of matrix metalloproteinase and cyclooxygenase-2, as well as by blocking mitogen-activated protein kinases and Nuclear factor kappa B (NF- κB) stimulation, HMW-HA has been demonstrated to decrease pro-inflammatory responses [82]. Owing to its anticancer qualities that were shown following external administration, HMW-HA is regarded as an attractive ingredient to supplement adjuvant cancer treatment [83]. Research has shown that HA protects human skin fibroblasts against oxidative stress's cancerous repercussions. NF-κB and caspase modulation play a role in HA's capacity to reduce damage caused by oxidative stress in cultures of fibroblasts [84].

Drug-coupled with HA positive outcomes include an increase in time of circulation, solubilization, and stabilization of the drug, and the capacity to target cancerous growth [85]. The carboxyl, hydroxy, and acetamido units are three functional components that can be modified chemically using HA [86]. To synthesize polymers that might be employed for a variety of biological purposes, including the manufacturing of prodrugs, the carboxylic position of HA might be utilized for targeted chemical functionalization with various hydrazides [87]. Another technique is to use carbodiimide to activate the hydroxyl moiety of paclitaxel so that it can be conjugated with 4-bromobutyric acid to synthesize 4-bromobutyric-paclitaxel with ester-linked which can easily conjugate with the HA [88]. Paclitaxel (PTX) and doxorubicin (DOX), the two most widely used chemotherapeutic medicines in the clinical setting, have also demonstrated excellent outcomes with conjugation with HA. One of the most effective substances and a compound with great anticancer potential is PTX. Nevertheless, PTX's lipophilicity and side effects make intravenous administration difficult. These constraints can be removed by PTX coupled with aqueous HA [89] (Fig. 3).

HA molecule consists of carboxylic, hydroxyl, and *N*-acetyl group which was used for structural modification. Anticancer activity of HA was demonstrated by its interaction with CD44 receptor which was overexpressed in cancer cells [91].

With a variety of special qualities, hyaluronic acid is an exceptionally versatile substance. Hyaluronic acid, a glycosaminoglycan that occurs abundantly in the body, has no sulfate bonding. Many alcohol and carboxylic acid groups, as well as a single amide functional group, are present in its chemical structure, which enables a variety of chemical changes. Fallacara et al formulated nasal insufflation of urea-crosslinked hyaluronic acid and sodium ascorbyl phosphate for the management of nasal inflammatory diseases. The formulation demonstrated no toxicity on human nasal septum carcinoma-derived cells (RPMI 2650 cells) [92]. Many studies have been conducted to check the cytotoxic effect of chitosan and hyaluronic acid. No investigation had demonstrated HA as toxic or carcinogenic [48,63–66,93].

5. Fabrication technologies for chitosan and HA-based nanocarriers

There are multiple formulation approaches for the development of chitosan nanoparticles or HA-nanoparticles or hybrid chitosan and HA-nanoparticles. The most commonly used techniques for the development of chitosan nanoparticles are ionotropic gelation, reverse micelles, and desolvation techniques. These techniques are described in detail as follows:

Ionotropic gelation method: This method involves the development of nanoparticles based on the electrostatic interaction of a polymer, such as CS or HA with an anion such as tripolyphosphate that serves as a cross-linking agent [93]. The characteristics of the NPs formed by this method are affected by the concentration of polymer, ratio of anion and polymer, pH, stirring time and speed, and centrifuge time and speed [94]. This method has multiple advantages it is easy and does not require the use of organic solvents or any fancy equipment. This method has one disadvantage as well, which is that it is not easy to form NPs of uniform size [95].

Reversed micelles method: This method is based on covalent cross-linking where in the aqueous phase containing the polymer and glutaraldehyde is mixed with the organic phase containing an organic solvent and surfactant using high-speed stirring [96]. This method has the advantage that it can be used to produce NPs smaller than 100 nm, but it also has the disadvantage of using organic solvents and glutaraldehyde which can be harmful to the body [97].

Desolvation method: In this method, two water-in-oil emulsions coalesce in the presence of a precipitating agent like NaOH, which results in the precipitation of NPs. After this, the NPs can be separated using centrifugation, washing, and drying. This method is suitable for fabricating NPs in the size range of 600-800 nm. This method has the advantage of encapsulating a higher percentage of hydrophobic drugs [98].

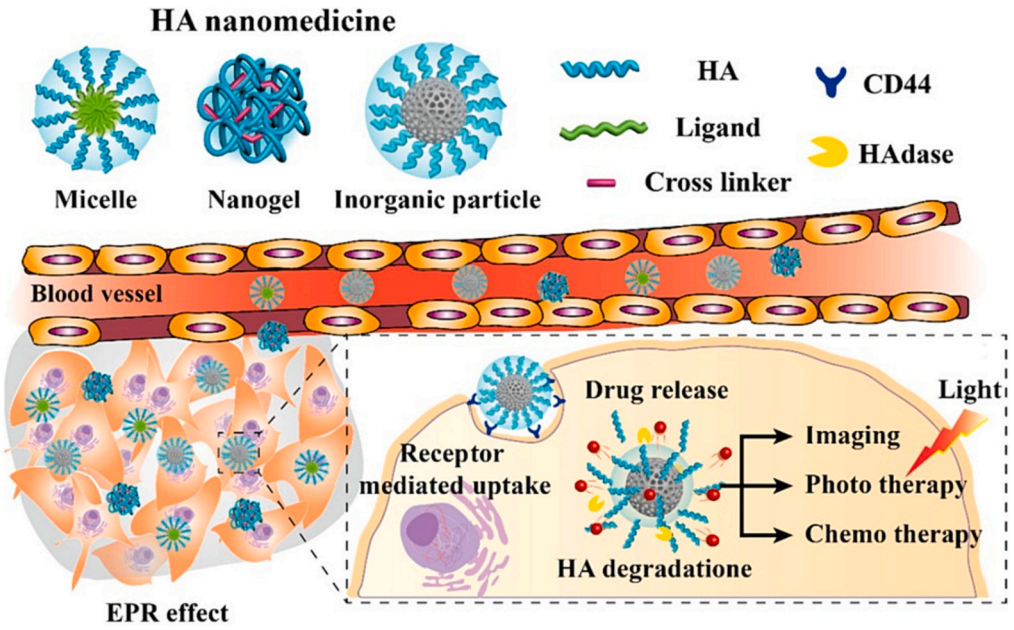


Fig. 3. Illustration depicting the role of hyaluronic acid in different formulations as an anticancer polymer.
Figure adapted with permission from [90].

5.1. Fabrication approach for hybrid chitosan and HA nanoparticles

Template method: This method involves the development of chitosan nanoparticles first using the ionotropic gelation method followed by mixing this template with a solution of HA under constant stirring [98]. This method can result in the formation of larger-sized NPs. It gives the advantage to control the morphology, structure, and size of the NPs [99].

Direct Complexation method: This method involves creating an aqueous dispersion of chitosan along with the drug and then adding it to a solution of HA under constant stirring. In this process the nanoparticles nucleate which results in increased particle size [98].

6. HA and chitosan to devise nanocarriers in the management of cancer

Drug delivery systems are designed to attain a balance between side effects and therapeutic efficacy with precise drug release patterns to avoid off-target release. Now, the focus turned toward biodegradable nano-formulation from nonbiodegradable nano-formulation, as it may accumulate in vital organs and lead to long-term toxicity. So, HA and chitosan-based drug delivery systems fascinated the major attention of researchers due to their excellent biocompatibility, and physicochemical and biological properties. Based on their suitable characteristics, HA and chitosan have been widely used in combination or alone in pharmaceutical formulations for the treatment of cancer. This section deliberates on the progress of preclinical research in the management of different types of cancer using these two natural polymers. Additionally, it will provide insight and recent research trends of chitosan and HA in cancer treatment (Table 1).

6.1. HA and/or chitosan-based formulations for the effective control of gastric cancer

The fourth most prevalent type of cancer and one of the most aggressive is gastric cancer. It has a high metastasis and local recurrence prevalence, making it the second most common reason for cancer-related deaths. Other features of gastric cancer include a low rate of early detection, a 5-year survival rate, and radical resection. Chemotherapy and gastrectomy combined with radiation therapy have been done as effective measures of gastric cancer. However, due to the low detection rate in the early stage, some of the advanced cases do not have the chance of surgery with a high risk of metastasis thus leading to poor prognosis. The majority of formulations of antitumor drugs in current practice have low bioavailability, target specificity, and therapeutic effectiveness. Therefore, to treat and diagnose gastric cancer, chitosan-based anti-metastatic and angiogenesis medicines have been created [119]. The excellent performance of chitosan was observed in terms of accumulation at the target site, low toxicity, and biological activity when it was combined/conjugated with small molecular drugs. Moreover, the improved biological property was shown by carboxymethyl chitosan due to its better water solubility. The concept was investigated by Chi et al. where conjugation of norcanctharidin (NCTD), a chemotherapeutic drug derived from blister beetle (Mylabris), with carboxymethyl chitosan was approached to treat gastric cancer [100]. In treating colorectal and stomach cancer, NTCD demonstrates substantial anticancer activity. Past studies have shown that NTCD can suppress metastasis and cause cell death in many human cancerous cells, but its use is restricted due to its short half-life, low intestinal absorption, and significant nephrotoxicity. Additionally, NTCD is injected at a high dose, which results in significant activation at the injection site. Thus, the authors examined the inhibitory effect of NTCD-chitosan conjugates on the proliferation of SGC-7901 cells and migration of HUVECs via transwell assay. The results revealed the significant inhibitory effects along with triggering apoptosis of SGC-7901 cells compared to free drug. When used on BALB/c nude cancer-induced mice, the findings demonstrate that norcantharidin-conjugated carboxymethyl chitosan has better antitumor activity than the bare drug. Fig. 4 demonstrates the outcomes of the in vitro cell line studies. In Fig. 4(c), with an extended time frame for incubation, the FITC-CNC was gradually endocytosed inside the cells. The SGC7901 cells showed modest fluorescence after 12 h of treatment. Following 1 day and 1.5 days of the incubation procedure the fluorescence enhanced and intensified, showing that the conjugate's cellular absorption in SGC-7901 cells was time-dependent [100].

The underlying mechanism for treating gastric cancer is increased TNF-α (tumor necrosis factor) and Bax and decreased gene expression of VEGF, MMP-2, MMP-9, and Bcl-2 [100]. In another study, Jiang et al.

Table 1
Compilation of chitosan and HA applications in the management of various cancer.

Disease	Drug	Polymer	Formulation	Cell Lines	Animal model	Reference
Gastric cancer and colorectal cancer	Norcanctharidin	Carboxymethyl chitosan	Drug-carboxymethyl chitosan conjugates	SGC-7901 cells	BALB/c naked cancer-affected mice	[100]
Gastric cancer	Cisplatin and sorafenib	HA	Lipid nanoparticles	MKN28 and SGC7901 cancer cells	BALB/cA nude mice	[101]
Gastric adenocarcinoma	Selenium	Chitosan oligosaccharide	Chitosan oligosaccharide (COS)-conjugated selenium (Se)	SGC-7901 and L-929 cells	BALB/c-nude mice	[102]
Breast cancer	(3-Aminomethyl phenyl) boronic acid (AMPB), Manassantin B (MB)	HA oligomer	AMPB-installed HA-ceramide (HACE)-based nanoparticles	MDA-MB-231 cells	MDA-MB-231 tumor-xenografted mouse model, BALB/c nude mice	[103]
Breast cancer	Doxorubicin	Carboxymethyl chitosan (CMC) and HA	Graphene oxide-CMC-fluorescein isothiocyanate-HA-complex	CD_{44}-positive Hela Cells	–	[104]
Breast Cancer	Curcumin	Chitosan and Protamine	Curcumin-chitosan-protamine nanocarriers	MCF7, ATCC HTB-22TM	–	[105]
Cervical and breast tumor	Paclitaxel	HA and pluronic 85	Paclitaxel HA decorated pluronic 85 coated solid lipid nanoparticles (SLN)	HeLa and human breast carcinoma cell line	Tumor-bearing Female BALB/c mice	[106]
Connective tissue growth factor (CTGF)-overexpressing breast cancer	Breast tumor cell-penetrating peptide	HA	Breast tumor cell-penetrating peptide and HA Nanoparticles	CTGF-overexpressedMDA-MB-231 cells and MCF-7 cells	Female nude mice	[107]
Melanoma	Doxorubicin	HA	Doc-Common liposomes (cLip) and DOX-HA-Liposomes	B16F10 cells and MCF-7 cells	Male C57BL/6 mice	[108]
Skin cancer	Capecitabine	Chitosan, Transcutol, Pluronic F-127	Chitosan nanogel	HaCaT cells	–	[109]
Skin cancer lesions	Oxorubicin and Camptothecin Tailored at Optimal Ratios (DOCTOR)	HA	DOCTOR-HA Conjugates	Human epidermal keratinocytes and cancerous A431 cells	UV-induced model of spontaneous cSCC in immunocompetent SKH1-E mice	[110]
Skin cancer	Quercetin	Chitosan	Chitosan nanoparticles	HaCaT cells	C57BL/6 mice	[111]
Lung cancer	Cisplatin and gemcitabine	Chitosan	Chitosan nanoparticles	NCl-H460 cells	Mice bearing NCl-H460 cells xenografts	[112]
Non-small cell lung cancer	Cyanine 3-labeled siRNA	Chitosan and HA	HA-modified chitosan nanoparticles	NSCLC A549 cells	Tumor-bearing mice	[113]
Non-small cell lung cancer	Doxorubicin and Celecoxib	Chitosan and HA	HA decorated glycol chitosan nanoparticle	A549 cells	NSCLC xenograft mice	[114]
Lung cancer	Paclitaxel	Chitosan	Chitosan liposomes	A549 cells	A549 tumor-bearing mouse	[115]
Pancreatic cancer	Celastrol	Chitosan	Chitosan oligosaccharide conjugate	BxPC-3 and HL7702 cells	BxPC-3 tumor-bearing mice	[116]
Pancreatic ductal adenocarcinoma	Quercetin and gemcitabine	HA	HA-decorated nanoparticles	Mia-PaCa-2 and PANC-1	–	[117]
Pancreatic cancer	Curcumin	Chitosan	Chitosan/PEG blended PLGA nanoparticles	Mia-PaCa-2 and PANC-1	–	[118]

developed chitosan oligosaccharide–conjugated selenium (COS-Se) as an alternative to selenium–oligosaccharides due to its insufficient natural production. An essential source of selenium for dietary supplements is selenium-oligosaccharides. According to research, COS-Se has no negative side effects on healthy fibroblast cells and can stop human gastric cancer cells from proliferating and metastasizing. COS-Se may considerably inhibit gastric cancer growth by lowering matrix metalloproteinase-9, vascular endothelial growth factor levels, and CD34 [102].

It is known that CD44 is expressed by gastric cancer cells, and HA targets these cells via CD44 as a receptor. Keeping this in mind, Yang et al. developed lipid nanoparticles containing HA loaded with the drugs cisplatin and sorafenib for combination therapy. They evaluated the nanoparticles for their morphology, which was found to be satisfactory. The formulation exhibited a prolonged release and had a cytotoxic effect on the cancer cells, according to the in vitro study findings on human stomach cancer cells. Also, the in vivo study done on mice showed that the formulation reduced the volume of the tumor significantly with no body weight loss while the free drug showed a loss in body weight. This makes these nanoparticles a novel approach to treating gastric cancer [101].

Based on the available reports, it could be inferred that the efficacy of the chemotherapeutics can positively be augmented while treating gastric cancer if delivered using chitosan or an HA-based drug delivery system.

6.2. *Targeting breast cancer using HA and/or chitosan-based drug delivery systems*

The second most prevalent cancer in women is Breast cancer. Traditional chemotherapy resistance is a problem in the fight against breast cancer. Upregulated CD44 receptors also highlight the various subtypes of breast cancer. Further, Jeon et al. produced lignin from the *Saurusus chinensis* roots called Manassantin B (MB) that was combined with nanoparticles of HA ceramide (HACE) and 3-aminomethylphenyl boronic acid (AMPB) for the treatment of breast cancer. Inhibiting DNA Topoisomerase-I, and II and hypoxia-induced factor-1 in overexpressed CD44 receptor human breast cancer cells allowed HACE-AMPB nanoparticles with MB to exert anti-neoplastic effects. One of the key binding pathways was created when salicylic acid upregulated on cancerous cells reacted with the boronic site of phenylboronic acid located on the HACE-AMPB/MB nanoparticles surface. The final nano

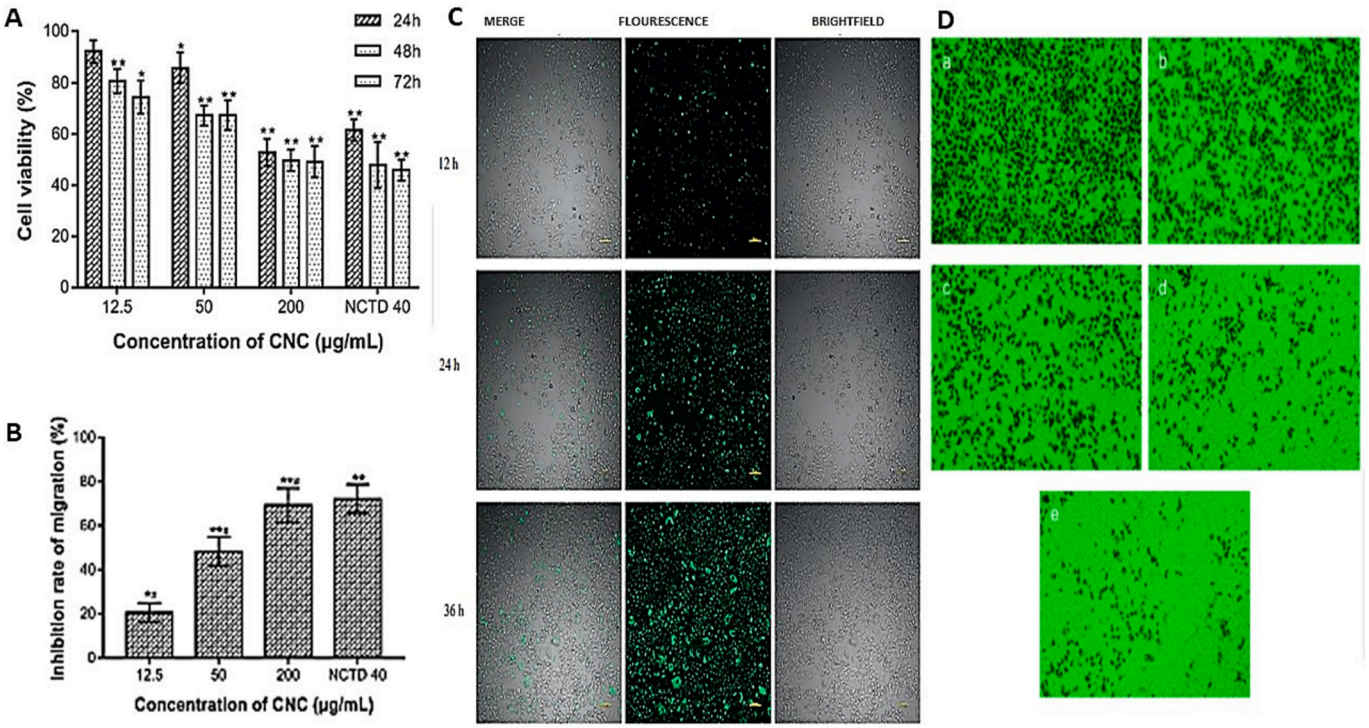


Fig. 4. (A) Cell viability studies of CNC on SGC-7901 cells. (B) Migration inhibition rate of HUVECs. (C) Uptake of CNC in SGC-7901 cells at varying time points (D) HUVECs migrated via transwell chambers.
Figure adapted with permission from [100].

assemblies exhibited an enhanced amount of HA-APMB-MB compared to free MB inside the tumor cells. HACE-AMPB nanoparticles are a promising carrier for imaging and treating CD44 receptor-expressed malignancies because of the improved penetration and retention impact that is provided by phenylboronic acid-salicylic acid interaction together with the interaction of HA-CD44 receptor (Fig. 5) [103].

To overcome the drawbacks associated with novel anticancer drug carriers like lack of ability to control drug release and targeted delivery, insufficient uptake of drugs by cells, and nonspecific accumulation of drugs in normal tissue a new modified drug carrier system for doxorubicin (DOX) a chemotherapeutic drug has been developed by Yang and Bremmer et al. Graphene oxide an effective drug carrier was first modified with carboxy methyl cellulose (CMC) which acts as a mediator. Later, they undergo a series of modifications. The first involves targeting the ligand HA, which has a high affinity for binding to cell-specific markers such as CD44 receptors, and the second involves tracing the ligand fluorescein isothiocyanate (FI). The DOX encapsulation in GO-CMC-FI-HA carrier was attributed to π-π stacking interaction. The complex demonstrated a pH-dependent DOX release pattern with a greater amount being released in pH 5.8 of the tumor cell microenvironment than in physiological pH of 7.4, confirmed by H-NMR spectroscopy, TEM, and zeta potential measurements. According to the findings, the GO-CMC-HA-FI/DOX compound can precisely attack and stop the upregulation of CD44 receptors. Thus, the newly modified complex is an ideal drug carrier for the controlled release and targeted delivery of antineoplastic drugs [104]. Further Wang et al. developed HA-conjugated, paclitaxel-loaded pluronic P85 solid liquid nanoparticles by hot homogenization approach to overcome the paclitaxel (PTX) drug resistance of P-glycoprotein. P-GP develops multidrug resistance against antineoplastic agents by pumping drugs out of the cells causing decreased intracellular drug concentration and cytotoxicity. The ability of final nano assemblies was evaluated in overexpressed human cervix cancer cell lines and human breast tumor cell lines. Results indicated that developed SLN, prolongs the mean residence time, and cytotoxicity as well as increased accumulation of paclitaxel as compared to free PTX making it a potential drug delivery vehicle [106].

Breast tumors have higher expression levels of congestive tissue growth factor (CTGF), a physiological component that contributes to multidrug resistance. The real drug resistance-inducing factors, Bcl-xl, and apoptosis protein 1 (clAP1) are strengthened by the overexpression of CTGF. Small interference RNA (siRNA) inhibits cIAP1 and Bcl-xL, which causes CTGF to be downregulated and prevents corresponding drug resistance. The mesoporous silica nanoparticles were developed and subsequently coupled with PEGA-pVEC, a peptide for breast cancer cell navigation and enhanced penetration and HA, to decrease the emergence of multidrug resistance, enhance the site-specific administration of anticancer drugs, and promote antitumor activity. These siRNA and DOX-loaded nanoparticles were intended for breast cancer cells. The data revealed that siRNA, which had been introduced to the target tumor cells through HA and penetrating peptide, showed its anticancer impact by blocking the anti-apoptotic proteins [107].

Other than the synthetic drug, curcumin, a natural polyphenolic compound found in *Curcuma longa* is another potent compound against breast cancer. In the last decade because of its bio-functional significance in anti-inflammatory, antitumor, and antimicrobial activities, curcumin has gained significant interest as a chemotherapeutic agent. However, curcumin's use is constrained by its lipophilicity and low bioavailability. Curcumin-loaded chitosan nanocarriers were fabricated by Abdel-Hakeem et al. and utilized to treat the MCF-7 breast cancer cell line. The outcome showed that using this method considerably boosted the stability and bioavailability of curcumin. Additionally, the therapeutic impact, i.e., its inhibition of NF-κB, which is crucial for the start of inflammation and the production of TNF-α and IL-6, has been improved. Consequently, for the treatment of breast cancer, chitosan-curcumin nanoparticles may be a promising future formulation [105].

Overall, the management of breast cancer condition can be controlled well with the concept of advanced drug delivery systems while the development of such formulations carrying chemotherapeutic

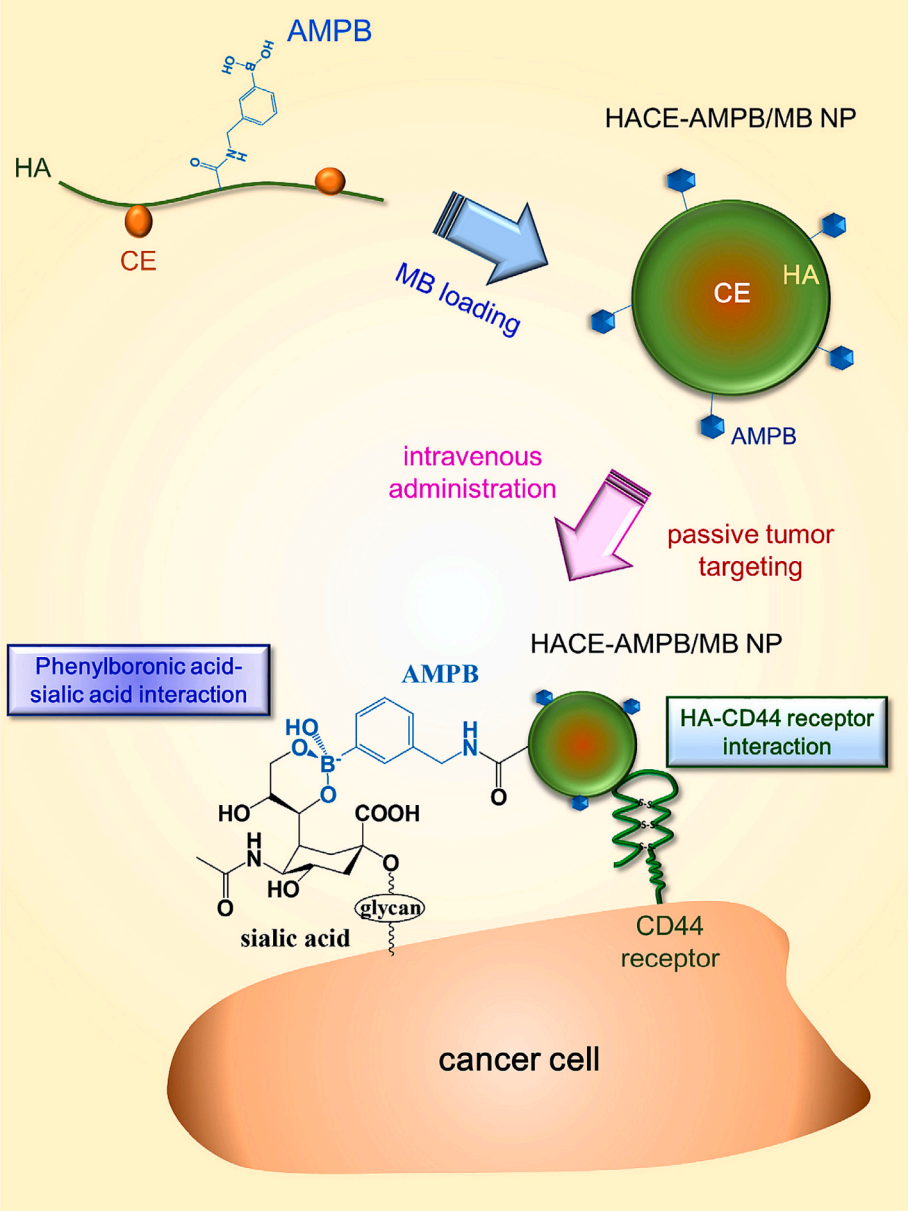


Fig. 5. Strategy of penetration and tumor targeting of HACE-AMPB/MB NPs. Figure adapted with permission from. [103].

agents used HA and chitosan.

6.3. *HA and/or chitosan-based drug delivery systems to target skin cancer:*

Malignant melanoma is another aggressive and metastatic skin tumor. However, because of its poor prognosis and resistance to conventional chemotherapeutics, new methods for melanoma therapy are required. Moreover, high interstitial fluid pressure, multilayer of tumor cell, and short blood circulation is the main setback for nano to accumulate in tumors. Therefore, the strategy to target skin cancer is slightly different from other cancers. In this regard, HA-modified liposomes (HA-Lip) with iRGD (cancer permeating peptide) were prepared to improve cellular uptake of the anticancer drug into B16F10 skin cancer cells rich with CD44. The results revealed that HA-Lip has lower liver distribution with good stealth in the bloodstream and higher internalization in cancer cells compared to common liposomes. Enhanced accumulation and retention of HA-Lip was observed in B16F10 melanoma-bearing mice after systemic administration. Furthermore, the tumor penetration ability of HA-Lip was significantly improved with the coadministration of iRGD. This phenomenon is attributed to the binding of iRGD with integrins αv located at deep tumor regions. To achieve increased tumor apoptosis and to reduce tumor size in mice, DOX-loaded conjugates were prepared. The findings demonstrated that HA (oligomeric) molecules possess higher compatibility to high CD44-expressing melanoma cells than to CD44–deficient expressing cells, which lowers DOX-HA-Lip distribution in healthy organs like the kidney and liver. DOX-loaded liposomes decorated with HA oligomer showed prolonged circulation time. The co-administration of DOX-HA-Lip with iRGD extended the DOX distribution in tumors[108].

Similarly, Krishnan et al. developed a topical HA-conjugated formulation containing doxorubicin and camptothecin which they called DOCTOR. The authors performed in vivo studies of the formulation which showed that the formulation killed the cancer cells effectively and without any irritation or systemic absorption (Fig. 6). The reported IC50 value of DOX in DOCTOR R15, R5, and R2 were 7-fold,

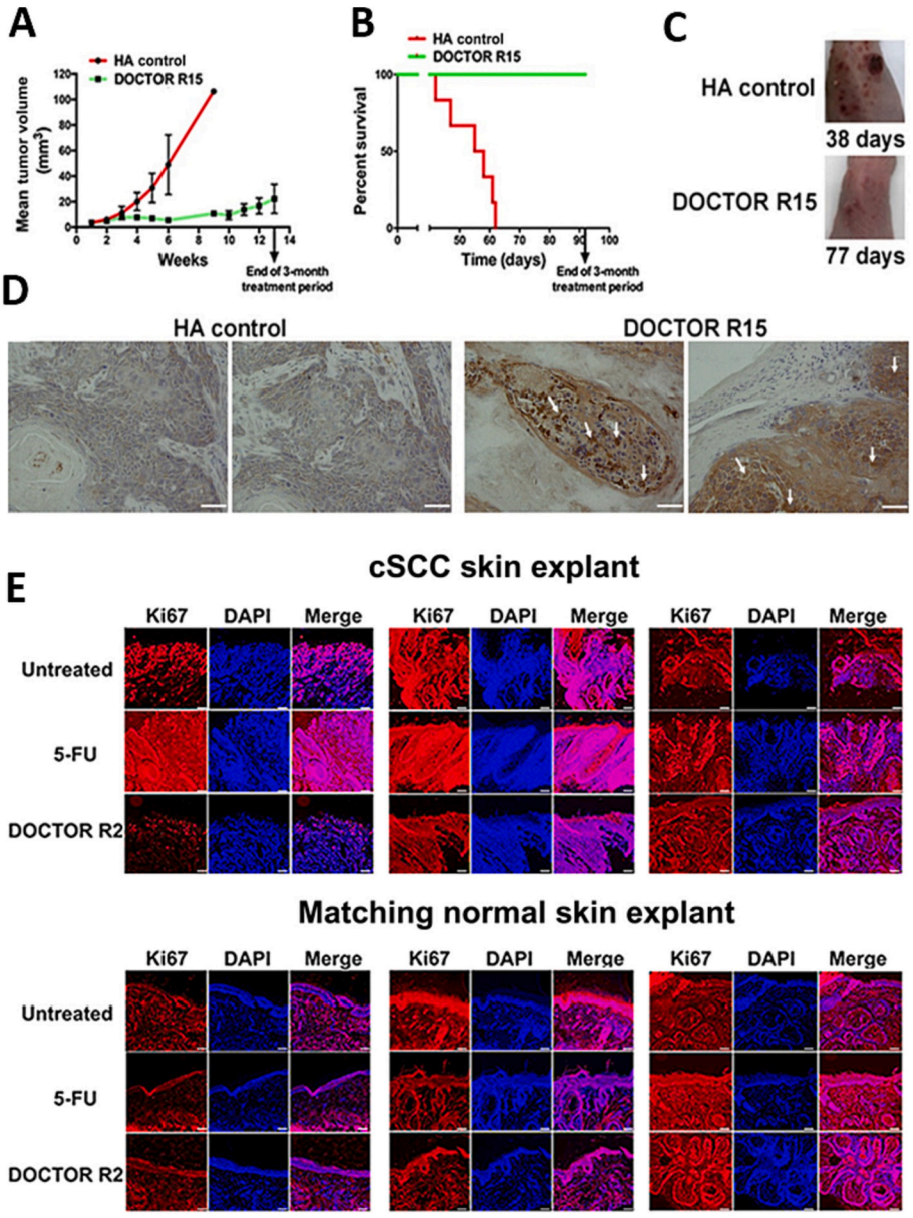


Fig. 6. At exceptionally low DOX concentrations in DOCTOR, the in vivo performance (A to C) of DOCTOR delays the development of cSCC lesions and 65 % survival. (D) After treatment with DOCTOR R15, cSCC lesions exhibited apoptosis for cleaved caspase-3 immunostaining in the nucleus and cytoplasm (golden brown) (scale bar, 50 m). (E) Safety and anticancer activity in patient-derived primary cells and live skin explants.
Figure adapted with permission from [110].

3.5-fold, and 3.6-fold respectively on HEKa cell lines compared to pure drug. The tape-stripping method revealed the penetration of DOX and camptothecin into deep skin layers. The authors also tested the formulation on both cSCC skin and noncancerous skin cells and found that DOCTOR did not affect the proliferation of normal skin cells and rather inhibited the growth of cSCC cells. In comparison with another clinical standard, the formulation was found to have superior anti-cancer activity and safety [110].

Ultraviolet radiation, especially solar ultraviolet-B (UVB) is a potent risk factor that can induce skin cancer, photoaging, and inflammation. Quercetin (Qu), one representative flavonoid, has shown properties including anti-tumor, anti-inflammation, and antioxidant against UVB radiation. However, poor transdermal absorption limits its topical application. In a recent study, Nan et al. developed Qu-loaded tripolyphosphate (TPP) chitosan nanoparticles (QTCs) which displayed better uptake by HaCaT cells, easy permeation through the epidermis low cytotoxicity, and better stability. The result indicated that chitosan carriers could significantly enhance the inhibitory effect of Qu on the NFκB/COX-2 signaling pathway, as high COX-2 levels are a major reason for cancer development. Further, they can mitigate UVB radiation-induced skin edema. Therefore, QTCs are potent chemotherapeutic agents against skin cancer [111].

On the other hand, Sahu et al. have developed pH-triggered transdermal systems by utilizing the acidic tumor microenvironment. The study focuses on developing and investigating the penetration capacity and toxicity of biocompatible chitosan-capecitabine nanogel (CPNL) for combating skin cancer. Capecitabine is an antimetabolite of fluorouracil a potent chemotherapeutic agent. The ionic gelation method was used to prepare CPNL from Pluronic F 127 with Transcutol. The onset of the cytotoxic effect of these pH-responsive nanogel lies under the mechanism of ionic interaction between the cationic CPNL and anionic cancer cell membrane which leads to acid degradation of chitosan and thus

exhibiting site-targeted release of capecitabine. The optimized nanogel demonstrated better penetration and transportation against skin cancer over free drugs. Overall, the studies indicated that CPNL exhibited less toxic and low-dose transdermal chemotherapy against skin cancer [109].

Improved research outcomes while skin cancer is treated with therapeutic agents fabricated in an advanced delivery tool using HA and chitosan.

6.4. *Lung cancer management using HA and/or chitosan-based drug delivery systems:*

Non-small cell lung cancer (NSCLC) is the most fatal and common lung cancer. The effective treatment of cancer with high therapeutic efficacy, the ability to setback drug resistance, and decreased side effects is combination chemotherapy. The first-line therapy for NSCLC is cisplatin plus gemcitabine (GEM). Both are potent antineoplastic agents that provide antitumor activity against pancreatic, ovarian, and lung, including NSCLC. However, its poor solubility, severe toxicity like neurotoxicity, renal toxicity, short half-life, and drug resistance have setback its clinical implications. A recent study has developed a prodrug of chitosan, platinum, and nano carrier-based formulations to reduce toxicity and drug resistance. Due to the different physicochemical properties of cisplatin and gemcitabine, it is difficult to deliver both in the same nanoparticles. To achieve synergistic effects GEM and platinum (IV) prodrugs were loaded in layer-by-layer nano-formulation (HA-GEM/CH-Pt NPs). In the layer-by-layer (LBL) technique platinum (IV) was complex with CH to produce conjugation with a positive charge, in contrast, GEM and HA conjugates produce a negative charge for effective layering of nanoformulation. The prepared LBL nanoparticles exhibit an average size of 187 nm with 90 % entrapment efficiency of drugs. Moreover, the in-vitro cytotoxicity results showed the best IC50 value when GEM to Pt ratio was 1/1 in HA-GEM/CH-Pt NPs. Nanoparticles also displayed no sign of significant toxicity on vital organs. Furthermore, HA-GEM/CH-Pt NPs efficiently reduce the tumor size on NCl-H460 cells of mouse models compared to drug solution and NPs loaded with a single drug. It was also observed the HA-GEM/CH-Pt NPs showed enhanced anticancer effects with low toxicity due to low off-target distribution [112].

Targeted gene therapy is another popular approach explored for the treatment of NSCLC. Chitosan is considered one of the best choices of vector for siRNA delivery due to the better interaction of the negative

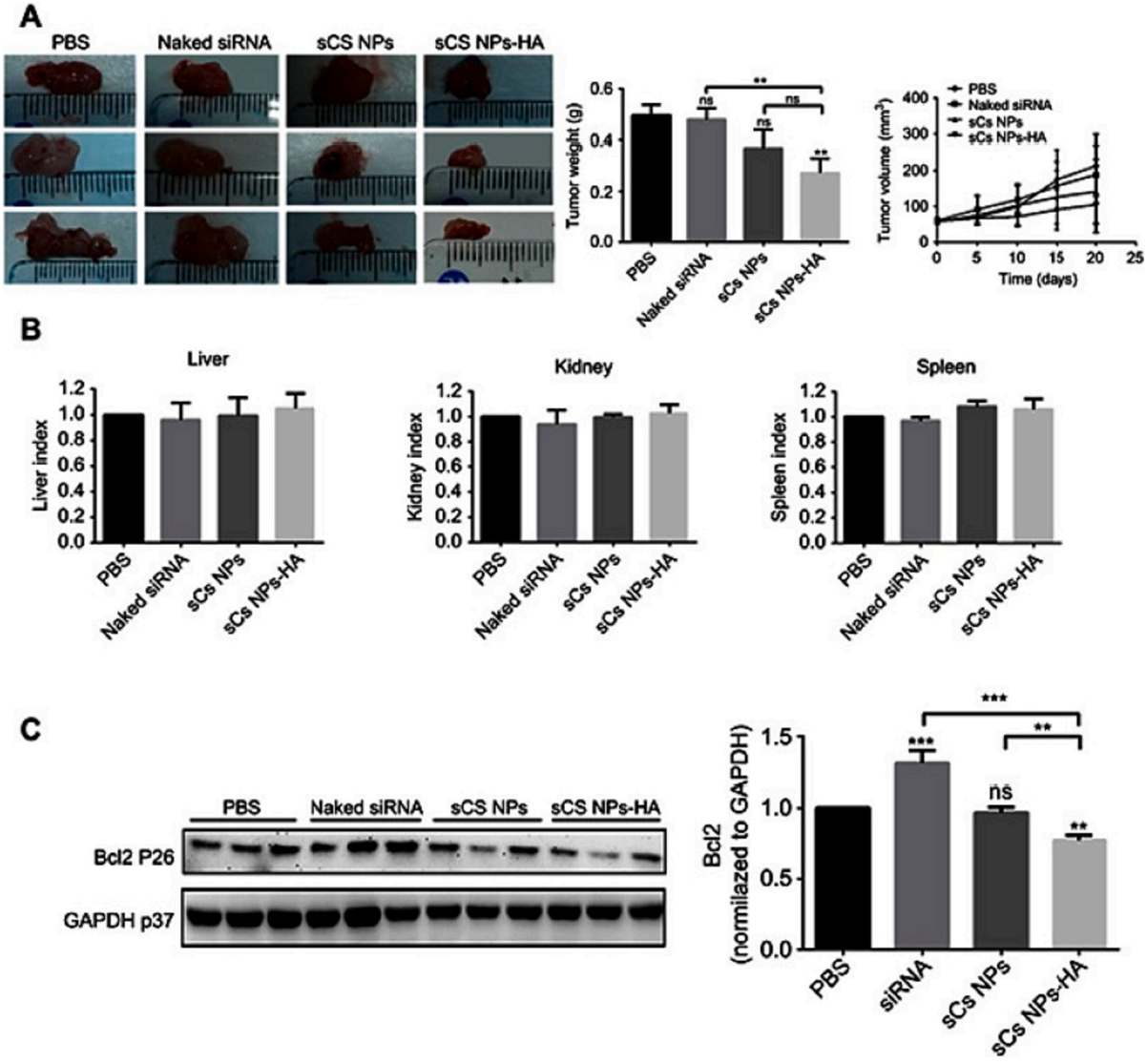


Fig. 7. (A) Tumors from all groups are isolated (B) The ratio of the weight of the kidneys, spleen, and liver, to all group body weight; (C) The Western blotting measurements of the BCL2 protein expression levels in mouse tumors normalized to GAPDH expression.
Figure adapted with permission from [113].

charge of siRNA with a cationic charge of primary amine groups on chitosan. Despite this chitosan has several limitations as a carrier such as low permeability, poor transfection efficiency, lack of targeting, etc. Therefore, to combat these shortcomings, Zhang et al. formulated chitosan nanoparticles modified with HA (sCS NPs-HA) for siRNA delivery. The formulation was checked for its cytotoxicity and antitumor effect. Effective transfection efficiency was evident by flow-cytometric analysis on A549 cells. Further effect on tumor growth was investigated on a mouse model injected with human A549 cells to develop a tumor. Later the mice were treated with PBS, naked siRNA, sCS NPs (chitosan NPs loaded with siRNA), and sCS NPs-HA. After 20 days animals were sacrificed and investigated. The results as shown in Fig. 7 illustrate the smallest tumor size and significant inhibition of the growth of subcutaneous tumors in the group treated with sCS NPs-HA. Moreover, the prepared nano formulation showed no toxicity and weight change in vital organs (kidney, liver, and spleen) compared to the control group. The in vivo studies data also showed siRNA delivery to the lung cancer cells via the CD44 receptor and prevented cell propagation and results on mice displayed that the formulation showed targeted delivery of siRNA to the tumor site more efficiently than the naked cyanine labeled siRNA and with enhanced inhibition of cell proliferation [113].

In a recent study, Lee et al. fabricated glycol chitosan nanoparticles decorated with HA and loaded with the drugs doxorubicin and celecoxib as a combination therapy with a pH-controlled release. The nanoparticles had optimum size, were stable at pH 7.4, and showed drug release at a slightly acidic pH. The in vivo xenograft of the mice treated with this formulation showed significant tumor growth inhibition and metastasis-related gene suppression in the cancer tissue related to the free drug. The formulation also improved the targeting efficacy, reduced inflammation, and showed no toxic effects [114].

Another nano delivery system comprised of chitosan oligosaccharide conjugated with Pluronic 123 polymers was synthesized to develop new biomaterials CP20 and CP50 and CSO-altered liposomes to improve the delivery of paclitaxel in lung cancer. Chitosan's enzymatic breakdown product is called CSO, which exhibits low water solubility, and the molecular weight is used to overcome the limitations of chitosan. When compared to un-modified liposomes, CP20-LSs, and CP50-LSs significantly boosted tumor accumulation *in* animal models and in vitro in A549 cells. CSO-modified liposomes show an increased affinity for lung tumor cells due to the abundant presence of receptors on cancer cells for high adsorption of collagen. With a suppression rate of 86.4 %, the PTX-CP50-LSs provided efficient anticancer performance. Therefore, CSO customization has wide potential for nanomedicine in the therapeutic management of lung cancer [115].

In a nutshell, it could be said that lung cancer can also be treated better if the chemotherapeutic agents are entrapped in a drug delivery system containing HA or chitosan.

6.5. Management of pancreatic cancer (PC) with HA and/or chitosan-based drug delivery systems

Unlike other cancers, PC has fewer diagnosed biomarkers, and rapid progress results in late diagnosis at an advanced stage. Then available chemotherapeutic options are not improving the prognosis of the patient due to the off-target release of cancer drugs, severe side effects along with heterogenicity, and complexity of PC. Furthermore, complex immunosuppressive microenvironment is another limitation of effective treatment. Therefore, finding a suitable vector for the targeted delivery of anticancer drugs with safeguard drugs from blood circulation degradation is the main research hotspot. In this regard, Zeng et al. developed chitosan oligosaccharide conjugate for the delivery of celastrol. The formulation showed an enhanced drug solubility along with its tumor inhibition ability. The formulation induced apoptosis and suppressed the metastasis of the tumor in human pancreatic cancer cells along with lower cytotoxicity than pure celastrol. While having great anti-cancer activity, the formulation enhanced the drug circulation time and subacute toxicity reduction, thereby rendering the formulation a reliable option in cancer therapy [116]. As depicted in the 19th-day tumor section staining (Fig. 8), there was a dose-dependent apoptotic index of celastrol-chitosan oligosaccharide conjugate against BxPC-3 cells. The apoptotic index with 2 mg/kg and 4 mg/kg were reported to be significantly higher when compared with saline control and free celastrol-treated groups. This result indicated that the carrier system effectively assisted in delivering the conjugated drug to the tumor microenvironment, which induced apoptosis in the tumor cells [116].

Combination therapy is a viable option for cancer treatment. Therefore Serri et al. developed poly (lactic-*co*-glycolic acid) HA nanoparticles to deliver gemcitabine and quercetin for pancreatic cancer treatment. Upon testing the formulation on pancreatic cancer cell lines, the authors found that the formulation increased the cytotoxicity along with cellular uptake of the drugs, and the improved anti-inflammatory effect of quercetin, thereby helping reduce the cellular levels of interleukin thus resulting in slowing the metastasis [117].

In another research, to improve the stability and solubility of curcumin in cancer therapy, Arya et al. developed PLGA nanoparticles coated with chitosan and PEG and loaded with curcumin. The authors were able to develop nanosized particles with a smooth surface and effective loading capacity. The in vitro studies results showed enhanced cytotoxicity, anti-migratory and anti-invasive activity along with more effective apoptosis-inducing ability as compared to pure curcumin. All these results made these nanoparticles a better approach for cancer therapy [118]. Finally, it can be concluded that pancreatic cancer can be controlled well if treated with chitosan or HA-containing drug delivery systems.

7. Hybrid chitosan and hyaluronic acid-based nanocarriers

In the area of drug delivery and biomedical applications, hybrid nanocarriers based on chitosan and hyaluronic acid (HA) have attracted a lot of interest. By combining these two polymers, it is possible to create nanocarriers with distinctive characteristics and future research possibilities. In an investigation, scientists attempt to evaluate the efficacy of alpha-mangostin administration using hyaluronic acid-coated chitosan nanoparticles for the treatment of breast cancer. Against numerous kinds of malignancies, alpha-mangostin has antiproliferative and cytotoxic properties, specifically in breast cancer, it upregulates Bcl-2-associated X protein and downregulates B-cell lymphoma 2. Chitosan nanoparticles were developed using sodium tripolyphosphate via an ionic cross-linking approach and subsequently coated with HA. The developed nanoparticles were in the range of 200 to 400 nm with spherical morphology, along with drug loading of more than 8 %. Encapsulation efficiency was found to be more than 90 %. The in vitro release profiles demonstrated a sustained release up to 96 h, with an initial burst release of 11 % in phosphate buffer saline (pH 5.5 and 7.4). The cytotoxicity studies were conducted on MCF-7 breast cancer cell lines which revealed significant cellular toxicity of HA-coated chitosan nanoparticles as contrasted with pure alpha-mangostin and uncoated chitosan nanoparticles. The developed anti-cancer formulation requires additional preclinical investigation to ascertain their extent of absorption, toxic effects, and chemotherapeutic efficacy [123]. Another research designed a combination therapy of doxorubicin and cisplatin for breast cancer. For the targeting of the cancer site, HER2 antibody was used to functionalize the nanoparticles made up of hydroxyethyl chitosan and aldehyde hyaluronic acid. Initially, doxorubicin and cisplatin were coupled with aldehyde hyaluronic acid via Schiff base reaction and complexation forming a central core. Later on, hydroxyethyl chitosan was coupled with the inner core and finally functionalized with the HER2 antibody. The developed nanocarriers were found to be 160 nm in size with high zeta potential. The drug release of both drugs was high at pH 5.5 (pH corresponding to tumor milieu) as compared to pH 7.4 (normal physiological pH) owing to the linkages connecting AHA with doxorubicin and cisplatin being ruptured due to the removal of ions of

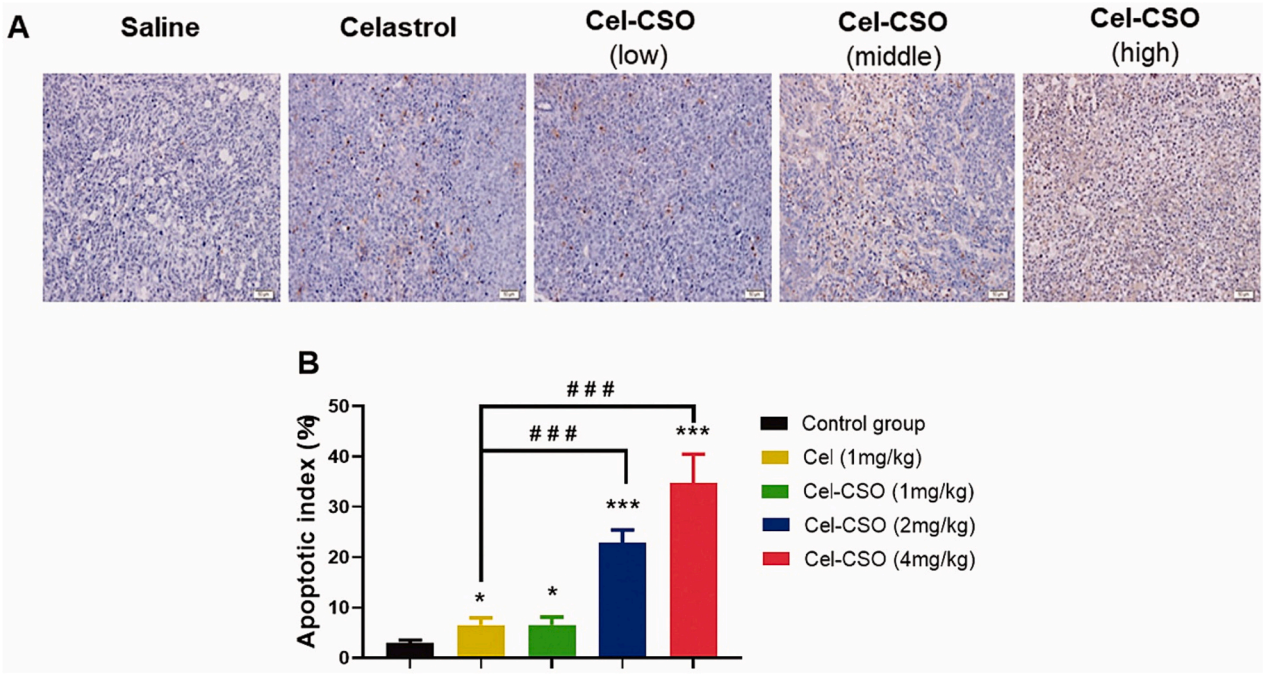


Fig. 8. (A) TUNEL assay in tumor tissues (×200); (B) Apoptosis index of tumor cells. Scale bar: 50 μm.
Figure adapted with permission from. [116].

the carboxyl chains in AHA and Schiff's base bond amid acidic circumstances, which demonstrates its pH-responsive behavior [124].

8. Proprietary technologies on chitosan and HA for cancer therapy

Various patents have been published utilizing chitosan and HA as drug delivery means for cancer therapy (Table 2). Lead inventors are from China, the United States, South Korea, and Japan during the last ten years of tenure. Jilin University, China had filed a patent application regarding the development of HA-based nanoparticles loaded with the photosensitizer drug verteporfin for the treatment of uveal melanoma. The formulation is intended to solve the problems of poor systemic availability. At the same time, it reduces the toxicity of the drug by increasing the targeting ability of the formulation toward the melanoma site. The mechanism of drug release from the formulation depends upon hyaluronidase enzymolysis after being irradiated from the laser of a specific wavelength. Preclinical and in vitro research demonstrates that the formulation has a more effective anti-tumor impact in an animal model of uveal melanoma while also realizing fewer toxic effects and improved specificity [125].

Changsha University of Science and Technology, China grants a patent based on nano-composite medicine comprised of manganese dioxide nanosheets, glucose oxidase, and HA. The aim was to develop novel material with excellent stability, specificity, and anticancer potential. This novel material had a core of manganese dioxide nanosheets functionalized with glucose oxidase on surfaces and the shell is HA. Studies reveal that the developed composite can efficiently increase the cancerous and normal cell specificity using novel components. In addition, the synergistic impact of the composite can accurately and more specifically kill the cancerous cells, and in low doses can induce cancer cell apoptosis significantly. Furthermore, the material does not affect healthy cell metabolism [126].

Another cohort from Xian Jiaotong University, China developed a nanocomposite i.e., HA-coated composite nanoparticles for tumor targeting by using sonodynamic therapy. The therapeutic molecule is attached to the radial nano polymer unit to form the composite nanoparticle. The altered dendrimers PAMAM, where the alterations refer to that portion of amino groups that are on the dendrimers PAMAM surface, make excellent sources for the nano-polymer molecules. Additionally, the formulation is appropriate for noninvasive/minimally invasive therapy of tumors due to its favorable acoustodynamic anticancer effect by adding sensitizers such as indocyanine green (ICG) and similar substances. Additionally, the formulation is simple to purify and features small, identical particles that possess excellent penetrability [127].

Chitosan has been found to enhance the water solubility, stability, and loading of drugs. Beijing Forestry University, China develops novel target-specific carboxymethyl chitosan-based nanopharmaceutical formulation that demonstrates excellent water solubility and stability. The steps include the conjugation of targeted folic acid (FA) with polyethylene glycol (PEG) using amide linkage to form FA-PEG conjugate, which is then linked to carboxymethyl cellulose. Ursolic acid is used for the esterification of folic acid, polyethylene glycol, carboxymethyl chitosan, and black bearberry acid conjugate, and another drug's self-assembly package containing 10 hydroxy camptothecin is prepared and obtained. These two chemotherapeutic drugs are then combined to create a nanoparticle. The formulation demonstrated extended ursolic acid and 10-hydroxycamptothecin half-lives and significantly increased drug loading rate [128]. The other group provides a type of chitosan-based antitumor hydrogel that is made by reacting crosslinking agent and chitosan with the load antineoplastic, which solves difficulties like low targeting efficiency and significant harmful side effects of conventional chemotherapy. At room temperature, a hybrid reaction was carried out to produce an injectable chemotherapeutic aqua gel for chitosan (85 %-99.9 % deacetylated), cross-linker, and adriamycin at a volume ratio of 5:4:1. After being injected inside a tumor, the hydrogel can be self-healing and a long-lasting slow-release, with potent targeting that additionally decreases the toxic effects on human normal tissue during antineoplastic treatments. The hydrogel's therapeutic activity has been contrasted to the direct injection of doxorubicin solution and is superior in the inhibition experiment of tumor-bearing nude mice [129].

In addition, inventors from the United States have also granted the

Table 2
Summary of the patent based on chitosan and HA formulation for cancer therapy.

Patent number	Country	Description	Publication date	Reference
CN114931563A	China	Describes a method of preparing HA nanoparticles of verteporfin for treating uveal melanoma.	2022-08-23	[125]
CN110755407B	China	Discloses preparation and application of an anti-cancer material having a core of manganese dioxide nanosheets modified with glucose oxidase on the surface and a shell made of HA.	2022-07-26	[126]
US11111316B2	United States	Discloses chitosan-derived therapeutic compositions used for treating tumors.	2021-09-07	[129]
CN109999197B	China	Describes the method of preparation and HA-based composite nanoparticle applications for tumor treatment by acoustic power.	2021-04-20	[127]
US10413612B2	United States	Discloses various drug delivery devices and HA-based compositions for anti-cancer therapy.	2019-09-17	[130]
CN108567764A	China	Describes a nanocarrier based on carboxymethyl chitosan with a double-layer structure to enhance the water solubility and stability of the anti-cancer drugs along with improving their tumor targeting ability.	2018-09-25	[128]
CN107349177A	China	Discloses a chitosan-based hydrogel potential for cancer therapy.	2017-11-17	[133]
JP6180436B2	Japan	Discloses a saccharified preparation of chitosan with improved viscoelastic properties for anti-cancer drug delivery.	2017-08-16	[134]
CN103751795B	China	Describes a preparation method, HA-antitumor drug conjugate application, and nanoparticle composition for tumor targeting.	2017-02-08	[135]
KR20140094775A	South Korea	Describes the preparation of water-soluble chitosan conjugated with an active ingredient having anti-cancerous, anti-oxidation, and anti-inflammatory properties.	2014-07-31	[131]
KR101323102B1	South Korea	Enumerates preparation method for nanoparticles prepared by encapsulating a hydrophobic antitumor drug inside a glycol chitosan-bile acid complex.	2013-10-30	[132]

patent for an aqueous solution of the viscoelastic glycated chitosan polymer that has been sterilised and filtered, for the treatment of malignancies, such as malignant kidney, thyroid, and lung neoplasms as well as other forms of malignant neoplasms, as well as other medical conditions. In addition, the other cohort from the United States developed compositions and devices for drug delivery including a hydrogel-like biomaterial e.g., HA, chitosan, alginate dextran, and gelatin, etc., an agonist of the stimulator of interferon genes (STING) protein which stimulates the innate immune response, a cytokine, and an adaptive immune system. To stop tumor recurrence and the spread of cancer cells the drug delivery device can be inserted in the tumor's empty volume [130].A medicinal formulation with anticancer or anti-inflammatory properties that contain water-soluble chitosan with three different molecular weight i.e., 8 kDa, 43 kDa and 67 kDa as a therapeutic component is the subject of the current invention from South Korea. The composition can be formulated in a variety of conventional oral dosage forms viz., granules, tablets and capsules, and sterile injectable formulation as well. The formulation claims to be anti-cancer, anti-oxidant, and anti-inflammatory as well [131]. In the other invention from South Korea, a glycol chitosan-bile acid conjugate is formulated which comprises a nano non-aqueous anticancer active covalently linked to glycol chitosan to form self-aggregates in water. More particularly, the present innovation can encapsulate an anticancer medication with substantial water solubility, minimal toxicology, and high effectiveness. The high-water solubility would facilitate injection formulation by sterile filtration technique. The formulation also demonstrates an excellent targeting effect on cancer cells [132]. The abovementioned patents describe the potency of chitosan and HA toward upgraded cancer management. More exploration is required to utilize their potential in different types of cancer.

9. Clinical trials of chitosan and/or HA nanoformulation for cancer therapy

When browsing different search engines, it was discovered that many in-vitro and preclinical studies on chitosan, HA, and their combination had been conducted. Only a small number of them do, however, enter to assess its efficacy and safety in humans. Table 3 demonstrates that just a few clinical trials have made use of these polysaccharides. However, their combination for cancer therapy has not been reported yet. Very few trials have been reported for the clinical assessment of chitosan and hyaluronic acid which have been disclosed in the given section. A randomized, parallel, double-blinded study is being conducted on 170

Table 3
Compilation of the clinical trials for chitosan and HA in the cancer management.

Clinical Trial.gov ID	Sponsors	Objective	Description of the trial	Disease targeted	Treatment offered	Status of the trial	Ref
NCT02967146	Samsung Medical Center, Seoul City, Korea,	To evaluate the safety and anti-adhesive effect of chitosan, poloxamer, and gelatin mixture (Mediclore®) on Axillary Dissection for Breast Cancer	• Double-blinded • Multicenter Randomized trial • 170 participants	Breast Cancer	Following axillary dissection, Injection of Medicare into the surgical site and stitching of the wound.	Recruiting	[136]
NCT04218188	National Taiwan University Hospital	To investigate the effect of pH-responsive chitosan on cell detachment ratio as a lung cancer prognostic factor	• Lung cancer patients • Administration of pH-responsive chitosan in lung cancer patients. • Determination of survival rate with cell detachment ratio	Lung Cancer	NA	Recruiting	[137]
NCT02753595	Eisai Inc. and Halozyme Therapeutics	Comparative evaluation of Study of Eribulin Mesylate alone and its combination with PEGylated Recombinant Human Hyaluronidase	• Open-label • Randomized trial • Phase 1b/2 • Multicenter, • 25 Subjects with High-Hyaluronan Metastatic Breast Cancer (MBC)	Human Epidermal Growth Factor Receptor 2 (HER2)-Negative, High-Hyaluronan Metastatic Breast Cancer (MBC)	Drug: Eribulin mesylate for Phase 1b Other: Biologic: PEGylated recombinant human hyaluronidase (PEGPH20) for Phase 2.	Recruitment Terminated	[138]
NCT03816735	Medical University of Graz	To compare HA Suppository and Yag Laser for GSM in Breast Cancer	• Randomized trial • Open study • Parallel assignment of 50 participants	Patients with a history of genitourinary symptoms of menopause and breast cancer.	Intravaginal HA suppository or Intravaginal Laser	Recruiting	[139]
NCT02563548	Halozyme Therapeutics	To investigate the combination of Pembrolizumab with PEGylated Recombinant Human Hyaluronidase (PEGPH20)	• Open-label study • Phase 1B • 56 participants	Patients with Selected Hyaluronan-High Solid Tumors	Pembrolizumab and PEGylated recombinant human hyaluronidase [PEGPH20]	Completed	[140]

participants who need axillary dissection for breast cancer. Intervention is an adhesive device made up of a mixed polymeric mixture i.e., poloxamer, gelatin, and chitosan that needs to be injected on the operation site and suture the site post axillary dissection. The primary outcome must be measured in terms of range of motion i.e., forward flexion and horizontal abduction for 24 weeks. Another outcome involves pain assessment and DASH (Disabilities of arm, shoulder, and hands) scoring. The role of chitosan and gelatin is to make the mixture mucoadhesive for the operated site [136]. Another trial demonstrates the pH-responsive nature of chitosan in lung cancer as a predictive parameter. The trial was observational, prospective, and cohort-based on 120 participants. The primary outcome measures the expression of surface markers which will be assessed by flow cytometry to determine the characteristics of the extracted and purified patient-derived tumor cells. This study aims to investigate the potential correlation between lung cancer patients' overall survival and the cell detachment ratio using pH-responsive chitosan. This initiative aims to offer an alternate approach for both early diagnosis and precise prognosis in cancer therapies through regulated cell-material engagement [137].

In addition, only three clinical trials have been found for HA having application in cancer. An open-label, randomized, parallel interventional clinical trial including 25 participants with metastatic HER-2 negative breast cancer. It is a Phase 1b/2 study of eribulin mesylate in combination with PEGylated Recombinant Human Hyaluronidase (PEGPH20). Phase 1 was conducted in two parts: Part 1 included 6 patients as the run-in safety cohort which aims to assess the safety of two drug combinations to ascertain the recommended Phase 2 dose as a primary outcome. In addition, dose-limiting toxicity was also evaluated. Part 2 comprises 12 more patients to further assess the safety profile [138]. Another trial compared laser with hyaluronic acid for genitourinary symptoms of menopause (GSM) in breast cancer patients which is evaluated by vaginal health index score. According to a scale of 1 to 5, the Vaginal Health Score Index rates the vaginal mucosa's suppleness, pH, discharge, integrity, and wetness as a primary outcome. The symptoms get worse as the score drops. The secondary outcome will be assessed via symptoms severity- visual analogue scale, therapy discomfort/pain, pelvic floor symptoms, and patient global impression of improvement and severity. The treatment includes two devices: one device is a laser (Juliet feminine laser) and the other one is an HA-based suppository ("Cikatridina" manufactured by the company Angelini). The results were significantly similar for both laser and HA-based suppositories in terms of urogenital atrophy, life quality, and sexual health [139]. Furthermore, an open-label, parallel, randomized, interventional clinical trial on 56 patients with Selected Hyaluronan-High Solid Tumors was conducted to study the effect of recombinant PEGylated recombinant human hyaluronidase (PEGPH20) in combination with Pembrolizumab. PEGPH20 was given intravenously as per the dosing schedule. The primary outcome assesses the number of subjects with dose-limiting toxicity, maximum tolerated dose, and objective response rate. The trial was carried out in two sections i.e., dose escalation and dose expansion [140].

These are the only trials reported in the literature for chitosan and HA being utilized for the treatment of cancer or its associated effects. Therefore, there is a need for more translational researchers.

10. Key challenges in nanocarrier delivery against cancer

Nanocarriers are increasingly prevalent in the medical field, however, the major problem with these nanometric foreign particles includes the formation of proteins corona on the surface of the nanocarriers spontaneously by the proteins available in the biological system. This process alters the physicochemical characteristics of the nanocarriers and their interactions with the biological system, thereby helping in the opsonisation of the nanocarrier to free the biosystem from foreign particles [141]. However, impregnation of polyethylene glycol or acetyl group on the nanocarrier surface slows the process of formation

of proteins corona [142], which can improve the circulating and targeting potential of the nanocarrier to the cancer microenvironment. Such improvement in the pharmacokinetic parameters of the chemotherapeutic agents, utilizing target-specific treatments and enhanced permeability and retention (EPR) effect, nanoformulations were found to increase the effectiveness of the therapy. The targeting potential of these nanocarriers often has a lengthy circulation time in the system and aggregates minimally in normal organs, which ultimately lessens the associated adverse effects of the chemotherapeutic agents.

The positive charge of chitosan and the negatively charged HA and their derivatives assemble themselves to form carriers of the nanometer range for chemotherapeutic agents from natural or synthetic sources that possess excellent biocompatible and biodegradable properties. Additionally, this HA is also used to target overexpressed receptors (CD44) on cancer cell surfaces. Also, the release of chemotherapeutics from the nanocarriers can be facilitated by the stimuli at the tumor microenvironment such as acidic pH environment, temperature, enzyme, etc., or can facilitate the release from the carrier through some external processes such as photo-, sound-, or thermal-irradiation. Although several formulation approaches are explored using chitosan and HA or their derivatives, their penetration into the cancerous tissue, stability of the formulation, and subsequent toxicities might restrict their use. For example, the layer-by-layer self-assembly of hydroxyethyl chitosan and aldehyde HA to approach photodynamic therapy together with chemotherapeutic effect. Due to exposure to excitation light in photodynamic therapy, this layer-by-layer self-assembly reported limitations in tissue penetration and stability issues, however, showed good anticancer effects. Moreover, these oppositely charged polysaccharides control the delivery of one or more therapeutics because of the simplicity, facileness, and versatile nature of the nanocarriers, where modifications of the surface of such nanocarriers facilitate uptake by the cancer cells.

Despite being thirty years old and having made significant advancements in the field of cancer therapy, cancer nanomedicine still has significant drawbacks that need to be resolved. Being the biodegradable and biocompatible nature, these two natural polymers are expected not to produce any toxic characteristics, however, the derivatives are not established for such characteristics. It would be necessary to establish the safety of the complex structures. Therefore, these nanomedicines may have unwanted side effects due to undesirable interactions of the excipients with biological systems or due to accumulation in the biological system. Therefore, analyzing the toxicity of these nanocarriers has become a crucial area of study.

Due to their intricate compositions and technologies, nanomedicines are particularly susceptible to the impacts of technological variations on efficacy and adverse effect profiles. Slight variations in the formula may lead to major changes in characterization parameters, including particle size, solubility, and stability. Thus, the commercial development of nanomedicine products is a major barrier because large-scale production is technologically challenging.

The substantial development of nanomedicine is coming up against an increasing number of difficulties in both the research and medical communities, such as development expenses, problems getting updated with advanced technology, and unsuccessful translation into clinical trials. Because of the complex biological system, one significant continuing problem is specifically the lack of in vitro/in vivo correlation of drug release features from the nanocarriers.

Another major obstacle to the commercialization of nanocarriers in chemotherapeutic delivery is its clinical translation because there isn't an adequate comprehension of how nano-bio interfacial interactions work. For animal and human clinical trials, nanomedicine is often utilized in very small amounts. Nevertheless, due to the possibility of batch-to-batch variation in their physical and chemical attributes, the large-scale manufacture of nanoformulations is rather difficult. Since the FDA has not established any special regulations for commodities using nanomaterials, the obstacles to regulatory approval of nanocarriers are another important concern. The parameters that are currently in use were directly adapted from bulk material recommendations. The regulatory decisions about nanomedicines are dependent on the unique examination of their benefits and risks, which makes evaluations labor-intensive and delays commercialization.

11. Theranostics aspects of chitosan and hyaluronic acid in cancer management

The use of radiation therapy and chemotherapy for cancer continues to experience issues with toxicity to the system, resistance to drugs, and low specificity, which leads to inadequate outcomes. The capacity of nanoparticles (NPs) to target tumors via passive deposition and active targeting procedures allows them to be commonly employed for loading diagnostics and therapeutic substances. Particularly, theranostic and multifunctional NPs that combine treatment plans with imaging for diagnosis have generated a great deal of interest. Chitosan and Hyaluronic acid, biodegradable and biocompatible polymers are of great interest to researchers, specifically for theranostics purposes. In addition, chitosan responds well to the acidic microenvironment of the tumor or cancer areas. Traditional chemotherapy-related cancer recurrence is caused by cancer stem-like cells that initiate new tumors. Investigators designed polymeric nanoparticles loaded with doxorubicin. The nanoparticle's surface is functionalized with chitosan-surface decoration that can target these cells' CD44 receptors with specificity. These nanoparticles are designed to release the doxorubicin in acidic microenvironment only. Specifically, when the nanoparticles are internalized and accumulated in lysosomes, the acidic environment there will trigger the drug release. When compared to free doxorubicin, this nanoparticle boosts the doxorubicin's cytotoxicity by a factor of six. This helps to get rid of CD44+ cancer stem-like cells that are present in 3D mammary tumor spheroids. Considering no apparent systemic toxicity, these nanoparticles decreased the size of tumors in an orthotopic xenograft tumor model [143].

In a literature review last year, it was found that chitosan has applications in photodynamic therapy, sonodynamic therapy, and magnetic field-mediated therapy. Photodynamic therapy is commonly used in the treatment of cancer. It is basically a light-mediated therapy that utilizes light-sensitive drugs/substances to induce cancer cell death. Before light activation, the light-sensitive drug is harmless but turns poisonous to the intended tissue after being activated by light. The likelihood of survival is increased by early detection of cancer using nanomaterials and light propagation characterization. To distinguish colon cancer cells from healthy cells, researchers looked into light diffusion as well as the fluorescence response to ashwagandha-loaded chitosan nanoparticles formulated by ionotropic gelation technique. The stability of the developed formulation was confirmed by a significant positive zeta potential value (+30 mV). Near-infrared light (NIR) irradiation was used to study the fluorescent effect of nanoparticles. The cells' transmittance and reflectance observations were made using a digital fiber spectrometer. The wavelengths of interest were 808 nm and 665 nm. The light propagation on malignant and healthy cell lines has been determined and the results of transmittance and reflectance between the healthy cells and the malignant cells were different, according to the data obtained. During the laser exposure, the manufactured nanoparticles illuminated the cells that were being imaged. Focusing on the optical properties of the tissue and the fluorescent Ashwagandha-loaded chitosan NPs, this investigation proposes a straightforward theranostic method for treating cancer [144]. Another research also studied the glowing effect of chitosan nanoparticles. To investigate how chitosan nanoparticles affect the ability to distinguish between cancer cells and normal cells, light propagation in both colon cancer cell lines (Caco-2) and normal cell lines (WI-38) was investigated as well. The wavelength of interest is 650 nm and 808 nm for cancer, and normal cells respectively. Spatially resolved constant-state diffuse reflectance data for each of the investigated cells were obtained using a Monte Carlo

simulation model. The results demonstrated that chitosan nanoparticles showed their luminescent effect when exposed to NIR during cell imaging [145]. The constraints of photodynamic therapy (PDT), an intriguing painless tumor therapeutic approach, are inadequate biological compatibility, high expenses, and challenging rare earth material upconversion procedures. Not only chitosan, HA has also been utilized for PDT in cancer diagnostics and therapy. Researchers have attempted to develop a novel formulation by integrating methotrexate and doxorubicin with the hyaluronic acid base. The formulation is a self-dual targeting prodrug conjugate aimed at effective targeting via imaging-guided chemical photothermal therapy (PTT). The manufactured nanoformulations showed an elevated photothermal effect and improved biological stability with a size of 200 nm. The nanoformulations significantly increased the accumulation in the tumor locations by improving the permeation and retention efficiency and CD44/folate receptor-mediated endocytosis, due to the HA-based backbone. The nanoformulations demonstrated accelerated breakdown and hastened release of drugs in response to acid stimulation. As a result, the nanoformulations showed outstanding tumor removal by chemotherapy and PTT combination upon NIR irradiation, as well as extremely effective cell death [146].

Natural melanin particles (MNPs), which are effective photothermal treatment (PTT) substances, have been demonstrated to dramatically increase the formation of reactive oxygen species (ROS) when cross-linked with C60 under 808 nm irradiation, in contrast to synthesized polydopamine, which lacked this feature. Based on this unique property of MNP, MNP-C60-hyaluronic acid (MC60H) nanoparticles have been developed for multifunctional therapy on a tumor under NIR irradiation, without the use of rare-earth up-conversion materials. After being specifically recognized by hyaluronic acid (HA), MC60H was able to activate the widely utilized immunotherapy and aid in the transformation of tumor immunosuppressed M2-type macrophages into antitumor M1-type cells while also effectively promoting tumor increase in temperature and ROS production. The presented research is the first to describe the characteristic of encouraging ROS generation from C60 by MNP and it offers a strong illustration of MC60H in the implementation of a synergistic anticancer approach beyond the dependence of conventional up-conversion material [147]. A molybdenum disulfide-based HA-functionalized nanosheets has been developed by researchers that enable the site-specific combined delivery of chemotherapy drug gefitinib and gadolinium-based contrast agents for magnetic resonance imaging (MRI) and collaborative chemo-PTT of tumors. Diethylenetriaminepentaacetic acid (DTPA) was used as a linkage to bind gadolinium ions to developed nanosheets, which was then followed by the integration of the drug. The final product had enhanced relaxivity which made it easier to do MRI in vivo. Additionally, the nanosheets efficiently transformed the absorbed NIR radiation into heat, which caused the photothermal destruction of cancerous cells as well as the expulsion of drugs from nanosheets, enabling synergistic chemo-photothermal treatment. The outcomes of laboratory and preclinical investigations showed that nanosheet upon NIR irradiation successfully hindered the phosphatidylinositol 3 kinase (PI3K)/protein kinase B (Akt) signaling process and triggered proteins associated with apoptosis to induce cell death and reduce cell differentiation, thus hindering the development of the tumor in mice bearing lung cancer cells [148]. It has been demonstrated that inorganic nanoparticles that respond to near-infrared light can improve the effectiveness of cancer photothermal ablation treatment. In this case, scientists present the development of a framework for photothermal ablation and immunotherapy combined with NIR-induced transforming nanoparticles. The framework was developed as empty CuS nanoparticles coated with chitosan which are employed for developing immunoadjuvant oligodeoxynucleotides with cytosine-guanine (CpG) sequences. Chitosan was employed to enhance the biocompatibility and biodegradability of inorganic nanoparticles. Additionally, following laser stimulation, these frameworks disintegrate, regroup, and change into polymer compounds that enhance immunotherapeutics tumor accumulation. Utilizing this "photothermal immunotherapy" technique, immunoadjuvants enhance patient immunity against tumors while photothermal ablation-induced tumor death of cells inhibits the growth of tumors and exposes tumor antigens to the surrounding tissue. The coating of chitosan has made the nanoparticles biodegradable and found to be excreted from the body after the laser stimulation [149]. Chitosan has also been utilized for its photoluminescence properties for the diagnosis and therapy of multiple diseases. In research, chitosan was specifically used for its excellent biocompatible nature to develop carbon quantum dots for cell labeling, diagnostics, and theranostics applications.

Chitosan-based carbon quantum dots have been functionalized with amino acids on the surface to make them more biocompatible. The developed nanodots exhibit excellent photoluminescence and lack of cellular toxicity on human dermal fibroblasts. The findings validated the development of biocompatible quantum dots with carbon nuclei that glow in the visible spectrum, thus suitable for diagnostics purposes [150]. Additionally, researchers have developed biodegradable chitosan nanoparticles using varying concentrations of the photosensitizer tetraphenylchlorin (TPC) in this study that incorporate the cytostatic medications mertansine (MRT) or cabazitaxel (CBZ). Owing to the interactions among the active substances and the aromatic photosensitizer positions of the polymers, the developed NPs exhibit an excellent capacity for loading and significant drug stability. In the breast cancer cell lines viz. MDA-MB-231 and MDA-MB-468, the developed nanoformulations loaded with MRT or CBZ demonstrated more cellular toxicity than the free drug solutions. Additionally, these breast cancer cells were strongly affected by photodynamic treatment after the nanoformulations were photochemically activated by light. The majority of the nanoformulations were deposited in the liver and lungs, according to biodistribution experiments in mice, but they additionally turned out to be confined in tumors made from HCT-116 cells. Regarding drug loading ability and NP stability, chitosan with 10 % of these side chains bearing TPC was shown to be the most suitable option [151]. Likewise, several therapeutic and diagnostic radioisotopes (64Cu, 68Ga, 90Y, 153Sm, and 166Ho) were complexed with chitosan to overcome the shortcomings of conventional chelation-based techniques of radiolabelling. The radiolabelled chitosan nanoparticles were formulated via an ionotropic gelation approach. In epithelial lung cancer cells, the radiolabelled nanoparticle's cellular uptake and cell toxicity were assessed. The developed radiolabeled nanoparticles had a good level of in vivo stability, according to biodistribution experiments in healthy C57BL/6 mice [152]. Furthermore, theranostics hybrid nanogels were formulated by modifying the surface of chitosan nanoparticles with polyacrylic acid and further conjugating them with cysteine-functionalized gold nanoparticles. Formulated nanogels exhibited superior loading of doxorubicin viz. 87 %. The rise in the concentration of lysozyme and the acidic pH in the microenvironment associated with the tumor caused the nanogels to break down and produce smaller nano blocks (30–40 nm) that successively released the drugs in a tumor-responsive fashion. Investigations on intracellular capture showed that nanogels make it easier for the drug to enter the cell's cytoplasm. Gold nanoparticle-mediated computed tomography visualizes the significant deposition of nanogels in the tumor. Findings from laboratory and preclinical experiments showed that hybrid nanogels permitted advanced doxorubicin cancer cell targeting, which was more effective and safe than the free drug [153].

Chitosan has a significant role in thermal drug delivery also. In an investigation, for the intracellular temperature-based delivery, scientists created Pluronic F127 and chitosan nanocapsules with a hollow center, and subsequently determined the wall penetration as well as the nanocapsule's thermal response. Furthermore, researchers established the viability of encapsulating small compounds in nanocapsules for thermal control within cell delivery. Furthermore, the wall-permeability of the nanocapsule in a non-swollen state is minimal at body temperature i.e., 37 °C, facilitating tiny molecules to be efficiently kept in the

nanocapsule for several hours while at 4 °C, when the nanocapsules are swollen, the wall-penetration is substantial, facilitating the free passage of tiny molecules viz. ethidium bromide with molecular weight of 394.3 Da through the wall. Both malignant (MCF-7) and noncancerous (C3H10T1/2) mammalian cells were used to study the wall penetration of the developed nanocapsules. Following a 45-minute incubation period at 37 °C, it was discovered that the malignant cells absorbed the nanocapsules significantly more quickly than the non-malignant cells. Additionally, it was discovered that the distribution method of nanocapsules had very low cytotoxicity [154].

Sonodynamic therapy (SDT) is a non-intrusive ultrasound-mediated therapeutic approach for the deep-penetration, location-specific therapy of tumors. There are currently no published studies on the design of nano-sonosensitizers that are appropriate for SDT of bladder cancer following intravesical administration. To achieve exceptionally efficient SDT, a transmucosal oxygen-self-production SDT nano platform is designed. In this approach, meso-tetra(4-carboxyphenyl)porphine-conjugated catalase (CAT-TCPP) is assembled with fluorinated chitosan (FCS). The formulated FCS-based nanosensitizer demonstrated superior transmucosal permeability and intratumor penetration. To substantially increase the therapeutic effectiveness of SDT to eradicate orthotopic bladder tumors under ultrasound, it may effectively alleviate hypoxia in tumor cells by the catalase-mediated O_2 synthesis from tumor endogenous H_2O_2 [155].

Not confined to PDT and SDT, another cohort had developed ferrimagnetic iron oxide nanocubes (FIONs) stabilized with chitosan oligosaccharide as a heat-mediator for magnetic field-induced cancer cell death. Chitosan has been coated over the multiple-FIONs which had a diameter of 30 nm. The total magnetic moment is enhanced due to the presence of multiple FIONs, resulting in specific retention in the tumor when a magnetic field is externally applied. The developed formulation was compared to marketed superparamagnetic Feridex nanoparticles and demonstrated superior results in the form of high specific loss power value. Under the influence of a magnetic field, the formulation has led to caspase-mediated cell death in A549 cells. With no severe toxic effects, the formulation revealed antitumor efficacy in male BALB/C nude mice [156].

As a new theranostic device, researchers attempted to create biocompatible hyaluronic acid (HA)-glutathione (GSH) conjugate stabilized gold nanoclusters (GNCs) in combination with graphene oxide (GO) and 5-fluorouracil (5FU) loading. The multifunctional formulation had exceptional fluorescence, sensitivity to light, and capability for targeting cancer cells specifically; yet, in tumor cells, lysosomal hyaluronidase (LH) was able to efficiently restore both their fluorescence and singlet oxygen generation after graphene oxide had drastically diminished LH. The enzymatic breakdown of HA and the light-induced heat impact caused by graphene oxide under the influence of laser light could both be used to successfully stimulate the sustained and full expulsion of 5FU from the nanoformulations [157].

12. Advantages of chitosan and HA-based nanocarriers over traditional technology

Chitosan and Hyaluronic Acid-Based Nanocarriers present a significant advancement in cancer therapy when compared to conventional treatment methods such as chemotherapy and radiation therapy. Unlike traditional therapies, which often result in non-specific drug distribution and widespread side effects, these nanocarriers offer targeted drug delivery, minimizing harm to healthy tissues while precisely reaching cancer cells [61]. They address the challenge of poor drug solubility by encapsulating hydrophobic drugs, increasing their bioavailability. Moreover, nanocarriers enable controlled and sustained drug release at the tumor site, ensuring a consistent therapeutic concentration [158,159]. Their biocompatibility, derived from natural polymers, reduces adverse reactions. The customization options for specific drugs and cancer types, as well as the potential for combination therapy, make them versatile and effective [160]. Additionally, the positive charge of chitosan facilitates targeting and binding to the negatively charged cancer cells for effective internalization of the carriers and simultaneous release of the entrapped chemotherapeutic agent with the help of lysozyme. Concurrently, the negatively charged hyaluronic acid can be functionalized to the positive chitosan, which has shown improved targeting of the overexpressed CD44 receptors on cancer cells. Expression of CD44 receptors has been recorded in many cancer cells, which is responsible for the progress of cancer cell growth along with infiltration and metastasis. Additionally, the surface of such polymeric nanoparticles can further be modified with PEG to facilitate the EPR effect, whereas overexpressed receptors on cancer cells can also be targeted via surface functionalization with RGD, folic acid, etc. (Fig. 9). Furthermore, integrated imaging capabilities in these nanocarriers allow real-time diagnosis and treatment monitoring. They can also overcome biological barriers, such as the blood-brain barrier, facilitating drug delivery to sensitive or hard-to-reach areas. In contrast, traditional cancer therapies like chemotherapy and radiation therapy struggle to offer these advantages, resulting in less precision, broader side effects, and limited drug solubility [161,162].

13. Conclusion and future perspective

A variety of nanocarriers containing chitosan and HA are gaining attention from researchers for the development of targeted drug delivery systems for the treatment of cancer owing to their biodegradability and biocompatibility nature. Their application is not limited to delivering small molecules from natural or synthetic sources, these polymers have also been employed to deliver nucleic acid and protein/peptides. The possibilities of derivatization on the basic structure of these natural polymers (chitosan and HA) lead to the enhancement of their drug loading and targeting ability. There are numerous nanocarriers (like nanoparticles, micelles, NLCs, nanocages, nanohydrogels, carbon nanotubes, and nanoconjugates) can be approached using compositions of HA and chitosan to deliver antitumor drugs, which have exhibited effective cytotoxic properties, particularly to the cancer cells. Their ability to increase the residence time following surface modification, and enhanced cellular uptake with more effective access to the tumor microenvironment as compared to the conventional treatment strategies have increased their application as a component of novel drug delivery systems. Their characteristics to carry an enhanced load of therapeutic agents due to their high surface area to volume ratio, ability to penetrate the leaky cancer microenvironment, and to deliver the entrapped drug subsequently to the cancer cells using the EPR effect made them suitable for application to cancer therapy. Besides possessing a plethora of benefits of both polymers, there are some practical drawbacks that chitosan and HA-based nanoformulations may suffer. Considering cancerous tissues often have a lower pH than healthy tissues, chitosan-based nanocarriers can be engineered/conjugated with ligands and antibodies to release their payload in response to this difference. The solubility of chitosan is dependent on pH; it is soluble only in acidic environments. The neutrality of physiological pH situations can be a challenge when designing nanocarriers for drug delivery. Furthermore, drug loading is also a big issue that should be therapeutically effective, but is difficult to achieve in the case of nanocarriers. Depending on the nature of the drug and preparation technique, the drug loading capability may change. In addition, owing to the aggregation properties of nanocarriers, formulations may not be as stable in biological settings. Variations in shape, size, and release of drug characteristics might result from stability problems. Maintaining batch-to-batch uniformity while increasing the output of chitosan nanocarriers might be difficult. During production, variables including consistent loading of drug and particle size dispersion must be managed.

Recently, several researchers have been adopting the Design of Experiment (DoE) to identify the influential variable and their impact on the formulation quality parameters. It comprised various mathematical

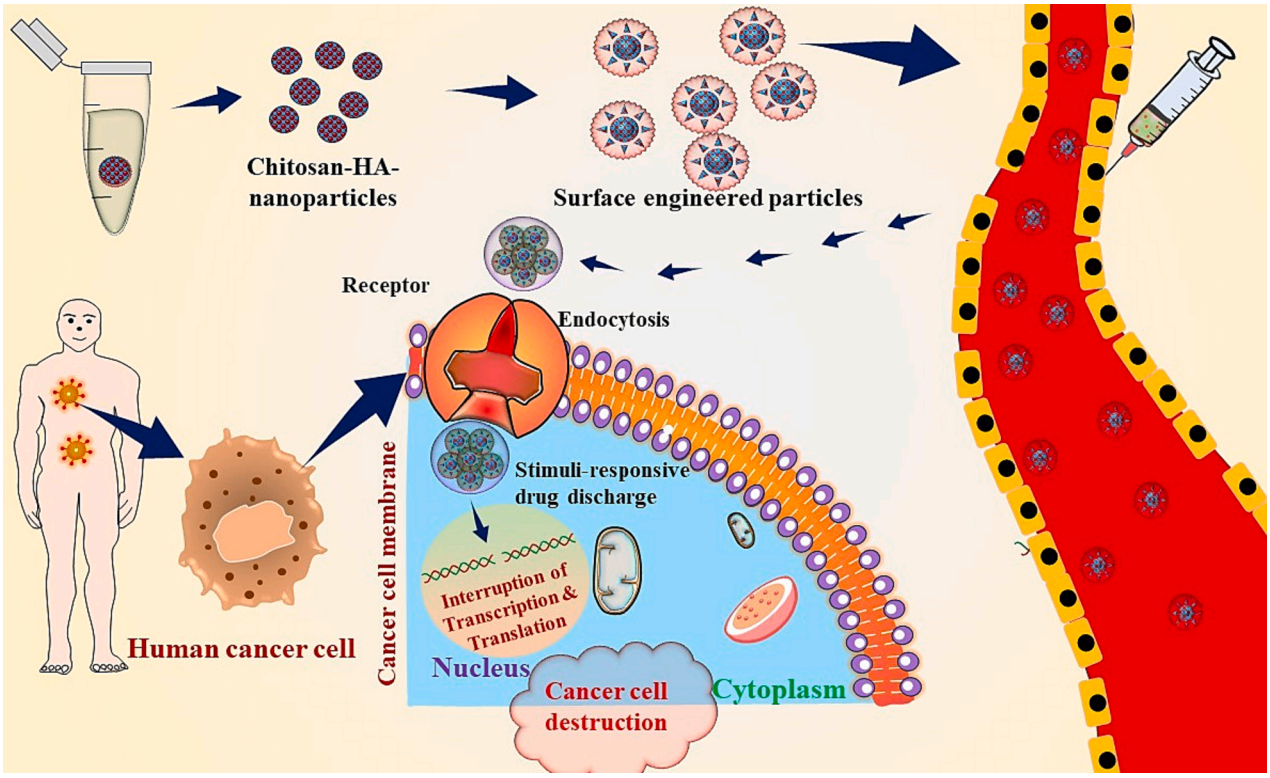


Fig. 9. Pictorial presentation of chitosan-HA-based nanoparticles integrated anticancer activity through the ligand-mediated internalization in cancer cells

models to produce graphical outcomes and the correlation between the factors was estimated by model-based response surface methodology. Similarly, the "quality by design" (QbD) approach encompasses a quality target product profile (QTPP) that focuses on the quality attributes of the nanoformulation and its preparation methodology affecting the desired quality of the final product. These quality attributes are selected based on the intended marketed product with enhanced therapeutic outcomes.

Although, both chitosan and HA-based formulations are under development and confront substantial gaps in clinical use, which include but are not limited to fewer safety data for the chitosan and HA nanocarriers and low aqueous solubility and chemical adaptability of chitosan, lack of information of targeting mechanisms in cancer patients, causing difficulty to accept them scientifically for clinical application. Furthermore, laboratory research of the nanocarriers is facing challenges during commercial scale-up, thus the characteristics of the nanocarriers alter, expecting an altered behavior in humans if applied. Hence, extensive research evidence is mandatory for the clinical translation of these preclinical successes. An exhaustive investigation into these prospects will help the researchers synthesize nanoformulations for the safe delivery of anticancer drugs in a controlled manner with targeted delivery for superior control of cancer patients.

CRediT authorship contribution statement

Parul Rohtagi: Writing – original draft. **Unnati Garg:** Writing – original draft. **Triveni:** Writing – original draft. **Neha Jain:** Conceptualization, Supervision, Writing – review & editing. **Manisha Pandey:** Conceptualization, Supervision, Writing – review & editing. **Mohd Cairul Iqbal Mohd Amin:** Writing – review & editing. **Bapi Gorain:** Writing – review & editing. **Pradeep Kumar:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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