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Advances in the delivery of anticancer drugs by nanoparticles and chitosan-based nanoparticles

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ABSTRACT

Cancer is the leading cause of death globally, and conventional treatments have limited efficacy with severe side effects. The use of nanotechnology has the potential to reduce the side effects of drugs by creating efficient and controlled anticancer drug delivery systems. Nanoparticles (NPs) used as drug carriers offer several advantages, including enhanced drug protection, biodistribution, selectivity and, pharmacokinetics. Therefore, this review is devoted to various organic (lipid, polymeric) as well as inorganic nanoparticles based on different building units and providing a wide range of potent anticancer drug delivery systems. Within these nanoparticulate systems, chitosan (CS)-based NPs are discussed with particular emphasis due to the unique properties of CS and its derivatives including non-toxicity, biodegradability, mucoadhesivity, and tunable physico-chemical as well as biological properties allowing their alteration to specifically target cancer cells. In the context of streamlining the nanoparticulate drug delivery systems (DDS), innovative nanoplatform-based cancer therapy pathways involving passive and active targeting as well as stimuli-responsive DDS enhancing overall orthogonality of developed NP-DDS towards the target are included. The most up-to-date information on delivering anti-cancer drugs using modern dosage forms based on various nanoparticulate systems and, specifically, CSNPs, are summarised and evaluated concerning their benefits, limitations, and advanced applications.

1. Introduction

Cancer remains the leading cause of death worldwide (Siegel et al., 2022). Most cancers result from environmental factors, such as exposure to radiation and pollutants, but most importantly, from an unhealthy lifestyle, including lack of physical activity, poorly balanced diet, tobacco smoking, and stress (Wu et al., 2018). Breast, lung, colorectal, cervical, and thyroid cancers are the most common types of cancer in women. Prostate, lung, colon, liver, and stomach cancers are most common in men (Bukowski et al., 2020).

Various radiotherapy and chemotherapy approaches have been devised to diagnose and treat cancers (Valencia-Lazcano et al., 2023). Chemotherapy is still the mainstay of cancer treatment, using cytotoxic agents to suppress the growth of cancer cells for curative or palliative purposes. Despite advances in screening and the development of new anticancer agents, chemotherapy in clinics still needs improvement for many reasons (Li et al., 2023a). Since the development, complete testing, and approval of new anticancer chemotherapeutics are highly costly and labor-consuming, it makes sense to improve existing therapies (Sztandera et al., 2019). The main drawbacks of conventional cancer treatment are the non-specific delivery of the drug, the low concentration of the drug in cancer cells, and the severe side effects caused by the action of chemotherapeutic drugs on normal cells (Rizwanullah et al., 2021). To effectively treat tumors, a drug carrier should be designed to accumulate in tumor tissues and protect normal healthy cells, thereby increasing the therapeutic efficacy of the drug while sparing healthy organs from toxicity (Raheem et al., 2023).

Recent research has focused on how NPs could function as drug delivery systems (DDS). NPs, when used as drug carriers, can enhance drug efficacy by improving solubility, and half-life, delaying drug circulation, and aiding in stimulated, controlled, and sustained drug release (Raheem et al., 2023). NPs offer several advantages, such as size and shape variability, formation of stable interactions with ligands, high carrier capacity, and convenient binding of both hydrophilic and hydrophobic drugs (Gupta et al., 2023). An advantage of using nanotechnology in medicine is the improved ability to distinguish between

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pathological and normal tissues. This ability is based on using distinct interactions and processing pathways within the organism that are unique to NPs (Wolfram and Ferrari, 2019).

Nanomedicines can alter the pharmacokinetics of free drugs and reduce systemic toxicity by targeting tumor sites and increasing anticancer activity (Chen et al., 2023). NPs can accumulate in tumors due to a process called the "increased permeability and retention effect." Due to the hyperpermeability of blood vessels and impaired lymphatic drainage of tumor tissues, NPs, and macromolecular drugs preferentially accumulate in the tumor interstitium. They are retained in tumor tissues for a long time (Sun et al., 2022). Ideally, nanomaterials should efficiently penetrate tumor tissues and release their payloads in response to specific exogenous or tumor-specific endogenous stimuli, thereby improving *in vivo* pharmacokinetic profiles to obtain balanced antitumor efficacy and safety (Li et al., 2023a). However, it is now recognized that not all patients respond to therapy similarly. Given that inter- and intratumoral variability may influence the architecture of the tumor neovasculature and microenvironment, passive targeting of NPs to tumors may be more complex than initially thought (Bertrand et al., 2014). Numerous studies have shown that ligand-directed, actively targeted modified NPs can accurately enhance tumor drug accumulation. However, actively targeted NPs still passively secrete blood vessels into tumor tissue. (Chen et al., 2023). Multifunctional or multi-reactive NPs, considered potentially ideal drug carriers for cancer therapy, are considered state-of-the-art (Xie et al., 2022). Stimuli-responsive DDS are another attractive systems in which the drug is released or delivered by different mechanisms according to the internal environment or an external stimulus (Dinakar et al., 2022).

Many types of compounds are used to prepare NPs for drug delivery. These include polymers, metal particles, lipids, and many others (Pourmadadi et al., 2023). Generally, organic polymers, in contrast to inorganic nanomaterials, have good biocompatibility, stability, and processability, as well as highly tailorable degradability and responsiveness to external stimuli (*e.g.*, light, temperature, pH, acoustic waves, and biological signals) (Yang et al., 2021). One of the highly studied natural polymers is CS, whose properties predispose it for use in the treatment of cancer. CS is a linear multilayered, positively charged polysaccharide. CS consists of randomly distributed (beta-1-4) *N*-acetyl-D-glucosamine units. It is commercially produced by deacetylation of chitin. Chitin is abundantly available on Earth in terrestrial vertebrates and crustacean shells in the sea, and its properties are highly dependent on the degree of deacetylation, polydispersity, and molecular weight (Hamedi et al., 2022), (Mukhtar et al., 2021). The significant amount of naturally produced chitin makes this biopolymer a sustainable, inexpensive, and accessible source for the production of CS (Wang and Zhuang, 2022). CS is biodegradable, biocompatible, and often used in drug delivery (Mukhtar et al., 2021). CS may be an excellent carrier for the delivery of anticancer drugs due to its non-toxicity and several improved modifications (Tian et al., 2021). Since CS is pH-sensitive, allowing pH-responsive drug delivery in the cancer microenvironment, CSNPs can be used as intelligent carrier systems (Dongsar et al., 2023).

Some recent review papers discussed the CSNPs for anticancer drug delivery. Yadav et al. (Yadav et al., 2023) presented advances and challenges in using CS and its derivatives in biomedical fields. Afra et al. (Afra et al., 2024) authored a review article on crosslinkers CS compounds and their applications in 3D bioprinting for the development of CS-based bioinks. Furthermore, Shakil et al. (Shakil et al., 2021) summarise the use of CS itself and its derivatives in treating cancer. Naskar et al. (Naskar et al., 2019) reviewed two decades of research on CS-based NPs as DDSs and Zaiki et al. (Zaiki et al., 2023) discussed functionalized CS for cancer nano drug delivery. Elkomy et al. (Elkomy et al., 2022) dealt with CS on the surface of NPs for enhanced drug delivery. Tian et al. (Tian et al., 2021), (Tian et al., 2023), Narmani and Jafari (Narmani and Jafari, 2021), and Rostami (Rostami, 2020) summarise recent advances in CS-based nano delivery systems for cancer therapy. Last but not least, Kantak and Bharate (Kantak and Bharate, 2022) prepared a review analysis of clinical studies of biomaterials and therapeutic applications of CS.

The aim of this review was to uniquely summarise all the basic knowledge needed for both newcomers and experts to understand the issue of cancer treatment using innovative carrier systems for the transport of anticancer drugs. It highlights nanoplatform-based cancer therapy pathways such as passive and active targeting and stimuli-responsive systems. In this context, various organic as well as inorganic nanoparticulate carriers of anticancer drugs are discussed. Within them, special attention is given to the discussion and critical evaluation of using chitosan and its derivative-based nanoparticles as an onco-specific carrier system. Thanks to comprehensive evaluation of a wide scale of different nanoparticulate systems in drug delivery, it is possible to critically evaluate a position of CS among other reviewed nanoparticulate systems concerning their character (biocompatibility, biodegradability, toxicity, stability, homogeneity, structural variability, *etc.*) and potentialities for implementations into biological systems (drug solubilization, drug preservation, target drug delivery, route of delivery, degree of implementation, *etc.*). The benefits, limitations, and advanced application potentialities of nanoparticulate systems based on CS and its derivatives are discussed in the context of recently developed anticancer drug delivery approaches.

2. Anticancer drugs: approaches in anticancer therapy

Usually, healthy cells are tightly regulated, while stimulatory and inhibitory signals are in a delicate balance. For normal cells to develop cancer, it is thought that a physical, chemical, or biological factor must harm the cell and induce a genetic and/or epigenetic change that subsequently spreads during cell division. Later, cancer cells undergo several changes that lead to uncontrolled proliferation, invasion, and metastasis. Tumors can be divided into benign or malignant. Benign tumors are non-cancerous growths that are often encapsulated and localized. Malignant tumors, however, invade and damage the surrounding tissue. Such cancerous cells are genetically unstable, losing their typical cellular architecture, eventually leading to cells atypical for their tissue or cell of origin (Cordes and Cuellar, 2023). Classical theory indicates that the oncogenic mutations of malignant cells cause cancer initiation. They surround untransformed cells after recruiting and adapting, accompanied by the release of various chemokines, cytokines, growth factors, and vesicles, and this leads to the formation of a tumor microenvironment (TME). As cancer progresses, the TME is trained and modified by the cancer cells to facilitate the development of cancer hallmarks, respond to internal or external stresses, and respond to stimulation and treatment, thereby promoting the survival and migration of cancer cells in the body (Fan and Guo, 2023). Tumors are highly complex (heterogeneous) with nucleus-like structures in which various loosely arranged tissues with highly proliferative cells surround a hypoxic, tightly circumscribed tumor core (Marusyk and Polyak, 2010). Notably, these loosely bound, unorganized proliferating cells are highly vascularised with a disrupted epithelial lining, often leading to marginal gaps. These gaps, referred to as the enhanced permeability and retention (EPR) effect, are commonly applied for passive targeting during drug delivery (Cheng et al., 2021).

2.1. Classification according to the mechanism of action

There have been ongoing efforts to treat this life-threatening disease since the first discoveries of modern anticancer drugs in the 1940s (Anand et al., 2022). Despite considerable advances in cancer treatment methods in recent decades, chemotherapy remains the leading cancer treatment method (Bukowski et al., 2020). Anticancer drugs can generally be divided into phase/cell cycle specific (they ensure the destruction of rapidly proliferating cells, usually during a defined phase of the cell cycle) or non-cell cycle specific (they have the same effect on cancerous and normal cells, irrespective of growth phase or rate of

division). These include alkylating agents, heavy metals (cause cross-linking between DNA strands, leading to inhibition of DNA replication and induction of apoptosis), antimetabolites, cytotoxic antibiotics, spindle poisons (affect microtubule assembly and disassembly), and topoisomerase inhibitors (Miles and David, 2018). Groups of anticancer drugs are illustrated in Fig. 1.

2.1.1. Alkylating agents

Alkylating agents represent a core group of anticancer drugs. Synthetic alkylating agents are widely used to treat solid tumors and leukemia/lymphoma (Biersack, 2019). They are electrophiles that react with the ring nitrogen and other cyclic oxygen atoms of DNA bases to form covalent adducts, which in turn lead to DNA strand crosslinking, abnormal base pairing, or DNA strand breaks (Chiorcea-Paquim and Oliveira-Brett, 2023). Alkylating agents are classified broadly into six classes; nitrogen yperites (mechlorethamine, cyclophosphamide, ifosfamide, melphalan and chlorambucil), ethyleneamine and methyleneamine derivatives (altretamine, thiotepa), alkylsulfonates (busulfan), nitrosoureas (carmustine, lomustine), triazenes (dacarbazine, procarbazine, temozolomide), platinum-containing antineoplastic agents (cisplatin, carboplatin, oxaliplatin), which are known as platinum coordination complexes. Although they do not alkylate DNA, these antineoplastic drugs are usually categorized as alkylating agents but cause covalent adducts of DNA in various ways (Alkylating Agents, 2012).

2.1.2. Antimetabolites

Antimetabolites are considered to be one of the oldest groups of anticancer agents whose mechanism of action is mediated by interaction with essential biosynthetic pathways (Nussbaumer et al., 2011). These molecules usually have a structure similar to a cellular metabolite or enzyme substrate that is generally identified and processed by an enzyme to satisfy a cellular need (Chu, 2021). Structural analogs of pyrimidine or purine are integrated into cellular components to interfere with nucleic acid synthesis. Other antimetabolites, such as methotrexate (MTX), interfere with basic enzymatic metabolic processes (Nussbaumer et al., 2011). Antimetabolites may be grouped into several classes: pyrimidine antagonists (cytarabine, 5-fluorouracil (5-FU), gemcitabine, and capecitabine), purine antagonists (fludarabine), purine analogs (6-mercaptopurine, azathioprine, and cladribine), antifolates (MTX, pemetrexed, and pralatrexate), and ribonucleotide reductase inhibitors (hydroxyurea) (Bukowski et al., 2020).

2.1.3. Spindle poisons

Microtubules (MTs) are fundamental in cellular organization, cargo transport, and chromosome segregation during cell division. In mitosis, microtubules constitute a macromolecular structure known as the mitotic spindle, responsible for precisely segregating chromosomes between two daughter cells. Moreover, recent studies suggest that MTs' dynamics are specifically tuned such that minor perturbations in the rate of MTs assembling in mitotic spindles can affect cell division and cancer development. (Vicente and Wordeman, 2019). Mitotic spindle inhibitors like taxanes (docetaxel (DTX)) and paclitaxel (PTX)) and vinca alkaloids (vincristine and vinblastine) alter spindle microtubule function/formation by suppressing nuclear division (arrest of mitosis in metaphase), resulting in cell death (Nussbaumer et al., 2011).

2.1.4. Topoisomerase inhibitors

DNA topoisomerases are enzymes controlling the topology of DNA in all cells. Two types, I and II, are classified according to whether they make transient single- or double-stranded breaks in DNA (Bush et al., 2015). Topoisomerase inhibitors inhibit topoisomerase activities in DNA replication and cause DNA strand breaks (Bax et al., 2019).

2.1.5. Other chemotherapeutic agents

Other chemotherapeutic agents are some enzymes (l-asparaginase), proteasome inhibitors (bortezomib), tyrosine kinase inhibitors (imatinib and erlotinib), and antibiotics (bleomycin, actinomycin D, and anthracyclines such as idarubicin, daunorubicin, doxorubicin (DOX)) (Bukowski et al., 2020).

2.2. Limitation on the use of anticancer drugs

Chemotherapy uses drugs that destroy cancer cells, stopping tumor development and cell division. However, no chemotherapy can affect malignant cells without causing side effects (Fong and To, 2019). These drugs can interfere with cell division pathways, DNA mimicry, and chromosomal segregation and are not specific to cancer cells (Osumi et al., 2019). Although chemotherapy has considerably increased

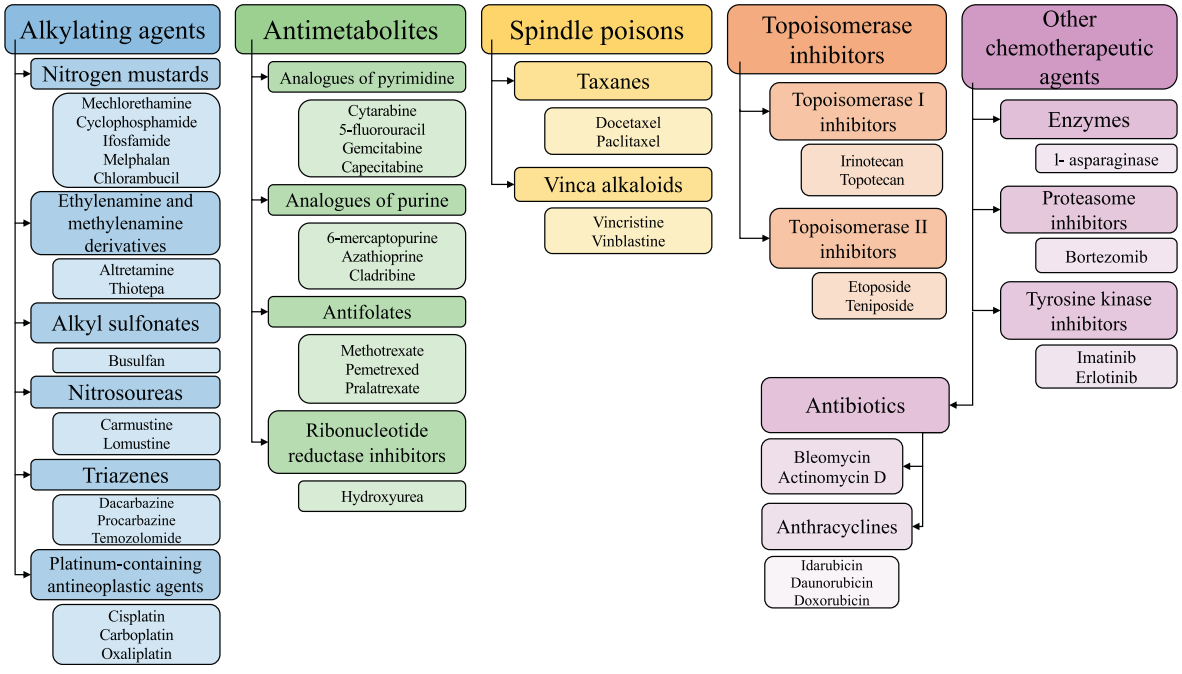

Fig. 1. Groups of anticancer drugs.

survival rates overall, patients still report experiencing a wide range of physical and psychological symptoms that affect their quality of life. These symptoms rarely occur in isolation. Hair loss caused by chemotherapy continues to be a severe problem that negatively affects the well-being of many cancer patients. Impaired appetite caused by cancer and chemotherapy is usually due to changes in taste, mouth ulcers, nausea and vomiting, increased satiety, side effects of medications, pain, exhaustion, depressed mood, and anxiety (Anand et al., 2022). Still, the selectivity of most drugs is limited, and such drugs are considered among the most harmful drugs used in treatment (Large et al., 2019). Cancer cells adjust to the challenges of conventional treatment by activating multiple molecular and cellular signaling pathways and often evolve into more violent phenotypes that are difficult to destroy with currently available therapeutic agents (Singh et al., 2023). Microenvironmental acidity is assumed to be involved in tumor progression and resistance to treatment. The pH of the microenvironment surrounding the tumor is typically lower than the pH of healthy tissues and blood due to the production of lactate and other acidic compounds. This can generate a potentially unfriendly environment for immune cells and other non-tumor cells, which can become less able to function and survive in such an acidic environment (Bhattacharya et al., 2023). The effect of most anticancer drugs is restricted by multidrug resistance (MDR) induced by cancer cells. Several mechanisms that can lead to drug resistance include mutation of the molecular target of the drug, changes in the tumor microenvironment, large-scale cellular changes, and changes in the drug-tumor interaction, or a combination of these factors (Rawal and Patel, 2019). MDR is now responsible for more than 90 % of deaths in cancer patients receiving traditional chemotherapy drugs or new targeted drugs. Many biomedical trials focus on designing chemotherapeutics to avoid or reverse MDR (Bukowski et al., 2020).

3. Innovative nanoplatform-based cancer therapy pathways

Routine methods for drug administration involve pulmonary, transdermal, transmucosal, ocular, and other delivery systems, among which injection and oral delivery are the most popular. In these methods, however, one of the main problems is the poor pharmacokinetics and/or untargeted delivery of the drugs; therefore, in some applications such as chemotherapy, there are increasing side-effects from the drug, and the effectiveness of therapy could decrease (Kazemzadeh and Mozafari, 2019). Given the highly toxic nature of anti-neoplastic drugs, off-target accumulation can result in severe adverse effects such as fatigue, nausea and vomiting, bone marrow hematopoietic dysfunction, cardiotoxicity, hepatotoxicity, nephrotoxicity and neuropathy (Zaiki et al., 2023). Nanomaterials have been developed to mitigate against conventional cancer chemotherapy's limitations. Nanomaterials exhibit particular properties that can overcome the drawbacks of conventional therapies, such as lack of specificity, high drug concentrations, and adverse drug reactions (Ahmadi et al., 2022). The results of the clinical studies, which Tian et al. (Tian et al., 2023) summarised in their review indicated that anticancer drugs-loaded NPs can provide more potent therapeutic effects than single drugs. Furthermore, combining different treatments may offer a more efficient approach to treating cancer.

Solubility is a governing parameter in deciding drug disposition and bioavailability. Also, most of the high molecular weight drugs and highly protein-bound drugs tend to have very poor tumor penetration owing to poor permeability across cellular and other biological barriers (Rawal and Patel, 2019). It has been proven that NPs may shield therapeutics from degradation, enhance their solubility, and extend bloodstream circulation time, increase permeability across biological membranes while, at the same time, providing targeted transport and controlled release of therapeutics (Sztandera et al., 2019).

Before understanding the action of NPs, it is required to understand their pathways compared to the conventional formulations delivering chemotherapeutics. The predominant approaches employed to deliver the drug through NPs include passive and active targeting approaches (Liu et al., 2020). According to Hoffman and Lai (Hoffman and Lai, 2020), three significant developments of the last decades in controlled drug delivery are (i) the concept of PEGylation of drugs and their carriers (acting in passive targeting), (ii) the application of monoclonal antibodies and their conjugates with drugs (antibody-drug conjugates, ADCs) (related to active targeting), and (iii) the use of the EPR effect (acting in passive targeting). The active and passive targeting, illustrated *via* CSNPs with or without a tumor-specific label (ligand), respectively, are represented in Fig. 2 and discussed in Subsections 3.1 and 3.2.

Despite 30 years of experimentation, adding targeted moieties to therapeutic NPs to increase their site-specificity has yet to yield clinically approved drugs. This may be because the addition of molecular targeting agents increases the specificity of recognition but at the cost of much more incredible difficulty in addressing biological barriers (Wolfram and Ferrari, 2019). Hence, other innovative nanoplatform-based cancer therapy pathways are represented by stimuli-responsive DDS additionally enhancing orthogonality of onco-specific nanoparticulate systems in a complex biological environment possessing characteristic attributes of healthy and cancerous regions and structures. Stimuli-responsive DDS, discussed in Subsection 3.3 and listed in Fig. 3, offers a rational solution to minimize an obstacle to the efficacy of a nanosystem in therapy consisting of the premature release of the drug from the NP pores during systemic circulation (*i.e.* before reaching the target tissue) and the unexpected uptake of drugs by normal cells (causing unwanted side effects) thanks to its possibility to respond to both internal (biological) and external (externally introduced) stimuli.

To implement innovative nanoplatform-based cancer therapy pathways properly, a knowledge of the basic building units of a nanoparticulate structure and the resulting structural and chemical types of nanoparticulate systems is a prerequisite. Therefore, the most important nanostructures based on organic materials such as polymers, dendrimers, solid lipids, micelles, and liposomes, and inorganic materials such as gold, silica, quantum dots, carbon NPs, and nanotubes, as well as hybrid nanostructures, are discussed in Subsection 3.4 and listed in Fig. 4.

3.1. Passive targeting via EPR effect and PEGylation

3.1.1. EPR effect

NPs are reported to accumulate in tumor tissues by a passive mechanism, the EPR effect, without the addition of targeted ligands (Li et al., 2018a). As the oxygen levels surrounding malignant tumors are low, cancer cells can produce angiogenic factors such as HIF-1α (hypoxia-inducible factor 1 alpha) and VEGF (vascular endothelial growth factor), which enhance angiogenesis. However, the blood vessel formation rate is insufficient to keep up with the growth of cancer cells, leading to blood vessels with a rough and leaky architecture. This leaky structure and the invasion of blood vessels by nearby cancerous tissues result in EPR (Jang et al., 2020). In particular, lymphatic obstruction at the tumor site causes the NPs to persist in the tumor tissue (Chen et al., 2023). Whilst there is evidence of the EPR effect in humans, the prevalence of this phenomenon is highly heterogeneous between individuals. Furthermore, an analysis of 17 clinical trials showed that the EPR effect was most significant in pancreatic, colon, breast, and stomach cancers, which exhibited intratumoral NP levels more than ten times higher than in normal tissue. In comparison, head and neck cancer and melanoma patients showed a twofold increase in preferential tumor accumulation. In metastatic breast cancer, NP-based therapies promise to overcome drug resistance and achieve site-specific delivery through approaches such as hemodynamic targeting (Wolfram and Ferrari, 2019).

The potential size of NPs is an essential indicator of nanomedicine, significantly influencing the accumulation of NPs in tumors and other organs. Although most NPs developed to date are spherical, different shapes can also be used for targeted drug delivery to tumors (Chen et al., 2023). The NPs' physical size should be kept below 200 nm for cancer treatment to meet the EPR effects (Adhikari and Yadav, 2018).

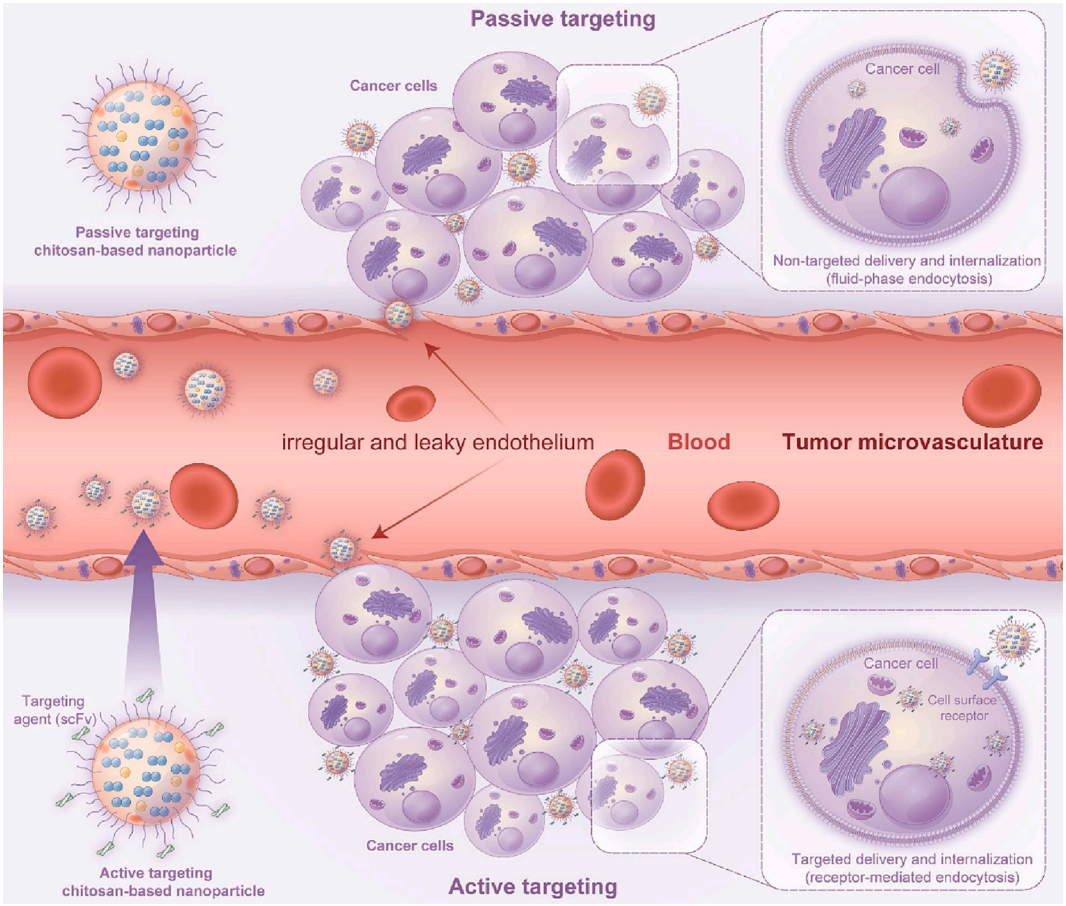

Fig. 2. Schematic diagram of intake and release of chitosan-based nanoparticles for drug delivery. Chitosan-based nanomaterials help transport nanomedicines to tumor tissues for a more extended period. The high-affinity ligand on their surface interacts with tumor markers on cell surfaces, triggering endocytosis. The drug is released inside the cells, killing the tumor cells in acidic, high ROS, and high GSH environments (Tian et al., 2023).

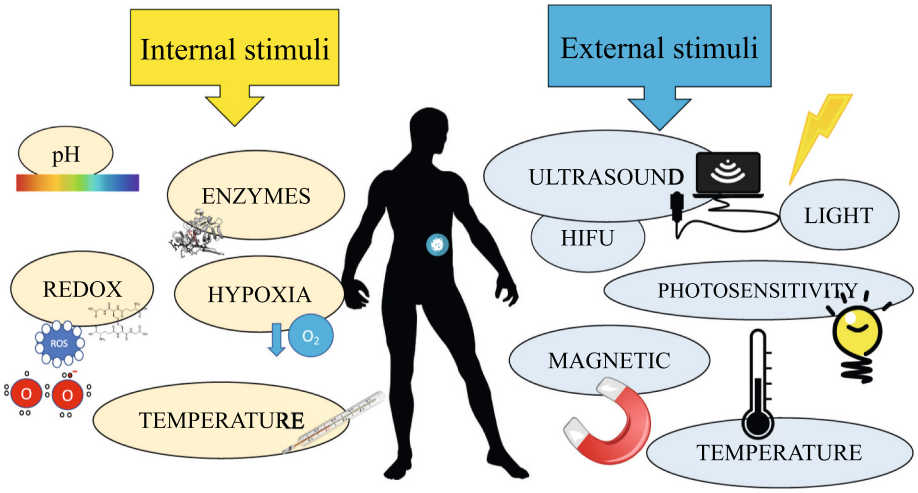

Fig. 3. The stimuli-responsive drug delivery systems.

3.1.2. *PEGylation effect*

NPs must circulate in the bloodstream for as long as possible to achieve adequate concentrations of the systemically administered drug in the target tissues (Suk et al., 2016). Once in the blood, opsonin proteins such as immunoglobulins, complement proteins, and macrophage receptors detect and bind to unprotected NPs, and these are then phagocytosed and finally removed. Hydrophobic and charged particles tend to be more readily opsonized because of a greater propensity to interact with opsonin proteins and are, therefore, highly susceptible to early removal (Zaiki et al., 2023). Coating the surface of NPs with polyethylene glycol (PEG) or "PEGylation" is a widely used technique to increase the efficiency of drug and gene delivery to target cells and tissues. (Suk et al., 2016). This steric layer serves as a barrier against the penetration of foreign molecules (*i.e.* proteins) and cells of the

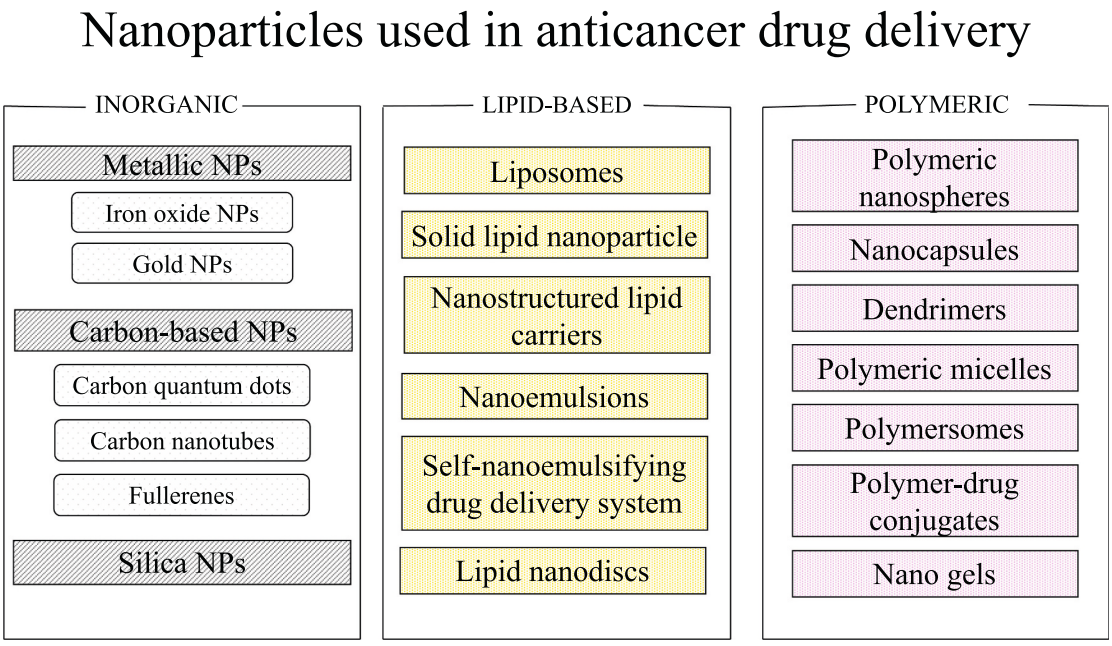

Fig. 4. Types of NPs used in anticancer drug delivery.

mononuclear phagocytic system (dendritic cells, blood monocytes, granulocytes, and macrophages) into the corona of the PEG (Echeverri-Cuartas et al., 2020). This modification exhibits biocompatibility, internalization, and sustained release of the drug (Khalaf et al., 2023). It is, therefore, understandable that most nanomedicines approved for clinical use and experimental nanotherapeutics contain PEG (Shi et al., 2022).

3.2. Active targeting via tumor cell-specific ligands

As previously indicated, one of the leading and essential aspects of therapeutic administration is to achieve maximum intracellular bioavailability of the drug, which leads to successful therapeutic efficacy over a prolonged period (Loira-Pastoriza et al., 2014). Due to many confounding factors, delivered drugs should not be released from the DDS before reaching the target tissue but to be delivered and released directly at the target site (Tang et al., 2012). One of the crucial characteristics of tumors that can be used in developing new nanotherapeutics is the overexpression of specific receptors compared to normal cells (Mazzotta et al., 2020). This allows us to decorate NPs with tumor cell-specific ligands such as folate, hyaluronic acid, transferrin, antibodies (*e.g.*, trastuzumab), peptides (*e.g.*, RGD peptide), and aptamers (*e.g.*, AS1411 DNA aptamers), thereby initiating active targeting of tumor cells and their subcellular organelles (Bajracharya et al., 2022).

Folic acid (FA) is also known as vitamin B9. FA can bind to folate receptors, which are overexpressed on the cell membranes of many tumors. We can further decorate NPs with biotin, also known as vitamin B7. It is a growth promoter that maintains the rapid proliferation of cancer cells, and therefore its biotin receptor is often highly expressed in many cancer cells compared to normal cells.

Another approach may be through aptamers, a highly stable single-stranded DNA or RNA with high specificity and affinity with various molecular ligands. Compared to normal cells, tumor cells often overexpress some polysaccharide receptors on the cell membrane.

N-acetylgalactosamine and β-d-galactose are specific ligands for asialoglycoprotein receptors commonly used for targeting liver cancer.

Hyaluronic acid (HA) is a widely used ligand for tumor cell-targeting modification, as many tumor cells overexpress CD44 receptors. HA can be fixed on the surface of NPs by chemical grafting or physical adsorption. We can also use the antibody-mediated approach.

Antibody-drug conjugates (ADCs) represent a typical class of anticancer drugs that directly conjugate cytotoxins and target antibodies. For example, rituximab and moxetumomab can specifically recognize receptors on the surface of tumor cells, and this leads to activation of the immune system or an increase in the effect of chemotherapy.

The transferrin (Tf) receptor is often highly expressed in malignant cells. Many Tf-modified NPs significantly enhance the phagocytosis of drugs by cancer cells and increase accumulation in the tumor (Chen et al., 2023).

3.3. Stimuli-responsive drug delivery systems

Tumor development is characterized by unregulated cell proliferation and abnormal dilation of blood vessels resulting in a high concentration of reactive oxygen species, low oxygen levels, and a slightly acidic pH in the TME (Bhattacharya et al., 2023). Although active targeting *via* ligand-mediated drug delivery to specific tissues is possible, an obstacle to the efficacy of such a nanosystem in therapy is the possible premature release of the drug from the pores during systemic circulation to reach the target tissue and the unexpected uptake of drugs by normal cells, causing unwanted side effects (Shen et al., 2019). On the other hand, stimuli-responsive carriers can respond to both internal biological stimuli (pH, redox, hypoxia, enzyme) and external stimuli (temperature, ultrasounds, magnetic field, radiation, and light) (Valencia-Lazcano et al., 2023). Thus they can eliminate the drawbacks of conventional carriers, such as premature drug release, and control the kinetics of drug release (Soleimani et al., 2021).

NPs that are negatively charged are less likely to be absorbed by the mononuclear phagocytosis system (MPS), which means they can circulate in the blood for longer periods. Particles with positively charged surfaces tend to attach to proteins in the plasma and cause clumping, which the MPS can clear out rapidly. To overcome this problem, a charge-reversal strategy was developed. This involves creating nanocarriers that remain neutral or negatively charged in the bloodstream to extend circulation time. Once they reach the tumor tissue, the charge reversal is triggered by changes in pH, enzymes, light, or temperature. This allows for better penetration into the tumor tissue and improved cell uptake (Chen et al., 2023). The stimuli-responsive DDSs are listed/illustrated in Fig. 3.

3.3.1. Internal stimuli in drug delivery systems

Appropriate nanosystems are capable of pH-responsive drug delivery because of the stark difference in pH levels between healthy and diseased tissues (Ray et al., 2021). pH-responsive polymers usually have ionizable acidic or basic residues that undergo ionization depending on the pH of the solution. Utilizing the fact that tumor tissues have a lower pH than normal tissues, pH-responsive polymers can be used for targeted delivery of chemotherapeutics (Soleimani et al., 2021). In acidic tumor environments, CS is more likely to undergo protonation and become soluble (Zaiki et al., 2023). The idea of redox-responsiveness relies on the distinction in the levels of a reducing agent, like glutathione (GSH), in the extracellular and intracellular environments (Soleimani et al., 2021). The disulfide bond is a type of linkage that can be used as a redox-responsive connector. It can be reduced to a thiol group when exposed to a reducing agent. Various redox-sensitive linkers containing disulfide have been created for building drug delivery carriers. When present in cancer cells, the high level of GSH causes the disulfide bonds to break down, leading to swift drug release (Mollazadeh et al., 2021). Around 50–60 % of solid tumors exhibit a distinct pathological characteristic called “hypoxia”. This happens when the cells located deep within the tumor interior do not receive enough oxygen supply (Kumari et al., 2020). NP-based platforms have great potential for selective drug delivery in low oxygen concentration and highly reducing environments. Bioresponsive NPs use hypoxia to activate hypoxia-responsive NPs or vectors. Although several hypoxia-activated prodrugs (HAPs) are currently undergoing clinical trials, none have produced promising results that would warrant approval for patient treatment (Shahpouri et al., 2023). It is fascinating to note that nanorobots can now serve as drug carriers that move independently to aid in tumor penetration and increase drug accumulation in tumor tissues. As an illustration, natural nanorobots like Escherichia, Clostridium, and Salmonella bacteria can track and colonize in hypoxic tumor tissues (Chen et al., 2023). Stimuli-responsive nanomaterials have been also successfully created through the use of enzymes like proteases, phosphatases, kinases, and oxidoreductases. (Mu et al., 2018). Enzymes are often found in high levels in cancerous and inflamed tissue. To target these diseased areas, drug-loaded NPs can be attached to enzyme-specific ligands, which can improve the effectiveness of the treatment (Gupta et al., 2023).

3.3.2. External stimuli in drug delivery systems

A technique that can be used to increase the amount of drugs that reach a tumor is by locally treating it to increase its vascular permeability. Various methods can be used to achieve this effect, such as microwave, radiofrequency ablation, ultrasound, hyperthermia, radiotherapy, sonoporation, and photodynamic therapy (PDT). These techniques improve the permeability of blood vessels, which allows NPs to penetrate and accumulate more efficiently (Chen et al., 2023).

Temperature-responsive polymers are commonly used to construct smart materials for drug delivery purposes. These polymers change their physical properties when exposed to changes in temperature, making them an effective and safe option for medical applications like hyperthermia, which can kill cancer cells. Temperature change is a valuable stimulus due to its simplicity and effectiveness (Nakayama and Okano, 2011). Thermo-responsive polymers can control and sustain drug delivery through their LCST (lower critical solution temperature) (Sanoj Rejinold et al., 2015). Some of the most commonly used thermo-responsive polymers in drug delivery are N-isopropyl acrylamide (NIPAM), poly(N-isopropylacrylamide-*co*-acrylic acid), and poly(N-isopropylacrylamide-co-*N*-hydroxymethyl acrylamide). These polymers have been extensively studied and utilized (Mustafa et al., 2023). Ruan et al. developed a thermoresponsive nanocarrier that enhances the cytotoxicity of DOX and reverses drug resistance in tumor-bearing mice (Ruan et al., 2022). Targeted ultrasound techniques *e.g.* high-intensity focused ultrasound (HIFU) can destroy nanocarriers filled with medicine at the exact location. However, its effectiveness is limited and absorption could be more consistent, which are significant drawbacks in drug delivery. Safety concerns also restrict the number of ultrasound pulses used in a single session, making a long-lasting release of chemotherapy drugs beneficial for solid tumors. Larger tumors may require more complex and lengthy treatment (Bachu et al., 2021). Magnetic NPs (MNPs) are synthesized from ferrous, nickel, cobalt, and rare earth metals. (Gupta et al., 2023). MNPs offer significant advantages when compared to traditional nanocarriers. Utilizing an internal or external magnetic field, they can be easily guided throughout the body. By applying an alternating magnetic field, they can produce targeted hyperthermia, triggering drug release at the delivery site. Additionally, their ability to be visualized and tracked through magnetic resonance imaging (MRI) makes MNPs indispensable in drug delivery (Al-Rawi et al., 2020). Photosensitivity is not only achieved through photochemical or thermal reactions but also photoisomerization. The applications of photosensitive NPs are vast, particularly in the realm of controlled drug release. These NPs can react to light of specific wavelengths through physical or conformational changes in the delivery system. This photoresponsive DDS has a distinct advantage over other stimuli-responsive formulations due to its ability to react when it receives photons (Pan et al., 2021). The absorption of light (photons) by the photosensitive material triggers a significant change in the shape of the nanocarriers. Consequently, the enclosed substance is released at the intended location, and this process can be controlled with precision over time and space (Sanchis et al., 2019).

Both specific TME conditions and the approaches potentiating onco-specificity of nanosystems, like passive and active targeting and stimuli-responsivity, must be considered when designing new DDSs. The following chapter provides an overview of the currently most important nanoconstructs used for innovative anticancer drug delivery.

3.4. Structural and chemical types of nanoparticulate anticancer drug delivery systems

Nanostructures consist of organic materials such as polymers and lipid-based materials, or inorganic materials such as gold, silver, silica, and carbon. Furthermore, hybrid nanocarriers exploit the benefits of both organic and inorganic materials (Habeeb et al., 2023). Structurally they can be spheres, tubes, micelles, rods, cages, and other diverse configurations, allowing for tailored properties suited to specific drug delivery requirements. Thanks to the advanced techniques used in their creation, NPs can possess qualities such as uniformity, reduced toxicity, manageable distribution, and more (Paramasivam et al., 2021). NPs can also transport a variety of medications, DNAs, RNAs, and imaging agents (Zain et al., 2022). Types of NPs used in anticancer drug delivery are represented in Fig. 4. Recently developed nanoparticulate DDS for anticancer drugs are listed in Table 1 and critically evaluated concerning advantages and limitations of these systems, potential applications (related to a cancer type), and state of the implementation of such system in practice.

3.4.1. Inorganic-based nano-assemblies

The significant attention garnered by the increasing use of inorganic NPs in DDSs can be accepted. With this approach, there is better control of drug release, directing it towards the malignant site (Darroudi et al., 2021). Inorganic nanoparticles have high surface-to-volume ratios, making them ideal for combining treatment and drug delivery applications. They possess unique properties such as optical, electrical, catalytic, and magnetic properties that are not found in organic nanoparticles. Additionally, inorganic nanoparticles can be precisely manufactured to optimize drug delivery, localization, and biodistribution by controlling their size, shape, surface charge, and composition (Amaldoss et al., 2022). The nanocarrier can even be guided using an external magnetic field stimulation (Darroudi et al., 2021). Different kinds of inorganic-based nano-assembled structures, as discussed in the following subsections and their paragraphs, are illustrated in Fig. 5.

Table 1
Structural and chemical types of nanoparticulate anticancer drug delivery systems.

Chemical type of nanoparticulate anticancer DDS	Structural types of nanoparticulate anticancer DDS	Anticancer drug	DDS composition	Advantages of DDS	Limitations of DDS	State of implementation in practice	Potential application	Ref.
Inorganic-Based Nano-Assemblies	Iron oxide NPs	doxorubicin	DOX/EDT-IONPs (doxorubicin-loaded, trimethoxysilylpropyl-ethylenediamine triacetic acid-stabilized iron oxide nanoparticles)	biocompatible, an accelerated release in acidic microenvironments, overcome blood-brain barrier and multidrug resistance, site-specific magnetic targeting	iron oxide nanoparticles show a quenching effect of DOX fluorescence intensity, necessary *in vivo* tests	*in vitro*	brain cancer	(Norouzi et al., 2020)
	Gold NPs	5-fluorouracil	5-FU/G-AuNPs (5-fluorouracil-loaded, guar gum-capped gold nanoparticles)	green synthesis	necessary *in vivo* tests	*in vitro*	pancreatic cancer	(Chinnaiyan et al., 2019)
	Carbon quantum dots	5-fluorouracil	5-FU-CQD (5-fluorouracil-loaded, carbon quantum dots)	good physicochemical properties, photostability, pH-dependence, reduced drug-associated toxicity	necessary *in vivo* tests, exhibits an antitumor effect to MCF-7 cells analogous to free 5-FU	*in vitro*	lung cancer, breast cancer	(Cutrim et al., 2021)
	Carbon nanotubes	5-fluorouracil	5-FU/PEG@OCNT (5-fluorouracil-loaded, polyethylene glycol-coated, oxidized multi-walled carbon nanotubes)	pH-dependence	necessary *in vitro/in vivo* tests	theoretical and molecular insight into the factors that affect the pH-responsive binding	cancer; unspecified	(Solhjoo et al., 2021)
	Fullerenes	doxorubicin, paclitaxel	DOX/TMC@fullerene (doxorubicin-loaded, trimethyl chitosan-coated fullerene) PAX/TMC@fullerene (paclitaxel-loaded, trimethyl chitosan-coated fullerene)	pH-dependence, stable carrier	necessary *in vitro/in vivo* tests	molecular dynamics simulations	cancer; unspecified	(Maleki et al., 2020)
	Silica NPs	doxorubicin, β-elemene	β-elemene-encapsulated in the pore of mesoporous silica nanoparticle, doxorubicin electrostatically adsorbed on the surface of mesoporous silica nanoparticle by hyaluronic acid	synergistic chemotherapy, accumulate in tumor sites, *in vivo* long-term circulation, continuous drug release	further investigation needs to be carried out	*in vitro/in vivo*	esophageal cancer	(Zhan et al., 2020)
Lipid-Based Nanoplatforms	Liposomes	5-fluorouracil	5-FU/CS@L (5-fluorouracil- loaded, chitosan-coated liposomes (chitosomes))	sustained 5-FU release, good physical stability, chitosomes improved the cytotoxicity	necessary *in vivo* tests, increased erythrocyte hemolysis was observed with the chitosome formulations	*in vitro*	colorectal cancer	(Alomrani et al., 2019)
		5-fluorouracil	5-FU/FA-L (5-fluorouracil- loaded, folic acid-decorated liposomes)	active targeting, higher cellular uptake, lower IC50 and higher ROS production than free drug on cancer cells, better tumor inhibition than free drug, blood biocompatibility	further investigation needs to be carried out	*in vitro/in vivo*	colon cancer	(Moghimipour et al., 2018)
		doxorubicin	Doxil® (doxorubicin-loaded, PEGylated liposomes)	prolonged drug circulation time, high and stable remote loading of doxorubicin, passive targeting	higher price	FDA-approved	ovarian cancer, AIDS-related Kaposi's sarcoma, multiple myeloma	(Barenholz, 2012), (D'Angelo et al., 2022)
	Solid lipid NPs	5-fluorouracil	5FU/PEG-SLN (5-fluorouracil-loaded, PEGylated solid lipid nanoparticles)	significantly inhibited tumor growth in comparison to 5-FU	further investigation needs to be carried out	*in vitro/in vivo*	colorectal cancer	(Smith et al., 2020)
	Nanostructured lipid carriers	lapachone, doxorubicin	DOX + Lapa/PEG-NLCs (doxorubicin and lapachone- loaded, PEGylated nanostructured lipid carriers)	increases stability and circulation time	further investigation needs to be carried out	*in vitro/in vivo*	breast cancer	(Li et al., 2018b)

(*continued on next page*)

Table 1 (*continued*)

Chemical type of nanoparticulate anticancer DDS	Structural types of nanoparticulate anticancer DDS	Anticancer drug	DDS composition	Advantages of DDS	Limitations of DDS	State of implementation in practice	Potential application	Ref.
Polymeric Nanoparticles	Nanoemulsions	letrozole	letrozole/PEG-SNE (letrozone-loaded, PEGylated solid nanoemulsion)	technology helps to enhance the solubility of poorly water-soluble drugs	necessary *in vivo* tests	*in vitro*	breast cancer	(Tarik Alhamdany et al., 2021)
	Self-nanoemulsifying DDS	resveratrol	RES/SNDDS (resveratrol-loaded self-nanoemulsifying drug delivery system)	more cytotoxic than resveratrol alone in PANC-1 cells, prevents tumor progression	necessary *in vivo* tests	*in vitro*	pancreatic cancer	(Md et al., 2021a)
	Lipid Nanodiscs	cancer stem cells antigen peptide	synthetic high-density lipoprotein (sHDL) nanodisc vaccine formulated with Sox2, Nanog (cancer stem cells associated transcription factors), and ALDH antigen peptides together with CpG, a Toll-like receptor 9 agonist	immunotherapeutic strategy, reduced tumor growth, extended animal survival without toxicity towards normal stem cells, cost-effective	nanodiscs elicit immune responses to target cancer stem cells (only a small fraction of the cells found in the tumor)	*in vivo*	melanoma	(Aikins et al., 2022)
	Polymeric nanospheres	doxorubicin	DOX/PNIPAM@PAA (doxorubicin-loaded, poly(N-isopropylacrylamide)-coated poly (acrylic acid) nanospheres)	dually temperature/pH-responsive drug delivery system	necessary *in vivo* tests	*in vitro*	breast cancer	(Ghalehkhondabi et al., 2023)
	Nanocapsules	paclitaxel	PTX/nanocapsules (paclitaxel-loaded polymeric nanocapsules)	reduction of IC50 concentration, increased apoptosis of cancer cells	necessary *in vivo* tests	*in vitro*	ovarian cancer	(Ara et al., 2023)
		docetaxel	DTX/PEG@nanocapsules (docetaxel-loaded, polyethylene glycol-coated nanocapsules) or DTX/CS@nanocapsules (docetaxel-loaded, chitosan-coated nanocapsules)	oral chemotherapy, sustained release, significantly higher cytotoxicity than free DTX, significant uptake by gastric and intestinal tissues	further investigation needs to be carried out	*in vitro/in vivo*	gastrointestinal cancer	(Daşkın et al., 2023)
	Dendrimers	doxorubicin	DOX/Apt#RBC@PAMAM (doxorubicin-loaded, Aptamer-grafted, red blood cell membrane-coated dendrimer)	controlled and sustained release, enhanced apoptosis, and significantly elevated uptake by the cancer cells as compared with the non-targeted preparation	further investigation needs to be carried out	*in vitro/in vivo*	breast cancer	(Sheikh et al., 2023)
	Polymeric micelles	paclitaxel	PTX/mPEG-CS-Bio-Hz-Q (paclitaxel-loaded, mPEG, biotin, rhodamine derivative, and hydrazone bonds modified chitosan)	near-infrared fluorescence imaging, active tumor targeting, pH-responsive drug release	further investigation needs to be carried out	*in vitro/in vivo*	cancer; unspecified	(Cheng et al., 2023)
	Polymersomes	doxorubicin	DOX/HA-PBAE polymersome (DOX-loaded, diblock copolymer of hyaluronic acid-b-pPoly (β-amino ester) polymersome)	pH-sensitive, active targeting, effective tumor growth suppression	further investigation needs to be carried out	*in vitro/ex vivo/in vivo*	breast cancer	(Borhaninia et al., 2023)
	Polymer-drug conjugates	methotrexate, 5-fluorouracil	(MTX + 5-FU)*HA (methotrexate and 5-fluorouracil- conjugated hyaluronic acid)	active targeting, high antitumor activity against CD44 receptors, synergistic chemotherapy	low hemolytic levels	*in vitro/ex vivo*	colorectal, liver, and breast cancer	(Shao et al., 2024)
	Nano gels	cisplatin, dasatinib	cisplatin+dasatinib/HA (cisplatin and dasatinib encapsulated hyaluronan nanogel)	facile preparation, active targeting, significantly increased the maximum tolerated dose, significantly less reduction of mice body weight, relieving the renal toxicity	no statistical difference in tumor growth between the formulation and pure drugs	*in vitro/in vivo*	breast cancer	(Liu et al., 2024)

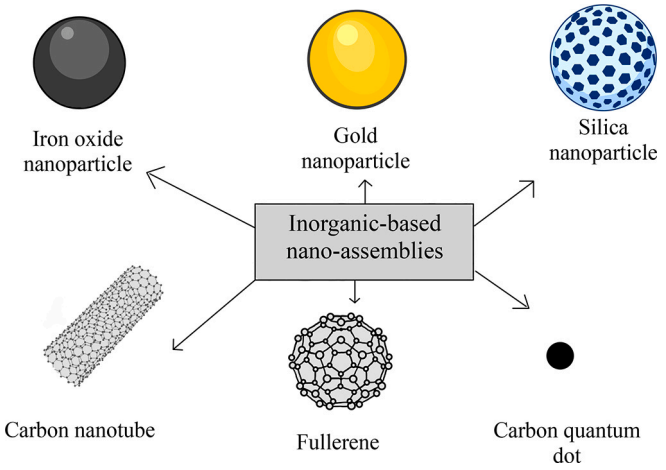

Fig. 5. Different kinds of inorganic-based nano-assembled structures.

3.4.1.1. Metallic nanoparticles. Iron oxide NPs. NPs made of metal oxide have been found to generate reactive oxygen species (ROS), which can inhibit the growth of tumor cells. Among the different types of transition metal oxide NPs, iron oxide is particularly advantageous due to its magnetic properties, varying oxidation state, and cost-effectiveness. By applying a magnetic field externally, magnetic iron oxide NPs can be targeted to the tumor cells. These particles have been deemed safe for clinical use as they are biocompatible, biodegradable, and non-toxic (Pillai et al., 2022). Iron oxide NPs in a spherical shape have been discovered to be extremely useful in treating brain and prostate cancer through magnetic tumor hyperthermia (Pillai et al., 2022). Norouzi et al. (Norouzi et al., 2020) prepared DOX-functionalized iron oxide NPs for targeting glioblastoma. Exploring the compatibility of different inorganic-based designs is necessary because many inorganic precursors have a significant drawback of accumulating and posing safety risks due to poor clearance (Duan et al., 2023). Garcia-Pinel and their team have created a range of hybrid thermoresponsive microgels that contain Fe_3O_4 NPs. These microgels were used to investigate the loading and release of 5-FU and oxaliplatin for drug therapy. The nanoformulations demonstrated high biocompatibility, an exceptional ability to load specific drugs that target colon cancer cells, and a high level of sensitivity to changes in temperature. This allows for increased drug release and improved cytotoxic therapy for this type of tumor (Garcia-Pinel et al., 2020). In 2012, a new form of cell death called ferroptosis was discovered. Ferroptosis is characterized by unrestricted lipid peroxidation (LPO) which ultimately destroys the integrity of the cell membrane and organelles. Fe^{2+} or Fe^{3+} (Fenton's reaction) catalyzes the conversion of intracellular H_2O_2 to a highly reactive radical •OH, which leads to high ROS stress and causes LPO. While cancer cells are resistant to apoptosis and traditional treatments, they are highly susceptible to ferroptosis, making it a promising new method for treating cancer (Li et al., 2023a).

Gold NPs. Scientists are currently focusing on the properties and potential applications of gold NPs (AuNPs) in clinical chemistry, bioimaging, and cancer therapy. Gold NPs are preferred over other nanomaterials due to their unique properties and the ability to produce them in varied sizes and shapes. The different shapes of gold NPs include rods, cages, and tubes, among others. The majority of research on covalent conjugates (anticancer drugs conjugated with gold NPs) has been conducted on spherical gold NPs due to their easy surface chemistry and efficient cellular uptake (Sztandera et al., 2019). Gold NPs can be hidden from the reticuloendothelial system (RES) by surface changes (Owens and Peppas, 2006). To functionalize gold NPs, ligands can be attached through physical adsorption or covalent binding, typically using thiol linkages. PEG is a commonly used compound for functionalizing nanogold, covalently bonded to gold particle surface atoms (Mishra et al., 2016). It is important to note that the distribution of PEG-coated gold NPs is dependent on their size, regardless of any surface modifications (Terentyuk et al., 2009). It has been determined that they have the distinct capability to cross the blood-brain barrier (BBB) based on the size of AuNPs (Schleh et al., 2012). Researchers Chinnaiyan et al. have devised an eco-friendly approach to creating AuNPs and have successfully produced 5-FU-G-AuNPs (5-fluorouracil-guar gum-gold NPs). These 5-FU-G-AuNPs have been shown to have a more significant cytotoxic impact on MiaPaCa-2 pancreatic cancer cell lines than 5-FU alone (Chinnaiyan et al., 2019). Extensive research is currently being conducted on using gold NPs in photothermal and radiofrequency therapy. These particles possess unique properties that allow them to effectively absorb and scatter electromagnetic radiation (Sztandera et al., 2019).

3.4.1.2. Carbon-based nanostructures. Carbon quantum dots (CQDs). Zero-dimensional carbon nanomaterials known as CQDs possess remarkable fluorescence capabilities. CQDs comprise ultra-fine, dispersed, quasi-spherical carbon NPs that are less than 10 nm in size (Yang et al., 2023). Numerous precursors possess robust chemical resistance and multiple surface carboxylic groups that can be employed to produce CQDs. These carboxylic groups render them highly soluble in water and enable the inclusion of organic, polymeric, inorganic, or biological species for additional functionality (Vallejo et al., 2023). CQDs are the ideal option for drug delivery methods when it comes to administering antineoplastic drugs (Palestino et al., 2020). CQDs possess outstanding characteristics such as superb biocompatibility and robust photostability (Barman and Patra, 2018). The nanoconjugates of 5-FU-CQD can enhance the efficacy of tumor treatment by minimizing the harmful impact of free 5-FU on healthy cells. This can decrease unwanted side effects while retaining the therapeutic benefits of the drug (Cutrim et al., 2021).

Carbon nanotubes (CNTs). CNTs are carbon allotropes with a cylindrical shape, composed of coiled graphene sheets with a diameter of less than one μm. Their dimensions are limited to micrometers (Shejawal et al., 2021). Carbon nanotubes (CNTs) come in three classifications: single (SWCNTs), double (DWCNTs), and multi-walled (MWCNTs). These nanotubes have various uses, one of which is acting as nanocarriers to transport anticancer drugs. Functionalized MWCNTs possess desirable qualities such as aqueous solubility, stability, and retention in blood circulation (Díez-Pascual, 2022). Drawbacks are high production costs, poor degradation, and improper *in vivo* studies (Gulati et al., 2020). It has been found that MWCNTs have the potential for pH-sensitive delivery of anticancer drugs. In experiments, it was discovered that 5-FU binds more strongly to the nanocarrier's surface at a pH of 7.4, which is the same as the body's natural pH and is released more readily in acidic conditions with a pH of 5.0. These findings, along with others, can be utilized to conduct further research on using CNTs as drug-delivery systems for anticancer medications (Solhjoo et al., 2021).

Fullerenes. Fullerenes are molecules made up of sp2 hybridized carbon atoms that are either spherical or ellipsoidal. They consist of polyhedral closed cages with a minimum of 20 carbon atoms, comprising 12 pentagonal faces and (n/2–10) hexagonal faces, where 'n' denotes the number of carbon atoms (Dastidar et al., 2022). C60 is the most common fullerene in the synthesized composition. Fullerene has the potential to transport multiple drugs and offer targeted drug delivery, potentially addressing various side effects of chemotherapy (Kazemzadeh and Mozafari, 2019). Researchers have recently developed a successful nanocomplex based on cationic fullerene and siRNA, which is effective for delivering siRNA to the lungs through inhalation. *In vivo* experiments on a mouse model with metastatic melanoma showed that the formulation could effectively inhibit the growth of metastatic lung cancer without any adverse effects or toxicity (Liu et al., 2023). Maleki and his team studied how the CS polymer could be used to functionalize the fullerene carrier. They also added carboxyl groups to the carrier to

enhance the loading and releasing properties of drugs like DOX and PTX. The research revealed that utilizing trimethyl CS (TM CS) and functionalizing the fullerene with carboxyl groups could enhance the biocompatibility of anticancer drugs like DOX and PTX while reducing their side effects (Maleki et al., 2020).

3.4.1.3. Silica NPs. Porous materials can be classified based on the pore size of the NPs. They can be categorized into microporous, mesoporous, and macroporous, having pore sizes less than 2 nm, between 2 and 50 nm, and greater than 50 nm, respectively (Jafari et al., 2019). Inorganic mesoporous silica NPs (MSNs) have been extensively studied for drug delivery due to their properties such as simple and diverse synthesis methods, uniform and controllable pore size, biocompatibility, high loading efficiency, and the existence of a large number of surface silanol groups that allow for easy surface functionalization (Gupta et al., 2023). MSN particles have a negative external surface charge due to the presence of surface silanol groups. This charge makes the particles hydrophilic, which helps in maintaining their colloidal stability. The repulsive surface charges play a crucial role in this regard (Jafari et al., 2019). The size of the pores in MSNs determines which drugs can be effectively absorbed. If a drug molecule is too large to fit through the pores, it will not be absorbed into the mesopores. Therefore, biomacromolecules like proteins and genes with a large molecular weight and volume require larger pores for proper absorption (Tang et al., 2012). Drug-loaded MSNPs tend to release drugs through their pores, which can lead to unwanted side effects on non-target organs. To improve drug delivery to the intended target, it is crucial to block these pores (Xu et al., 2013). Zhan and colleagues reported on creating biocompatible dual-drug loaded complicated mesoporous silica NPs that have a β-elemene encapsulated in the pore of mesoporous silica NPs, and DOX was electrostatically adsorbed on the surface of mesoporous silica NPs. These NPs were tested for their efficacy in treating esophageal cancer and showed promising antitumor effects both *in vitro* and *in vivo* (Zhan et al., 2020).

3.4.2. Lipid-based nanoplatforms

Lipid-based nanocarriers are undoubtedly one of the most versatile options for delivering therapeutic agents and vaccines. They possess numerous advantages, such as high biocompatibility and biodegradability, the ability to encapsulate both hydrophilic and hydrophobic drugs, increased bioavailability and stability of payloads, and surface modifications for targeting effects (Chen et al., 2022). Different kinds of lipid-based nanoplatform structures, as discussed in the following paragraphs of this section, are illustrated in Fig. 6.

3.4.2.1. Liposomes. It has been reported that liposomes are both nontoxic and biocompatible (Puri et al., 2009). They consist of an aqueous center encompassed by a lipid exterior (Yang and Merlin, 2020). Liposomes can integrate both hydrophilic and lipophilic medications (Chaudhari et al., 2020). Liposomes have unique properties that make them a promising candidate for DDS. These properties include high efficiency in drug entrapment, biocompatibility, affordability, and scalability (Mohanty et al., 2020). Liposome improvements have made them more selective, offer longer drug release, and reduce toxicity to healthy tissues. These benefits make them a valuable tool in cancer therapy (Yang and Merlin, 2020). Their use is limited due to their vulnerability to lipid peroxidation, instability, uncontrolled release of medications, and insufficient surface customization (Mohanty et al., 2020). The first generation of liposomes (conventional liposomes) mainly comprise natural phospholipids, sphingolipids, and cholesterol (Akbarzadeh et al., 2013). The challenge with these substances is their stability within the living organism. They tend to merge or clump together, leading to the premature release of the drug and rapid clearance from the body by the mononuclear phagocyte system (MPS) (Allen and Hansen, 1991). The second generation of liposomes incorporates hydrophilic-polymer-modified phospholipids in their outer layer, allowing them to avoid issues with self-fusion or self-aggregation and reducing their chances of being recognized by the MPS. The most common phospholipid modification is PEGylation (Zhang et al., 2016). The first Long-Circulating Liposomes (LCLs) that were well-characterized were the ones that had been PEGylated. Specifically, the liposomal DOX that was PEGylated (dox/PEG-L) had a half-life of circulation that was 2–3 days, which is hundreds of times longer and yielded a delivered drug concentration (in the tumor tissue) up to six times higher, compared to the results obtained using conventional DOX (Gabizon et al., 2016). It is possible to utilize hydrophilic polymers like CS to enhance the exterior of liposomes (Liu et al., 2015). CS, being positively charged, can be coated onto negatively charged liposomes through an ionic interaction (Kumar et al., 2020). The extent of erythrocyte hemolysis was found to increase in proportion to the positive zeta potential. CS-coated liposomes exhibited better cytotoxicity with a sustained effect for 5-FU compared to regular liposomes and 5-FU solution. They also proved to be more efficient in preventing 5-FU leakage than conventional liposomes (Alomrani et al., 2019). A different method involves the use of actively targeting liposomes. These liposomes were coated with FA and filled with 5-FU. The final product, FA-nanoliposomes, exhibited exceptional capabilities for targeting specific cells and improving drug absorption for different types of colorectal cancer cells (Moghimipour et al., 2018). Liposomes can be designed to respond to specific cancer environment triggers by using polymers that are sensitive to pH, temperature, or light. By modifying the surface charge of a liposome, cationic liposomes can be created to have a higher affinity for target cells and enhance uptake. These liposomes are commonly used in cancer gene therapy as a synthetic nano-platform for nucleic acid delivery. To increase the effectiveness of cationic liposomes, they can be coated with PEG to enhance their systemic residence time and improve the delivery of nucleic acids for gene silencing (Yang and Merlin, 2020). The DOX liposomal injection, also known as Doxil®, was developed by Janssen Cilag International as a nanosized DDS. It received clinical approval from the U.S. Food and Drug Administration (FDA) in 1995 for the treatment of Kaposi's sarcoma caused by acquired immune deficiency syndrome (AIDS) (D'Angelo et al., 2022).

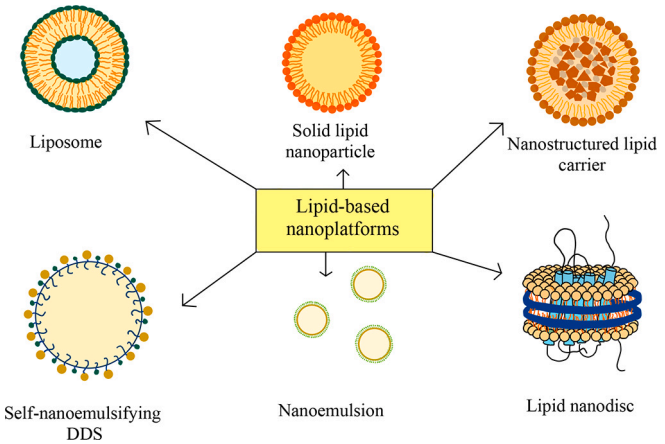

Fig. 6. Different kinds of lipid-based nanoplatform structures.

3.4.2.2. Solid lipid nanoparticles (SLNPs). SLNPs are nanoscopic carriers (ranging from 50 to 1000 nm) made up of a high melting fat matrix. They have been developed to address the limitations of traditional colloidal carriers, such as polymeric NPs and liposomes (Mardhiah Adib et al., 2016). It has been observed that SLNPs possess unique characteristics, including low toxicity, a large surface area, extended drug release, and better cellular uptake than traditional colloidal carriers. Furthermore, SLNPs made from biodegradable and biocompatible materials can effectively enhance drug solubility and bioavailability. Such

SLNPs can incorporate both hydrophilic and lipophilic bioactive substances, making them a promising option for targeted and controlled drug delivery (Mishra et al., 2018). Smith et al. created a smart solid lipid SLNP delivery system to improve the delivery of 5-FU into tumors for treating colorectal cancer. This system can release a high amount of the drug, enhancing *in vitro* activity. The developed formulation showed favorable therapeutic efficacy *in vivo* compared to free 5-FU (Smith et al., 2020).

3.4.2.3. Nanostructured lipid carriers (NLCs). NLCs offer an improvement over SLNPs due to their ability to carry more medication, have a more effective release rate, and maintain stability. This is achieved by combining solid and liquid lipids in their formulations (Nguyen et al., 2022). NLCs are composed of solid and liquid lipids mixed with surfactants that are dispersed in water. Researchers are exploring different methods to functionalize NLCs with targeting ligands and bioimaging markers to improve multimodal cancer therapy. Several investigations are underway to optimize these synthetic methodologies (Rizwanullah et al., 2021). Several studies have compared SLNPs and NLCs, and the findings suggest that NLCs are a superior carrier (Nguyen et al., 2022). NLCs effectively achieve higher load and controlled release for both hydrophilic and hydrophobic therapeutic agents, improving physical stability. Studies have shown that they offer high drug loading, reduced toxicity, enhanced therapeutic efficacy, increased bioavailability, and improved stability of active compounds compared to conventional DDS (Sartaj et al., 2021). One significant benefit of NLCs for delivering chemotherapeutic drugs is their ability to encapsulate multiple drugs with varying physicochemical properties. This is made possible due to the presence of two distinct lipids in the system (Rizwanullah et al., 2021). The study on the formulation of tamoxifen by NLCs revealed that it has a longer systemic circulation time, is absorbed better through lymphatic uptake, and bypasses hepatic metabolism for faster clearance (Sartaj et al., 2021). In the work conducted by Li et al., NLCs were developed to co-deliver Lapa and DOX as a strategy for overcoming MDR in breast cancer therapy. The formulation exhibited heightened DOX retention compared to Lapa-free NLCs when tested in MCF-7 ADR cells. Furthermore, *in vivo* anticancer assays demonstrated a notably augmented efficacy (Li et al., 2018b).

3.4.2.4. Nanoemulsions (NEs). NEs are a type of complex system where oil droplets are dispersed in an aqueous medium and stabilized using emulsifying agents. The droplets in NEs typically range in size from less than 200 nm, with some instances even smaller than 100 nm (Alshahrani, 2022). Some publications call NEs mini-emulsions, ultrafine emulsions, or submicron emulsions. Due to their small size, NEs are known as kinetically stable systems. Brownian motion effects dominate over gravitational forces, making NEs more resistant to droplet aggregation compared to conventional emulsified systems (Karami et al., 2019). Numerous efforts have been made to enhance the effectiveness of therapeutics by utilizing nanoemulsion platforms that improve their solubility and permeability while decreasing P-gp efflux. This aims to increase their oral bioavailability (Gorain et al., 2020). Nanoemulsions possess unique qualities that make them an ideal nanocarrier for delivering chemotherapy drugs. Their natural breakdown, compatibility with the human body, large surface area with small size, clear appearance, non-triggering of immune responses, gradual drug release, ease of creation, and stability under varying temperatures make them highly promising (Alshahrani, 2022). In recent years strategies have been developed to prepare next-generation emulsion nanomedicine with greater efficacy and safety for various cancers (Wilson et al., 2022). There have been successfully created oral nanoemulsion formulations that significantly improve the bioavailability of drugs with limited solubility in water. These formulations were particularly advantageous for chemotherapy drugs, such as PTX (Tarik Alhamdany et al., 2021).

3.4.2.5. Self-nanoemulsifying DDS (SNEDDS). SNEDDS is gaining a lot of attention because of its many advantages. SNEDDS is thermodynamically stable, easy to manufacture, can be scaled up for industrial use, and has the unique property of dissolving both hydrophilic and hydrophobic substances, making it a super-solvent (Kanwal et al., 2021). SNEDDS is a mixture of oil, surfactant, and hydrophilic co-solvent that forms a refined oil in water emulsion when introduced into the aqueous phase. This type of nanocarrier can improve the oral bioavailability of both lipophilic and hydrophilic drugs (Batool et al., 2020). The SNEDDS method is highly effective in enhancing the release profile and bioavailability of various types of physiologically distinct chemotherapeutic drugs (Parveen et al., 2023). The process of SNEDDS involves using nanosized oil droplets to improve the absorption of drugs by cells, resulting in more effective delivery of medication within the cells (Md et al., 2021a). When drugs are encapsulated in SNEDDS nanodroplets, it helps to prevent enzymatic hydrolysis. To make SNEDDS even more permeable across biological membranes, absorption enhancers can be included (Kanwal et al., 2021). Chaudhuri and colleagues have discovered that SNEDDS, which are based on omega-3-fatty acids, could be a highly effective means of enhancing the oral bioavailability of poorly bioavailable drugs, such as DTX (Chaudhuri et al., 2022b). The study in 2021 aimed to develop a SNEDDS formulation of resveratrol for potential use in pancreatic cancer therapy. Capryol 90, Cremophor RH, and Transcutol P were selected as the oil, surfactant, and co-surfactant, respectively. *In vitro* testing revealed rapid release of resveratrol from the resveratrol-SNEDDS, with 90 % release observed within 180 min. The study also demonstrated that resveratrol-SNEDDS exhibited enhanced cytotoxicity compared to resveratrol alone through cell viability studies and changes in mitochondrial membrane potential. Furthermore, the wound scratch method confirmed that resveratrol-SNEDDS significantly inhibited cell migration compared to the pure drug, suggesting that this formulation approach holds promise for addressing therapeutic challenges in pancreatic cancer treatment (Md et al., 2021b).

3.4.2.6. Lipid Nanodiscs. Nanodiscs are NPs shaped like discs, typically less than 50 nm in size. They consist of a lipid membrane and a belt, which is composed of a polymer or peptide that keeps the disc intact (Yang and Merlin, 2020). Irregularly shaped structures have the potential to alter the protein patterns adhering to the surface of the nanocarrier, which can significantly impact the proteins' structure and function, resulting in an entirely different approach to drug delivery within the body. Recent research conducted by Chen et al. has established that lipid nanodiscs can effectively transport drugs to the brain (Chen et al., 2022). Cancer stem cells (CSCs) are a significant factor contributing to unfavorable cancer results. A research team led by Aikins has developed a synthetic high-density lipoprotein (sHDL) nanodisc vaccine. The nanodisc vaccine has proven to be highly effective in reducing tumor growth and significantly extending the lifespan of animals without any harmful effects on normal stem cells. One way to achieve complete tumor regression is through combination therapy, such as using both a nanodisc to target CSCs and a chemotherapeutic to target differentiated tumor cells (Aikins et al., 2022).

3.4.3. Polymeric nanoparticles

The use of polymeric NPs for delivering drugs has been proven to be advantageous in the treatment of cancer. These NPs are made from polymers that are both biocompatible and biodegradable and range in size from 10 to 1000 nm. The drug is either trapped, dissolved, encapsulated, or attached to the NP matrix (Wang et al., 2021a). The structure of these particles varies depending on how they are prepared. If the drugs are contained at the center of the NP shell, they are classified as "nanocapsules". Alternatively, if the drugs are attached to the surface of the matrix, they are known as "nanospheres" (Adeyemi et al., 2023). These designs based on organic materials have numerous benefits,

including outstanding biocompatibility, biodegradability, simple synthesis and modification, the ability to be grafted onto surfaces, and being non-toxic (Duan et al., 2023). Natural polymers are the way to go for drug carriers. They are gaining recognition because of their biocompatibility, biodegradability, low toxicity, availability, and cost-effectiveness when compared to synthetic polymers (Bhatia, 2016). Animals provide materials like chitin (CS precursor), gelatin, collagen, silk, and hyaluronic acid, while plants offer pectin, starch, and cellulose. Similarly, algae provide alginate and agar, and microorganisms provide xanthan gum, dextran, and pullulan (Zaiki et al., 2023). There are also various synthetic polymer-based payloads such as polyvinyl alcohol, polycaprolactone, PEI, PLGA, and polylactic acid (Rehman et al., 2022) increasing the variability of usable polymeric nanoparticles. Different kinds of polymeric nanoparticulate structures, as discussed in the following paragraphs of this section, are illustrated in Fig. 7.

3.4.4. Polymeric nanospheres

Polymeric nanospheres are small, circular particles with uniform size and shape, which can be made from either non-biodegradable or biodegradable polymers (Pourmadadi et al., 2023). Nanospheres are made up of a round polymer matrix that contains a drug. The drug is generally spread evenly throughout the matrix and released into the surroundings through diffusion (Steichen et al., 2013). Nanospheres can range in size from 10 to 200 nm and are utilized as a delivery mechanism for enhancing drug entrapment and release. These minute particles also provide the advantage of evading rapid phagocyte removal while effectively penetrating tissue and cellular gaps (Hosseini et al., 2023). In a recent study, researchers synthesized a dual temperature/pH-responsive DDS with a core-shell structure to control the release of DOX at the target site. For this purpose, poly(acrylic acid) (PAA) nanospheres were synthesized, and then poly(N-isopropyl acrylamide) (PNIPAM) with thermo-responsivity properties was coated on the outer surface of PAA cores. The nanospheres containing drugs showed minimal leakage at neutral pH and body temperature. However, the release of drugs was significantly increased at an acidic pH of 5.5, indicating that the nanospheres respond to the tumor environment. The study found that including DOX in the nanospheres increased its ability to kill cancer cells compared to free DOX (Ghalehkhondabi et al., 2023).

3.4.5. Nanocapsules

Nanocapsules are a type of NP system that is commonly utilized for encapsulating drugs that have problems with solubility and bioavailability (Daşkın et al., 2023). Nanocapsules are composed of core-shell nanoscale structures that are coated with a polymeric layer, effectively trapping therapeutic payloads within their core's free space (Pourmadadi et al., 2023). The nanocapsules exhibit a two-phase release profile, starting with a rapid initial release, followed by a slower release. This gradual release is due to the drug molecules diffusing from the inner oily core to the outer phase (Daşkın et al., 2023). Nanocapsules have a solid/oil core that is more effective in increasing drug-loading efficiency than solid polymeric nanospheres. This also reduces the polymeric matrix content of NPs. Ara et al. have improved the chemotherapeutic activity of the drug PTX by encapsulating it within small polymeric nanocapsules (Ara et al., 2023). DTX-loaded polycaprolactone nanocapsules coated with CS and PEG can be effective in improving oral bioavailability and decreasing the dosing frequency. This approach reduces the occurrence of dose-related side effects and encourages patients to follow their treatment regimen (Daşkın et al., 2023).

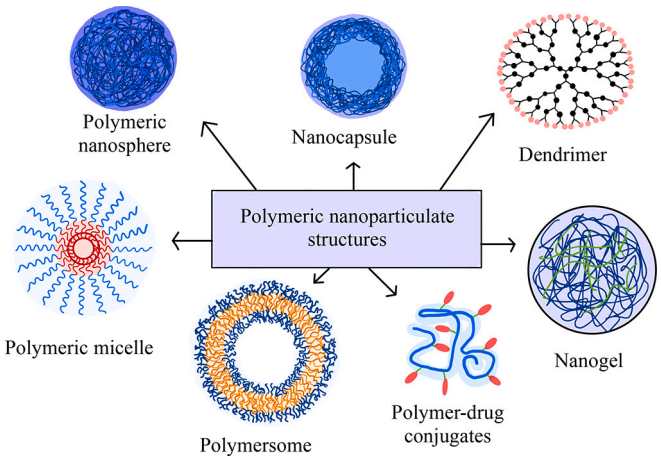

Fig. 7. Different kinds of polymeric nanoparticulate structures.

3.4.6. Dendrimers

Dendrimers possess an intricate molecular structure that is uniform in size and well-defined. Their multifunctional core is capable of effectively encapsulating drugs while providing extensive protection due to the numerous branches they possess (Steichen et al., 2013). In summary, symmetrical branching units are constructed with either a small molecule or a central linear polymer core as the foundation. The arrangement of these materials dramatically impacts their physical and chemical characteristics. The structure and end groups of these units can be customized and engineered as desired, and they can be utilized in various combinations (Hosseini et al., 2023). As the generation increases, the dendrimer's diameter grows linearly while the number of surface groups and molecular weight increases exponentially (Mignani et al., 2020). Scientists have succeeded in designing different classes of dendrimers which are classified as poly(amidoamine) dendrimers (PAMAM), poly (amido amine organosilicon) dendrimers (PAMAMOS), poly (propylene imine) dendrimers (PPI), *etc.* (Singh et al., 2021). When utilizing dendrimers as drug carriers, drug molecules can be enclosed within the dendrimer structure through either covalent encapsulation (a formulation technique) or physical interactions (a nanoconstruct technique) (Pourmadadi et al., 2023). Dendrimers can be effectively employed for accurate drug targeting and to develop delivery systems that react to both internal and external triggers to release a payload upon encountering specific stimuli (Dey et al., 2022). Different dendrimers are created by combining various antineoplastic medications, including DOX, DTX, imatinib, or tamoxifen (Singh et al., 2021). Sheikh et al. developed a targeted nanosystem for triple-negative breast cancer therapy. They used an Aptamer-grafted, red blood cell membrane-coated dendrimer loaded with DOX to target the epithelial cellular adhesion molecule (EpCAM). Red blood cell membranes express the CD47 glycoprotein and are easily taken up by phagocytosis. Coating the membrane by electrostatic interaction on PAMAM dendrimers could improve the pharmacokinetic profile. They observed a superior tumor inhibitory effect over non-targeted therapy, which is due to higher internalization through receptor-mediated endocytosis (Sheikh et al., 2023).

3.4.7. Polymeric micelles (PMs)

Polymeric micellar NPs contain multiple amphiphilic block copolymers (ABCs) that can create NPs through self-assembly. The hydrophobic segments of amphiphilic polymers interact positively to form the micelle core, while the micellar corona comprises the hydrophilic parts of the polymers (Indoria et al., 2020). The micelle's hydrophobic sections establish a core that serves as a hydrophobic drug storage space in polymers and copolymers. Meanwhile, the hydrophilic headgroups produce the micelle's shell. This shell can increase colloidal stability and prevent protein adsorption, resulting in a longer circulation time (de Pachioni-Vasconcelos et al., 2016). The hydrophilic surface of PMs provides a protective shield against non-specific absorption by RES. It is worth noting that PMs are generally smaller than 100 nm in size (Hosseini et al., 2023). PMs offer many benefits for drug delivery, making them a highly attractive option. They have an impressive capacity for drug loading and effectively protect drugs from harsh

environments. With a controlled drug release profile, PMs are an excellent choice for targeted drug delivery. Their physicochemical properties enable passive targeting of cancer cells, making them ideal for tumor targeting. Moreover, PMs can be modified with ligands or pH-sensitive moieties for active targeting, allowing them to match the biological characteristics of the diseased site with precision. In summary, PMs are a promising tool for drug delivery, and their unique properties make them an excellent choice for targeted treatments (Chaudhuri et al., 2022a). The PMs effectively encapsulated and delivered DOX to multiple cancer cell lines both *in vitro* and *in vivo*, resulting in a superior therapeutic outcome compared to using free DOX. A review article by D'Angelo and colleagues highlights the immense potential of DOX-PM nanoformulations as a promising alternative for anticancer therapy (D'Angelo et al., 2022). Genexol®-PM, a copolymeric micelle comprising mPEG-PDLLA (Poly(D, L-Lactic Acid)), has been specifically designed to enhance the water solubility and efficacy of PTX. Its clinical efficacy has been approved in multiple countries, including South Korea, Hungary, and Bulgaria. Currently, it is progressing through phase II of clinical trials in the USA (Chaudhuri et al., 2022a). A new method was developed that involves NIR fluorescence imaging, active targeting of tumors, and drug release that responds to changes in pH. This was achieved by self-assembling a hydrophilic segment of CS and a hydrophobic segment of rhodamine, which formed micelles in an aqueous environment and solubilized PTX in the hydrophobic core. The micelles were shielded by mPEG, allowing them to avoid quick clearance by the RES. Studies conducted *in vivo* showed that the micelles had high antitumor activity and were safe to use. Additionally, the micelles exhibited excellent fluorescence imaging capabilities (Cheng et al., 2023).

3.4.8. Polymersomes

Polymersomes are vesicles that are hollow and made up of amphiphilic copolymers. These vesicles have bilayer membranes as their shells, with hydrophilic polymers on both the inner and outer layers. Lipophilic polymers are placed in between the two hydrophilic layers (Pourmadadi et al., 2023). Using polymerosomes made of biodegradable and stimuli-sensitive block copolymers is crucial for cancer treatment. These carriers are designed to release drugs in response to external stimuli such as ultrasound, light, magnetic field, and temperature, as well as internal stimuli like ions, pH, glucose, and redox reactions (Rawal and Patel, 2019). Polymersomes are frequently used to deliver targeted bioactive biomolecules. They share similarities with liposomes in terms of their shape and how they are made from amphiphilic block copolymers. However, polymersomes offer distinct advantages over liposomes due to their high molecular weight and slower polymer mobility, resulting in a more complex and tangled polymeric membrane. Anticancer drugs can be loaded directly into the reservoir or incorporated into the membrane, depending on their solubility, for delivery to malignant sites. Singh et al.'s review provides information on taxane-loaded polymersomes as a new type of polymeric nanocarrier for cancer therapy (Singh et al., 2022). A pH-sensitive polymersome containing doxorubicin (DOX) was created using a diblock copolymer of hyaluronic acid-b-poly (β-amino ester) (HA-PBAE). This formulation displayed a faster release of DOX in an acidic pH of 5.4. It also exhibited significantly higher cytotoxicity and cellular internalization compared to free DOX when tested against 4 T1 cell line (which is CD44 positive). Furthermore, the polymersome demonstrated greater therapeutic effectiveness, favorable tumor accumulation, and lower systemic toxicity compared to free DOX (Borhaninia et al., 2023).

3.4.9. Polymer-drug conjugates (PDC)

The process of attaching pharmacologically active components to a polymer chain is known as polymer-drug conjugates (PDCs), and it is accomplished through covalent bonding (Javia et al., 2022). The covalent interaction between a biodegradable linker moiety and a drug can alter the drug's physicochemical properties. The success of the nanocarrier platform technology largely depends on the number of polymer-drug conjugates that have undergone clinical evaluation (Rawal and Patel, 2019). Nano-sized particles are ideal for drug delivery due to their ability to reduce antigenicity, biodegradability, and their flexibility to combine with both hydrophobic and hydrophilic drugs. Additionally, their improved pharmacokinetic parameters and specific targeting capabilities make them highly effective candidates for drug delivery (Thakor et al., 2020). The PEGylation technique has been widely used to create PDCs, which can accumulate more effectively in specific tissues through enhanced EPR effects. Additionally, various biodegradable polymers such as hyaluronic acid, chondroitin sulfate, polysialic acid, and dextran have also been studied as potential alternatives to synthetic PDC delivery. According to Javia et al.'s review article, the field of PDC has experienced significant growth over the last two decades, with an increasing number of PDC-based therapeutics being developed for clinical trials and entering the market. However, only traditional chemotherapeutic agents like DOX, PTX, camptothecin, and platinates have been clinically tested in anticancer drug conjugates (Javia et al., 2022). The researchers have developed a new nanodrug system that combines HA with MTX and 5-FU. This system aimed to overcome the toxic side effects associated with these two drugs. The study confirmed the effectiveness of the formulation in suppressing tumors. The nanodrug can effectively target cancer cells due to HA's ability to bind to CD44 receptors on the surface of cancer cells and its EPR effect (Shao et al., 2024).

3.4.10. Nano gels (NGs)

Nanogels are polymer networks that are cross-linked in three dimensions. They possess a large surface area, high mechanical strength, and the ability to trap and release therapeutic agents without any sudden release (Ghaffarlou et al., 2023). NGs utilize proprietary technology that employs hydrophobic polysaccharides to encapsulate and deliver drugs, therapeutic proteins, or vaccine antigens (Madkour, 2019). NGs offer the advantages of both hydrogels and NPs. Due to their porous structure, they can hold more medication than liposomes and polymeric micelles. Moreover, their ability to expand and contract can control the administration of drugs in reaction to changes in the surrounding environment, such as pH, temperature, or ionic strength.(Dastidar et al., 2022). Enhancing the efficacy of drug delivery can be achieved by functionalizing them with various ligands, ultimately leading to more precise and effective results (Iyer and Das, 2021). A review article from 2020 summarizes the use of DOX-loaded composite NGs for cancer treatment. The authors also discuss a study that aimed to use HA as the targeted substance. The NGs were designed to improve the effectiveness of the chemotherapeutic agent and reduce adverse effects on the body in a mouse model of lung cancer (Mohammadi et al., 2020).

Other researchers developed a hyaluronan nanogel that encapsulates cisplatin and dasatinib to improve the effectiveness of combination therapy for triple-negative breast cancer. This was achieved by allowing HA, cisplatin, and dasatinib to self-assemble. While there was no statistical difference observed in tumor inhibition between the nanogel and pure drugs, the formulation resulted in significantly less reduction in the body weight of mice, suggesting that the nanogel may help alleviate the side effects of the treatment (Liu et al., 2024).

4. Chitosan and its derivatives, preparations, properties and use

CS (see Fig. 8) is the only naturally occurring polysaccharide with a positive charge and can dissolve in acidic water-based solutions due to the abundance of free amino groups (Dimassi et al., 2018). CS has a pKa value of around 6,5, thanks to its amine groups. It has three distinct molecular weights: low (less than 50 kDa), medium (50–150 kDa), and high (greater than 150 kDa). As the molecular weight of CS increases, it dissolves less due to the numerous inter and intramolecular hydrogen bonds that form among its chains. The higher the DD% (deacetylation degree), the more will be its solubility in water due to the protonation of more amino groups in the chain. When added to an acidified aqueous

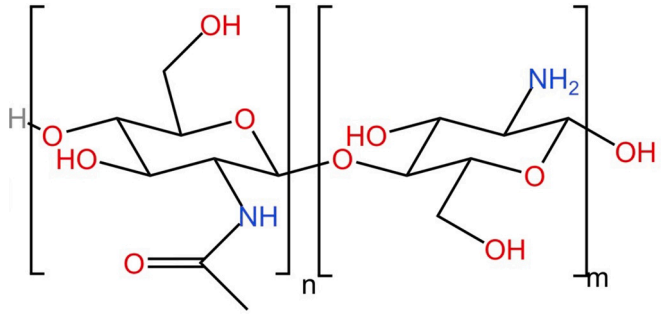

Fig. 8. Chemical structure of chitosan.

solvent, CS creates highly viscous solutions. Even a slight increase in the amount of CS can significantly increase the viscosity (Yadav et al., 2023). CS-based hydrogels are typically classified into covalently crosslinked and non-covalently crosslinked forms. Covalently crosslinked CS hydrogels often utilize crosslinking agents that can be cytotoxic or can have potentially unpredictable impacts on the body. Conversely, non-covalently crosslinked CS hydrogels, known for their enhanced safety and cost-effectiveness, have become a focal point of extensive research, particularly in the area of drug delivery (Xu et al., 2022).

CS is a readily available biopolymer with superior versatility in various biological activities. It is entirely biodegradable and biocompatible and possesses robust antimicrobial properties (Ding et al., 2021). When ammonium groups become positively charged through protonation ($-NH_3^+$), they can interact with the negatively charged phospholipids found in the bacterial membrane. This interaction can lead to bacterial cell wall leakage and block gene and protein transportation. These activities are crucial for bacterial function, making this an effective way to combat bacterial infections (Phuangkaew et al., 2022). CS demonstrates mucoadhesive properties and has been extensively utilized in developing mucoadhesive dosage forms (M. Ways et al., 2018). When the molecular weight increases, the mucoadhesion and permeation-enhancing effect may increase. However, studies show that as the molecular weight of CS increases, its biodegradability, as well as antioxidant and anti-tumor properties, decreases (Del Prado-Audelo et al., 2020). CS has intrinsic characteristics that promote hemostasis, regulate inflammatory cell activity, prevent bacterial contamination, support tissue growth, and stimulate fibroblast proliferation, collagen deposition, and angiogenesis. These properties are advantageous for wound healing (Miguel et al., 2019). It has been reported that CS exhibits antitumor activity by disrupting cell membranes and inducing apoptosis (Keawchaoon and Yoksan, 2011). CS is known for its ability to penetrate tumor cells and hinder their growth. This is achieved through its anti-angiogenic, immunomodulatory, antioxidant defense, apoptosis, and enzymatic modulation properties. When CS with low molecular weight is used, it triggers apoptosis and stops the cancer cells from progressing to the G1/S phase through NF-kB-mediated signaling pathways (Dongsar et al., 2023). In treating gastrointestinal cancers like colorectal and stomach cancers, oral DDSs often utilize CS. CS possesses mucoadhesive and cationic properties that enhance its interaction with mucous membranes, allowing for trans-mucosal drug delivery (Zaiki et al., 2023).

In the field of pharmaceuticals, it serves as a substance that is added to tablets to dilute them. Additionally, it is utilized as a disintegrant, drug carrier, and absorption enhancer. In today's market, there are numerous wound dressings composed of CS available in the form of nonwovens, nanofibers, composites, films, and sponges (Yadav et al., 2023). The ionic nature of CS is utilized to create different drug delivery complexes such as matrix tablets, microparticles, and NPs. Its cationic properties allow it to form polyelectrolyte complexes with other polyionic polymers (Wani et al., 2021). CS as a pH-sensitive biomaterial due to its amino functional groups has been used in smart DDS. Its thermosensitivity causes it to change to solid or semi-solid form at certain body temperatures, gradually releasing drug molecules to the desired targets (Nwabike Amitaye et al., 2024). CS is attractive for tissue engineering and 3D bioprinting, but its high water content makes 3D printing challenging. Modifications are needed to improve printing accuracy. CS is often combined with other polymers like gelatin, cellulose, or polyvinyl alcohol, and a crosslinker is used to induce gelation (Afra et al., 2024). Three-dimensional-printed multifunctional scaffolds, implants, and stents effectively treat localized and recurring tumors. Combining microfluidics with 3D bioprinting creates *in vitro* 3D tumor models replicating the tumor microenvironment (TME), improving drug screening and preclinical investigations. Researchers are now focusing on 4D and 5D bioprinting for designing scaffolds with dynamic morphological attributes (Ruchika Bhardwaj et al., 2024).

The properties of CS useful in medicine and pharmacy are illustrated in Fig. 9.

4.1. Derivatives of Chitosan

It is important to note that unmodified CS applications are severely restricted due to their water insolubility, high viscosity, and tendency to aggregate with proteins at high pH levels (Rostami, 2020). It is a fact that by modifying function groups and using different approaches, CS can be transformed into various structures of different shapes and sizes (Wang and Zhuang, 2022). To enhance the solubility and other characteristics of CS, various derivatives have been synthesized (Mukhtar et al., 2021). The derivatives of CS retain the original properties of CS while also exhibiting new or improved characteristics based on the type of additional functions (Shanmugam et al., 2016). The solubility of CS derivatives can affect the mechanism and intensity of bioactivities, which can vary based on different forms or morphologies. This can impact the efficiency of contact, interaction intensity, and movement of molecules. CS derivatives are occasionally utilized as matrices or modification agents to enhance biocompatibility or decrease the biotoxicity of other materials (Yilmaz Atay, 2020).

There are various ways to chemically modify native CS. Some of these methods include thiolation, phosphorylation, quaternization, blending with other polymers, adding inorganic fillers, and chemical cross-linking. The availability of free amine and hydroxyl groups in the CS skeleton allows for these modifications to take place (Rosli et al., 2022). The characteristics of CS-based hydrogels can be enhanced through covalent conjugation and/or combination with small molecules, other polymers, proteins, nanocomposites, or cells (Aguanell et al., 2022). It is essential to consider that the chemicals utilized in producing derived CS polymer, such as thioglycolic acid, sulfuric acid, HCl, ammonia compounds, and other crosslinkers, can pose a hazard if they are left within the synthesized polymer. Moreover, the use of these chemicals can harm the environment (Mukhtar et al., 2021).

4.1.1. Thiolated Chitosans (TCSs)

Thiolation is a highly effective method for producing functionalized polymers such as CS. This technique employs powerful violating agents that contain thiol groups, enabling the creation of high-quality polymers with numerous potential applications (Ways et al., 2018). Thiolated CS can be created through the covalent bonding of ligands that contain −SH groups, primarily to the polymer's primary amino groups, but also to its hydroxyl groups or both (Leichner et al., 2019). Afterward, the methods can be categorized into two groups depending on how thiol groups are created: either through direct substitution or by interacting with a ligand that carries an SH group (Alkabli, 2022). Thiolation can be accomplished through the utilization of different compounds, such as cysteine, thioglycolic acid, 2-iminothiolane, *N*-acetyl cysteine, and GSH, *via* cross-linking coupling chemistry or mercaptonicotinic acid (M. Ways et al., 2018; Mohammadi et al., 2021). These ligands have thiol groups that may already be present or require further chemical treatment to be

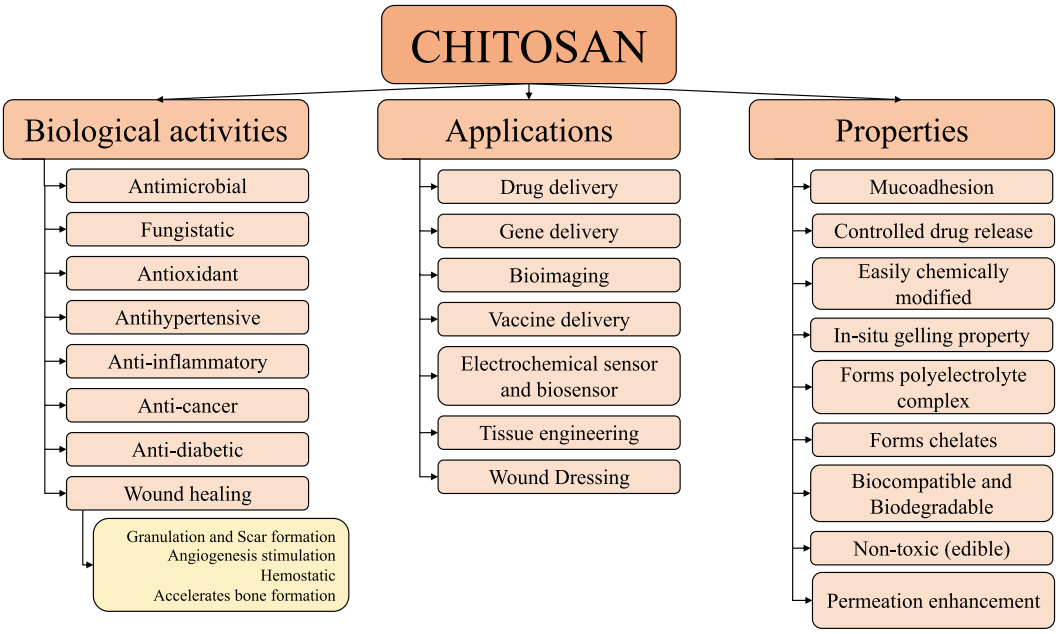

Fig. 9. The properties of CS helpful in medicine and pharmacy.

revealed. Additionally, the −SH groups that are formed can be protected or activated by reacting them with another ligand that also has thiol groups. This protects the free thiol groups from oxidation and enhances their reactivity across a wider pH range (Dünnhaupt et al., 2012). Different options for chitosan thiolation are illustrated in Fig. 10.

Compared to just CS, TCSs have enhanced solubility in water, increased mucoadhesion, and longer contact with mucosal epithelium due to their ability to form a disulfide bond with the intestinal epithelial cell that is enriched with cysteine (M. Ways et al., 2018). Increasing the number of thiol groups that are immobilized can enhance the mucoadhesive properties of a substance. Numerous studies have confirmed the beneficial features of these groups, including adhesion to biological surfaces, the ability to adjust cross-linking and swelling behavior, controlled release of drugs, improved permeation and cellular uptake, inhibition of efflux pumps and enzymes, complexation of metal ions, as well as antioxidative and radical scavenging properties. Additionally, these polymers are biodegradable and not associated with increased toxicity (Federer et al., 2020). TCS derivatives are frequently utilized as DDSs because they offer improved mucoadhesion and permeation, controlled and sustained drug release, and high drug retention capacity

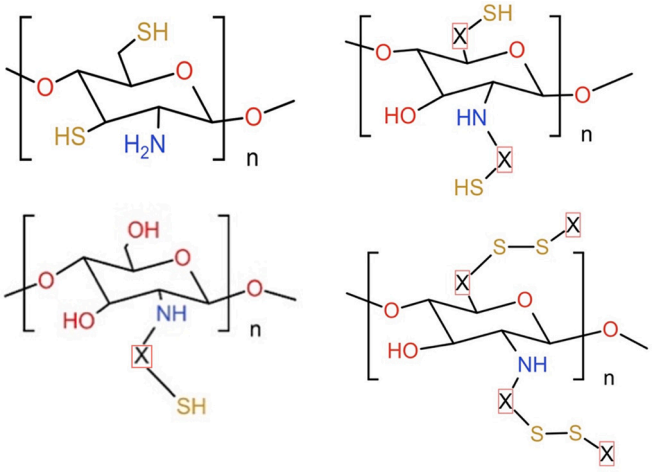

Fig. 10. Different options for chitosan thiolation.

(Yadav et al., 2023). One method to improve the binding affinity of CS to cancer cells is by thiolating it through covalent conjugation with available or activated thiol groups (Zaiki et al., 2023). TCSs were studied as nanoparticulate DDSs or an integral part of nanoparticulate DDSs of anticancer drugs, including doxorubicin, curcumin, docetaxel, alpha-mangostin, resveratrol and cisplatin, that are discussed in detail in the Section 5.6.1.

4.1.2. *Carboxyalkylated Chitosans*

Carboxyalkyl CS, particularly carboxymethyl (CM), is a crucial derivative of CS that is water-soluble and amphoteric. It has immense potential in medical applications due to its excellent water solubility, biocompatibility, biodegradability, and non-toxic nature (Zargar et al., 2015). To obtain CMCSs, one can directly alkylate CS with monochloroacetic acid (MCA) under varying reaction conditions. The selectivity of CS between N and O is influenced by temperature, with room temperature promoting substitution in hydroxyl groups and higher temperatures favoring substitution in the amine group. O-CMCS is considered amphiprotic due to the presence of both carboxymethyl and amino groups within the CS chains (Kono and Kato, 2021). This modification offers several physicochemical benefits, such as enhancing solubility, boosting stability, and removing positive surface groups of CSNPs to prevent binding with negatively charged biomolecules on cell surfaces. Additionally, it improves the binding ability of CSNPs to Ca^{2+} (excluding divalent ions in the extracellular matrix), which is essential for easy paracellular permeability (Khalaf et al., 2023). The cationic properties of N-CMCS are lower and its water solubility is higher compared to CS. N,O-CMCS has properties that fall in between those of O-CMCS and N-CMCS, depending on the degree of substitution (DS), which is the average number of substituent groups present per anhydroglucosamine unit (AGU) (Kono and Kato, 2021). The structures of N-CMCS, O-CMCS, N,O-CMCS, N,N-CMCS are illustrated in Fig. 11.

The field of CM CS has garnered significant attention for its applications in areas such as antimicrobial activity, biosensors, wound healing, the food industry, and bio-imaging (Negm et al., 2020). The process of carboxylation can impede the effective absorption of drugs into cells, leading to a reduction in the drug concentration within the cell. This can pose a significant challenge to the success of tumor therapy (Huo et al., 2020). After 13 days of treatment, the CM CS has a stronger ability to inhibit tumor growth in mice with H22 hepatic carcinoma than

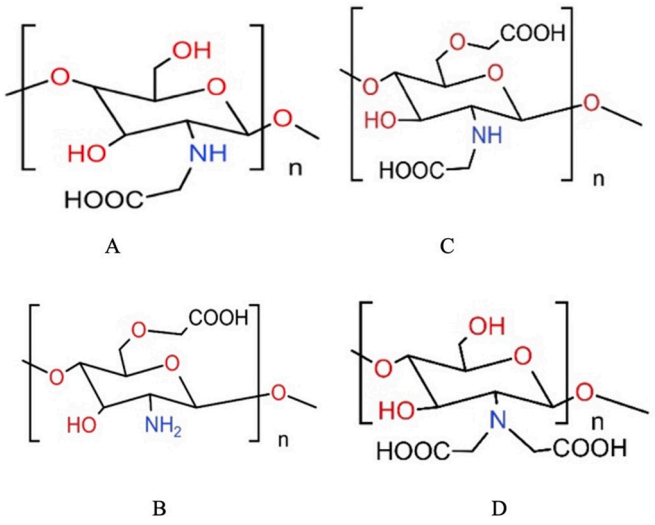

Fig. 11. The structures of N-CM-chitosan (A), O-CM-chitosan (B), N,O-CM-chitosan (C), N,N-CM-chitosan (D).

CS (Zaiki et al., 2023). Caroxyalkyl CSs were studied as nanoparticulate DDSs or an integral part of nanoparticulate DDSs of anticancer drugs, including docetaxel, curcumin, oxaliplatin, resveratrol, cinnamaldehyde and doxorubicin, that are discussed in detail in the Section 5.6.2.

4.1.3. Quaternized Chitosans

When CS is quaternized, it becomes more/permanently positively charged and can interact more effectively with the cell membrane. This interaction is crucial for enhancing local drug release and promoting cellular uptake (Zaiki et al., 2023). To enhance the water solubility of CS, various chemical modifications have been utilized, including the addition of a quaternary ammonium moiety to the CS chain. This method involves reacting CS with quaternized reagents like methyl iodide and glycidyltrimethylammonium chloride (GTMAC) to obtain a quaternized CS (Pakzad et al., 2020).

Studies have demonstrated that quaternization of CS improves the antibacterial properties of this biopolymer by strengthening the electrostatic interaction between positively charged quaternary ammonium groups and negatively charged components within bacterial cells (Cohen et al., 2022). After undergoing quaternization, highly substituted CS loses its ability to form a film (Huang et al., 2016), so it is important to carefully consider the degree of substitution and specific chemical modifications when designing and using chitosan-based materials for a variety of purposes such as drug delivery, wound healing, or tissue engineering. The structures of *N,N,N*-trimethyl ammonium chitosan (A), N-[(2-hydroxy-3-trimethyl ammonium) propyl] chitosan are illustrated in Fig. 12.

N-methylation of amino groups results in the creation of water-soluble *N,N,N*-trimethyl ammonium CS (TMCS), which is ideal for drug delivery purposes (Zaiki et al., 2023). TMCS boasts remarkable solubility at neutral and alkaline pH levels, thanks to the unrelenting positive charge on its amine group (Khalaf et al., 2023). Through amine functionalization using either methyl iodide or formic acid-formaldehyde methylation (Eschweiler-Clarke), TMCS was created with enhanced chemical stability that performs well in various ionic conditions, solubility, biological adsorption, porosity, and non-antigenic properties (Malik et al., 2018). This modification can significantly facilitate the transcellular and paracellular penetration of CSNPs. It is an essential element in novel treatment approaches (Khalaf et al., 2023). Compared to pristine CS, TMCS exhibited more significant antimicrobial activity against the *Staphylococcus aureus* (*S. aureus*) (Sahariah et al., 2019). Quaternized CSs were studied as nanoparticulate DDSs or an integral part of nanoparticulate DDSs of doxorubicin, quercetin, Survivin CRISPR/Cas9expressing plasmid, Survivin shRNA-expressing plasmid and siRNA, that are discussed in detail in the Section 5.6.3.

4.1.4. Glycol Chitosan

GCS (glycol CS) is a derivative of CS that is soluble in water. It is created by attaching ethylene glycol to CS. This process adds glycol groups to the CS, which weakens the hydrogen bonds between the molecular chains. The end result is a polymer that can dissolve in water (Chang et al., 2022). When GCS is altered, its amine groups stay unchanged, preserving the desirable characteristics of CS, including biocompatibility, non-toxicity, biodegradability, and mucoadhesive properties (Sahiner et al., 2023). This has emerged as a promising option for drug delivery and the controlled release of medications (Huang et al., 2022a). GCS polymers have the potential to be an excellent foundation for creating different cell surface probes with desirable traits. This is due to their high water solubility, simple chemical modification, and

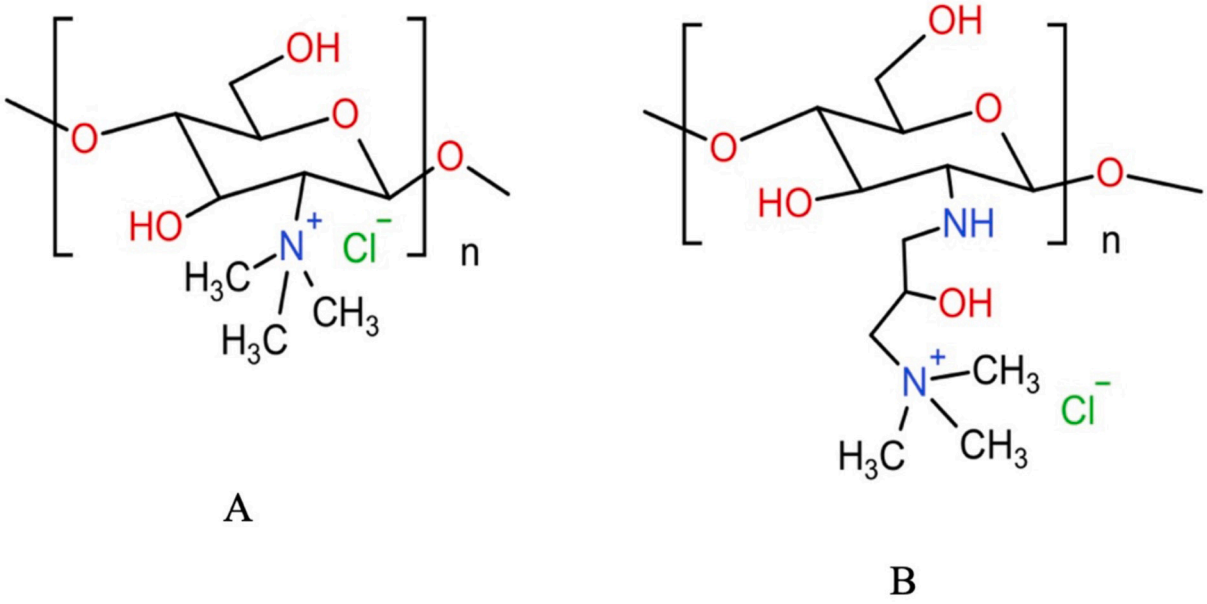

Fig. 12. The structures of *N,N,N*-trimethyl ammonium chitosan (A), N-[(2-hydroxy-3-trimethyl ammonium) propyl] chitosan.

minimal toxicity (Lin et al., 2019). The structure of GCS is illustrated in Fig. 13.

NPs made from GCS have proven to be highly effective in cancer treatment due to their ability to be easily taken up by cells, stability in the body, and compatibility with living tissue. Multiple preclinical studies have demonstrated their exceptional ability to target tumors, making them the ideal choice for delivering various chemotherapy drugs (Song et al., 2023). A groundbreaking type of anticancer prodrugs has been developed by combining the toxic effects of platinum complexes with the drug-carrying properties of GCS polymers. After synthesizing a range of platinum analogs of cisplatin, carboplatin, and oxaliplatin coordinated to dGCS (degraded GCS) polymers with varying molecular weights, researchers were able to produce 15 conjugates with different levels of platinum loading. The study's results unequivocally demonstrate that the conjugation of platinum complexes to dGCS polymers significantly increased their cytotoxicity. One oxaliplatin-dGCS conjugate was further tested in a biodistribution experiment in non-tumor-bearing Balb/C mice, where it exhibited enhanced accumulation in the lungs compared to the free oxaliplatin analog. These promising findings suggest that this new class of drugs holds enormous potential for treating various types of lung cancer and lung metastases (Lerchbammer-Kreith et al., 2023).

Amphiphilic GCS derivatives can be engineered from GCS, which can self-assemble into NPs when combined with hydrophobic molecules in a water (Lin et al., 2019). Glycol CS was studied as nanoparticulate DDS or an integral part of nanoparticulate DDSs of anticancer drugs, including paclitaxel, doxorubicin, celecoxib, and anti-PD-L1 peptide, which are discussed in detail in the Section 5.6.4.

5. Chitosan and its derivatives in nanoparticulate anticancer drug delivery systems

Designs incorporating nanotechnology possess immense potential to tackle various challenges that arise with conventional therapeutic approaches. These challenges include low bioavailability, insufficient targeting capabilities, subpar therapeutic efficacy, indiscriminate distribution, and excessive toxicity (Kankala et al., 2017). CSNPs are considered excellent DDS due to their affordability, biocompatibility, and biodegradability (Dongsar et al., 2023). Thanks to the unique properties of CS, its derivatives, and its composites, it can be utilized to create various forms of DDSs. These include hydrogels, beads, tablets, capsules, NPs, and nanofibers (Yadav et al., 2023). In acidic environments, the presence of amine groups on the CS backbone results in a high concentration of positive charge, which causes a significant increase in attention (Hassanpour et al., 2021). CS can interact effectively with the negatively charged membranes of cancer cells and the endothelial cells of tumor vasculature. This is due to the overexpression of anionic surface moieties such as phospholipids, glycoproteins, and proteoglycans.

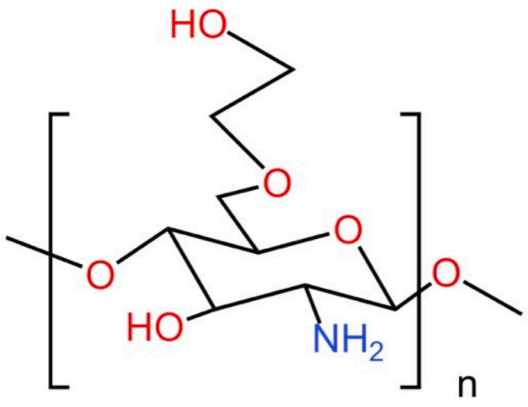

Fig. 13. The structure of glycol chitosan.

However, at a neutral physiological pH, CS has a lower affinity towards normal cells. This suggests that CS is selective in targeting cancer cells (Zaiki et al., 2023) which will also be reflected in CS nanoparticulate systems.

Various techniques are available to produce CSNPs, such as emulsification, crosslinking, complexation with polyelectrolytes, self-assembly, and drying processes (Shoueir et al., 2021). Mikušová and Mikuš (Mikušová and Mikuš, 2021) provide a detailed analysis of the synthesis methods for CSNPs in their review article. When producing CSNPs, cross-linking agents like polyaspartic acid, glutaraldehyde, and tripolyphosphate (TPP) are commonly used. It is important to note that ionic gelation is the most widely researched method and is commonly utilized for creating CSNPs (Hassanpour et al., 2021). It is possible to additionally modify CSNPs *via* attaching a proper ligand (*e.g.* HA, FA, Tf, antibody,*etc.*) to exhibit specific functions towards certain cells. This allows for the targeted delivery of drugs, which can prevent unwanted interactions, increase local drug concentration, and reduce toxicity and side effects (Mazzotta et al., 2020). Numerous studies have been carried out to evaluate the efficacy of CSNPs in providing various forms of treatments (Madamsetty et al., 2022).

The types of CSNPs designed for anticancer drug delivery are illustrated in Fig. 14, and they, as well as their combinations, are discussed in Sections 5.1–5.5 (for DDSs with native CS) and 5.6 (for DDSs with CS derivatives). Recently developed DDS of anticancer drugs with native or derivatized CS are listed in Tables 2 and 3, respectively, and critically evaluated concerning advantages and limitations of particular drug-CSDDS system, potential application (related to a cancer type), and state of the implementation of such system in practice.

5.1. Single polymer CSNPs

CS is a promising substance for delivering drugs in cancer treatment. Moreover, CS itself also can fight against cancer cells and improve the effectiveness of drugs. This shows that CS has great potential in developing anticancer DDSs (Sachdeva et al., 2023). Sun et al. utilized an ionic gelation method to create straightforward CSNPs that sustain the release of 5-FU. This approach solves the issue of 5-FU's short half-life in clinical settings while reducing the side effects resulting from frequent administration. To optimize drug loading and entrapment efficiency, the researchers examined 5-FU and CS at a 1:1 mass ratio. The NPs' drug release profile had two parts: a "sudden release" from the drug adsorbed on the NP surface and a "sustained release" caused by the drug's slow diffusion from the polymer matrix and carrier matrix degradation. *In vitro* cytotoxicity tests demonstrated no significant difference between the 5-FU/CSNPs group and the 5-FU injection group after 24, 48, and 72 h. This outcome may have resulted from incomplete drug release from the NPs. However, the results confirm that creating NPs can prolong drug release time (Sun et al., 2017).

Other scientists have effectively addressed the side effects of treating colorectal cancer by trapping imatinib molecules within the CS polymer through an ionic gelation technique. The formulation with the highest efficacy for entrapment (68.52 ± 0.01 %) contained maximum surfactant and a minor polymeric concentration (3 % mg/mL). Findings suggest that when polymeric concentration is lower, the drug can be more easily trapped due to the higher gelation properties of CS in minimal concentration. Conversely, higher concentrations of CS failed to produce effective entrapment due to increased viscosity in acetic acid aqueous solution, which enhances the crosslinking effect of CS. Following an initial burst effect, the formulation displayed zero-order release kinetics and significantly sustained *in vitro* anticancer activity against CT26 cell lines. In histopathological evaluation, no damage was observed in tissues indicating the final formulation can be safely administered through the i.v. route (Bhattacharya, 2020).

Berberine (BBR) is a natural isoquinoline alkaloid with various pharmacological benefits. However, its use is hindered by various obstacles, such as low absorption rates of bioactive BBR, low site-specific

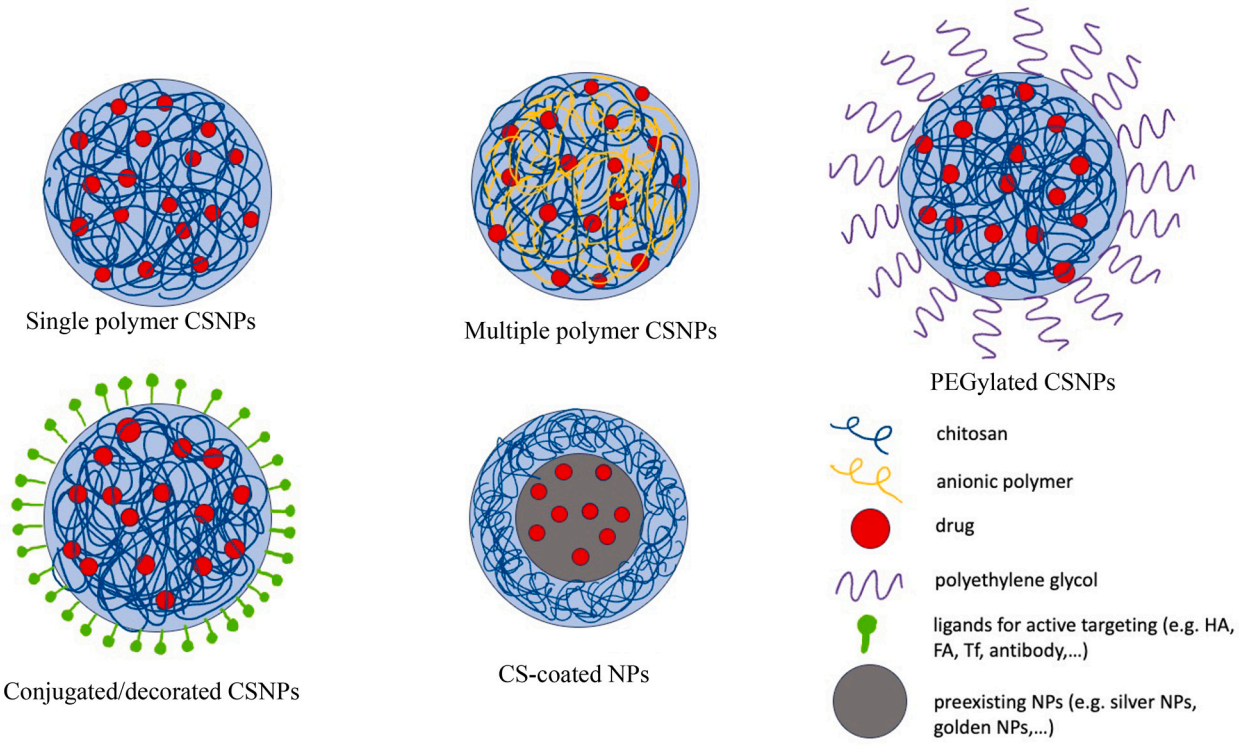

Fig. 14. The types of CSNPs designed for anticancer drug delivery.

delivery, and stability issues. When ingested, only 0.5 % of BBR is absorbed in the small intestine, and this proportion decreases further when it enters systemic circulation. Mahmoud et al. conducted a study to address these issues by synthesizing and characterizing BBR-loaded CSNPs for passive delivery systems. By increasing retention time, the study aimed to evaluate the protective effects of these NPs against urethane-induced lung cancer. The researchers used the ionic cross-linking method for synthesis. Treatment with BBR/CSNPs suppressed lung cancer growth, promoted apoptosis, and inhibited tumor angiogenesis. A photomicrograph of a lung section showing the histological appearance and architecture of alveolar cells with thin interalveolar septa composed of simple squamous epithelial cells after different treatment approaches is shown in Fig. 15. These NPs also protected liver enzyme activities (ALT and AST) and kidney function (urea and creatine). Therefore, BBR/CSNPs have potential applications in oral drug delivery formulations for lung cancer therapy (Mahmoud et al., 2022).

Another study conducted in 2022 found that CSNPs combined with the epigenetic drug, cromolyn, had an anticancer effect against breast cancer. The researchers used an ionic gelation method, utilizing CS as a polymeric nanocarrier and TPP as a chelating agent. The study showed the effectiveness of cromolyn CS NPs compared to plain CSNPs in both *in vitro* and *in vivo* studies (Motawi et al., 2022).

Recent studies have demonstrated that administering chemotherapeutics together enhances their ability to fight cancer. As a solution to the limitations of this approach, researchers have developed a nanocarrier that combines a chemotherapeutic drug with a natural bioflavonoid. The nanocarriers were created through the ionic gelation method, utilizing sodium TPP as a cross-linking agent in a 5:1 ratio. The resulting dual therapeutic CS carrier system displayed significant toxicity against human breast adenosarcoma cells, inhibited cell migration, and generated more ROS than the single carrier system (Radha et al., 2023).

For even more advanced multiple polymer CSNPs, see Section 5.3 PEGylated CSNPs and Section 5.4 Ligand-conjugated/decorated CSNPs.

5.2. Multiple polymer CSNPs

CS is a positively charged polymer that can be utilized to encase drugs with negative charges. This process involves an electrostatic interaction between CS and the drug, which helps to prolong the drug's release. Additionally, CS can be combined with anionic polymers like polyacrylate, HA, alginate (AL), pectin, or carrageenan through ionic coacervation to create dense, stable complexes that can also encapsulate drugs and regulate their release (Zaiki et al., 2023). Studies have shown that incorporating CS and its derivatives into PLGA NPs can result in decreased premature drug leakage, improved drug release based on pH levels, and increased cellular uptake (Lu et al., 2019).

Sohail and Abbas created a substance called AL-CS polyelectrolyte complex (PEC) by using the ionic properties of carboxylic groups of AL and amino groups found in CS, respectively. This process made the complex less porous, which helped to protect the drug inside and slow its release. Through experimentation, they found that they could create stable and effective amygdalin-loaded AL-CSNPs with high drug encapsulation efficiency and good colloidal stability. When tested on cancer cells, these NPs also showed increased cytotoxicity and uptake compared to pure amygdalin (Sohail and Abbas, 2020).

A new nanocarrier for drug delivery has been created by crosslinking CS with mucic acid (MA). This carrier can hold both DOX and quercetin (QUE) simultaneously. NPs containing MA are more effective at encapsulating drugs than pure CSNPs. This new material can also lower the dosage of both drugs significantly (Chaitra et al., 2022).

For even more advanced multiple polymer CSNPs, see Section 5.3 PEGylated CSNPs and Section 5.4 Ligand-conjugated/decorated CSNPs.

5.3. PEGylated CSNPs

Coating CSNPs with PEG can increase their circulation time in the bloodstream and improve the drug delivery approach (Helmi et al., 2021). Khalaf et al. have discovered that PEGylated CSNPs effectively reduce immunogenicity in the human body and can assist in the penetration of the nanocarrier through the EPR effect (Khalaf et al., 2023).

Table 2
CSNPs in drug delivery systems of anticancer drugs.

Type of CS based NP system	Anticancer drug	DDS composition	Advantages of DDS	Limitations of DDS	State of implementation in practice	Potential application	Ref.
Single polymer CSNPs	5-fluorouracil	5-FU/CSNPs (5-fluorouracil-loaded, chitosan nanoparticles)	sustained release, drug loading was higher than that of the previous reports, good stability	encapsulation efficiency was not high, the release of drug from nanoparticles was incomplete, the same inhibitory effect as the 5-FU injection	*in vitro/in vivo*	gastric cancer	(Sun et al., 2017)
	imatinib	IMT/CSNPs (imatinib-loaded, chitosan nanoparticles)	sustained release, harmless to RBC (red blood cells) membrane integrity, less hemolysis compared to IMT, less toxicity as compared to the drug	minor variations of the critical quality attributes after 6 months of studies	*in vitro/ex vivo/ invivo*	colorectal cancer	(Bhattacharya, 2020)
	berberine	BBR/CSNPs (berberine-loaded, chitosan nanoparticles)	inhibited tumor angiogenesis; oral administration formulations; more efficient than raw BBR		*in vivo*	lung cancer	(Mahmoud et al., 2022)
	cromolyn	cromolyn/CSNPs (cromolyn-loaded, chitosan nanoparticles)	treatment with cromolyn CSNPs significantly reduced tumor growth by 71 % compared with those in the control and plain CSNPs groups; epigenetic approach	further studies are needed; the effect of cromolyn alone wasn't investigated in this study	*in vitro/in vivo*	breast cancer	(Motawi et al., 2022)
Multiple polymer CSNPs	5-fluorouracil; hesperidin	Hesp+5Fu/CSNPs (5-fluorouracil and hesperidin-loaded, chitosan nanoparticles)	co-delivery system; synergistic role; slow and sustained dual drug release	necessary *in vivo* tests	*in vitro*	breast cancer	(Radha et al., 2023)
	amygdalin	amygdalin/A + CSNPs (amygdalin-loaded, alginate–chitosan nanoparticles)	sustained drug release; mucoadhesion; pH-dependence	necessary *in vivo* tests	*in vitro*	lung cancer	(Sohail and Abbas, 2020)
	doxorubicin; quercetin	DOX + QUE/CS NPs ~ MA (doxorubicin and quercetin-loaded, chitosan nanoparticles, cross-linked by mucic acid)	co-delivery system	necessary *in vivo* tests	*in silico/in vitro*	colon cancer	(Chaitra et al., 2022)
	methotrexate	MTX/mPEG@CSNPs (methotrexate-loaded, methoxy polyethylene glycol-coated chitosan nanoparticles)	enhanced blood circulation time in the body and decreased accumulation in the liver, spleen, and lung		*in vitro/in vivo*	undefined	(Ait Bachir et al., 2018)
PEGylated CSNPs	doxorubicin	DOX/PEG CS + PLGANPs (doxorubicin-loaded, PEGylated chitosan+PLGA nanoparticles)	pH-responsive hybrid nanoparticles; hydrophobic hybrid core surrounded by hydrophilic shells; appreciably suppressed TRAMP-C1 tumor growth compared to free hydrophobic DOX and non-responsive NPs	more *in vivo* studies needed	*in vitro/in vivo*	prostate cancer	(Huang et al., 2022b)
Conjugated/ decorated CSNPs	nano selenium; doxorubicin	Se + DOX/PEG@CSNPs (nano selenium and DOX-loaded, polyethylene glycol-coated chitosan nanoparticles)	co-delivery system; pH-dependent behavior; improved hydrophilicity	necessary *in vivo* tests	*in vitro*	breast cancer	(Li et al., 2023b)
	cytarabine	CTR/FA*CSNPs (cytarabine-loaded, folate-conjugated chitosan nanoparticles)	improved cytotoxicity in MCF-7 cells; a significant decrease in cell viability by FCCNP treatment than free cytarabine	cytarabine does not show cytotoxicity in A-549 cell lines in the studied concentration range	*in vitro*	breast cancer	(Geethakumari et al., 2022)
	siRNA	siRNA/HAD*CSNPs (siRNA-loaded, hyaluronic acid dialdehyde-conjugated chitosan nanoparticles)	simple preparation; high biological safety, good stability, good blood compatibility		*in vitro/in vivo*	bladder cancer	(Liang et al., 2021)
	docetaxel	DTX/FA + CTXmab*CSNPs (docetaxel-loaded, folate and	dual-receptor (folate and EGFR-epidermal growth		*in vitro/in vivo*	lung cancer	(Vikas et al., 2021)

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Table 2 (*continued*)

Type of CS based NP system	Anticancer drug	DDS composition	Advantages of DDS	Limitations of DDS	State of implementation in practice	Potential application	Ref.
		cetuximab-conjugated chitosan nanoparticles) CZT/FA + CTXmab*CS + ANPs (cabazitaxel-loaded, folate and cetuximab-conjugated chitosan-alginate nanoparticles)	factor receptor) targeted chitosan nanoparticles dual-receptor targeted CSA nanoparticles; enhanced anticancer efficacy of CZT with a prolonged survival rate				
	cabazitaxel				*in vitro/in vivo*	lung cancer	(Vikas et al., 2022)
	doxorubicin	DOX/Anti-hMAM*PEG@CSNPs (doxorubicin-loaded, anti-human mammaglobin-conjugated, polyethylene glycol-coated chitosan nanoparticles); DOX/Anti-HER2*PEG@CS NPs (doxorubicin-loaded, anti-human epidermal growth factor-conjugated, polyethylene glycol-coated chitosan nanoparticles)	sustained release; enhanced blood circulation time; high drug selectivity	*in vivo* studies needed	*in vitro*	breast cancer	(Helmi et al., 2021)
	5-fluorouracil	5-FU/CeO2 NPs-CSNPs (5-fluorouracil-loaded, cerium oxide nanoparticles decorated chitosan nanoparticles)	CeO2 NPs and CS-5FU NPs combined strategy notably enhanced the anticancer activity in HepG2 cells compared to treatment alone; enhanced the apoptosis in HepG2 cells; considerably inhibited DPPH and ABTS free radicals	necessary *in vivo* tests	*in vitro*	HepG2 cells	(Sathiyaseelan et al., 2022)
	doxorubicin	DOX/Ce6-CSNPs (doxorubicin-loaded, Chlorin e6 decorated chitosan nanoparticles)	photo-controlled smart DOX delivery system; sustained release	necessary *in vivo* tests	*in vitro*	breast cancer	(Bhatta et al., 2019)
	curcumin	CUR/CSNPs*MCM*(Au*Apt) (curcumin-loaded chitosan nanoparticles, conjugated with mesoporous silica conjugated with a conjugate of gold nanoparticles and aptamer)	"on/off" fluorescence biosensor, controlled and sustained release of curcumin	*in vivo* studies are needed; the threshold concentration of the aptamer should be optimized to form the highly sensitive biosensor; determine the effect of the introduced nanosystem on blood components	*in vitro*	MUC-1 positive tumor cells	(Esmaeili et al., 2022)
	nisin; 5-fluorouracil	5-FU/+NS*CS@AgNPs (5-fluorouracil-loaded and nisin-conjugated chitosan-coated silver nanoparticles)	effective drug peptide nano-combination; reduction of 79.37 and 6.66-fold in the required effective doses of nisin and 5-FU respectively	the anticancer role of chitosan cannot be ignored	*in vivo*	murine skin cancer	(Rana et al., 2022)
CS-coated NPs	paclitaxel	PTX/CS@IONPs~METAC (paclitaxel-loaded, chitosan-coated iron oxide nanoparticles, cross-linked by (methacryloyloxy) ethyl] trimethyl ammonium chloride polymer)	hydrophobic drug delivery; a new polymeric material; comparing the efficiency of the CS-IONPs-METAC with similar materials from literature hemocompatibility		*in vitro/in vivo*	breast cancer	(Manjusha et al., 2023)
	methotrexate	MTX/CS#PNIPAAm@Fe_3O_4NPs (methotrexate-loaded, chitosan-grafted poly(N-isopropylacryl amide)-coated iron oxide nanoparticles)	triple-responsive nanocarrier (pH, temperature, and magnetic field as stimuli); enhanced antitumor activity of MTX loaded on nanocarrier against A549 and MCF7 compared to free MTX	*in vivo* studies needed	*in vitro*	lung and breast cancer	(Moradi et al., 2022)

Table 3
NPs of CS derivatives in anticancer drug delivery systems.

Type of CS derivative in NP system	Anticancer drug	DDS composition	Advantages of DDS	Limitations of DDS	State of implementation in practice	Potential application	Ref.
Thiolated chitosan	doxorubicin, curcumin	Single polymer TCSNPs: DOX + CUR/CS + SANPs (doxorubicin+curcumin-loaded, thiolated chitosan-stearic acid-crosslinked nanoparticles)	for dual drug delivery; higher cancer cell killing efficiency in comparison to each free drug		*in vivo*	colorectal cancer	(Sood et al., 2022)
	docetaxel	Ligand-decorated TCSNPs: DTX/FA-TCSNPs (docetaxel-loaded, folic acid-decorated thiolated chitosan nanoparticles)	active targeting, improved mucoadhesion, remarkable enhancement of cellular uptake, carrier for oral drug delivery	necessary *in vivo* tests	*in vitro/ex vivo*	breast cancer	(Sajjad et al., 2019)
	docetaxel	Ligand-conjugated TCS' coated NCPs: DTX/FA*TCS@Ag NCPs (docetaxel-loaded, folic acid-conjugated with thiolated chitosan-coated silver nanocapsules)	oral administration, active targeting, sustained release, 6 folds higher half-life, and 9 folds higher bioavailability as compared to DTX suspension		*in vitro/in vivo*	breast cancer	(Sohail et al., 2018)
	alpha-mangostin, resveratrol	Coated TCSNPs: M + R/S@TCSNPs (alpha-mangostin and resveratrol-loaded, Eudragit® S100-coated thiolated chitosan nanoparticles)	pH-dependence, colon targeting, mucoadhesive delivery, synergistic activity of drugs	necessary *in vivo* tests	*in vitro*	colon cancer	(Samprasit et al., 2022)
	cisplatin	Ligand-decorated TCSNPs: Cis/HA-TCSNPs (cisplatin-loaded, hyaluronic acid-decorated thiolated chitosan nanoparticles)	active targeting, sustained release for up to 72 h, reduced off-target toxicity	necessary *in vivo* tests	*in vitro*	cervical cancer	(Kousar et al., 2023)
Carboxymethyl chitosan	docetaxel, curcumin	Ligand decorated CMCSNPs: DTX + CUR/T7 + BAPE-CMCSNPs (docetaxel and curcumin-loaded, T7 peptide and polyoxyethylene polyoxypropylene amine-decorated carboxymethyl chitosan nanoparticles)	T7-targeting-based pH/ROS dual-responsive nanocarrier	necessary to study their long-term safety and effectiveness in patients	*in vitro/in vivo*	lung cancer	(Zhu et al., 2021)
	oxaliplatin, resveratrol	Single polymer CMCSNPs: OXE/CMCSNPs (oxaliplatin-loaded, carboxymethyl chitosan nanoparticles), RES/CMCS NPs (resveratrol-loaded, carboxymethyl chitosan nanoparticles)	oxaliplatin combined with natural compounds; sustained release		*in vitro/in vivo*	colorectal cancer	(Wang et al., 2021b)
	doxorubicin, Tanshinone IIA	Single polymer CMCSNPs: DOX + TSIIA/CMCSNPs (doxorubicin and Tanshinone IIA loaded, carboxymethyl chitosan nanoparticles)	hypoxia-responsive nanoparticles, sustained release, for dual drug delivery		*in vitro/in vivo*	breast cancer	(Lu et al., 2023)
Quaternized chitosan	doxorubicin, Survivin CRISPR/Cas9expressing plasmid, or doxorubicin, Survivin shRNA-expressing plasmid	Ligand-conjugated TMCSNPs: DOX + sgSurvivin pDNA or DOX + iSur pDNA/FA + DPA*TMCSNPs (doxorubicin and Survivin CRISPR/Cas9expressing plasmid-or doxorubicin and Survivin shRNA-expressing plasmid loaded, folate and 2-(Diisopropylamino) ethyl methacrylate-conjugated trimethyl ammonium chitosan nanoparticles)	cooperative effect of a combination of chemotherapy and gene therapy		*in vitro/in vivo*	breast cancer	(Li et al., 2022)
	doxorubicin, siRNA	Ligand-conjugated TMCSNPs: DOX + siRNA/FA + CMβC*TMCSNPs (doxorubicin and siRNA-loaded, folate and carboxymethyl-β-cyclodextrin-conjugated trimethyl ammonium chitosan nanoparticles	effective protection of siRNA-survivin from degradation of serum RNAase for a long time, pH-dependent controlled sustained release, improved the efficacy of antitumor drugs	necessary *in vivo* tests	*in vitro*	lung cancer	(Zhang et al., 2021)
	doxorubicin, quercetin	Coated TMCSNPs: DOX + QUE/PEG@TMCSNPs (doxorubicin and quercetin-loaded, polyethylene glycol-coated	stimulus-responsive NPs, combinational therapy,		*in vitro/in vivo*	liver cancer	(Liu et al., 2021)

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Table 3 (*continued*)

Type of CS derivative in NP system	Anticancer drug	DDS composition	Advantages of DDS	Limitations of DDS	State of implementation in practice	Potential application	Ref.
		trimethyl ammonium chitosan nanoparticles)					
	paclitaxel	Ligand-conjugated, multiple polymer GCSNPs: PTX/ES*GCS + PDPANPs (paclitaxel-loaded, estrone-conjugated glycol chitosan and Poly(2-(diisopropylamino)ethyl methacrylate) nanoparticles)	active targeting, increased cellular uptake, enhanced tumor distribution, pH-responsive properties		*in vitro/in vivo*	breast cancer	(Yang et al., 2018)
Glycol chitosan	doxorubicin, celecoxib	Ligand-conjugated single polymer GCSNPs: CXB/HA + DOX*GCSNPs (celecoxib-loaded, hyaluronic acid and doxorubicin-conjugated glycol chitosan nanoparticles)	active targeting, pH-responsive properties, dramatically inhibited tumor growth, suppressed inflammation and metastasis-related gene/protein		*in vitro/in vivo*	lung cancer	(Lee et al., 2020)
	doxorubicin, anti-PD-L1 peptide	Ligand-conjugated single polymer GCSNPs: DOX/PP*GCSNPs (doxorubicin-loaded, anti-PD-L1 peptide conjugated glycol chitosan nanoparticles)	effective synergistic immunotherapy, complete tumor regression, efficiently minimize systemic toxicity *via* high tumor targeting, delayed drug release profiles		*in vitro/in vivo*	colon/lung cancer	(Song et al., 2023)

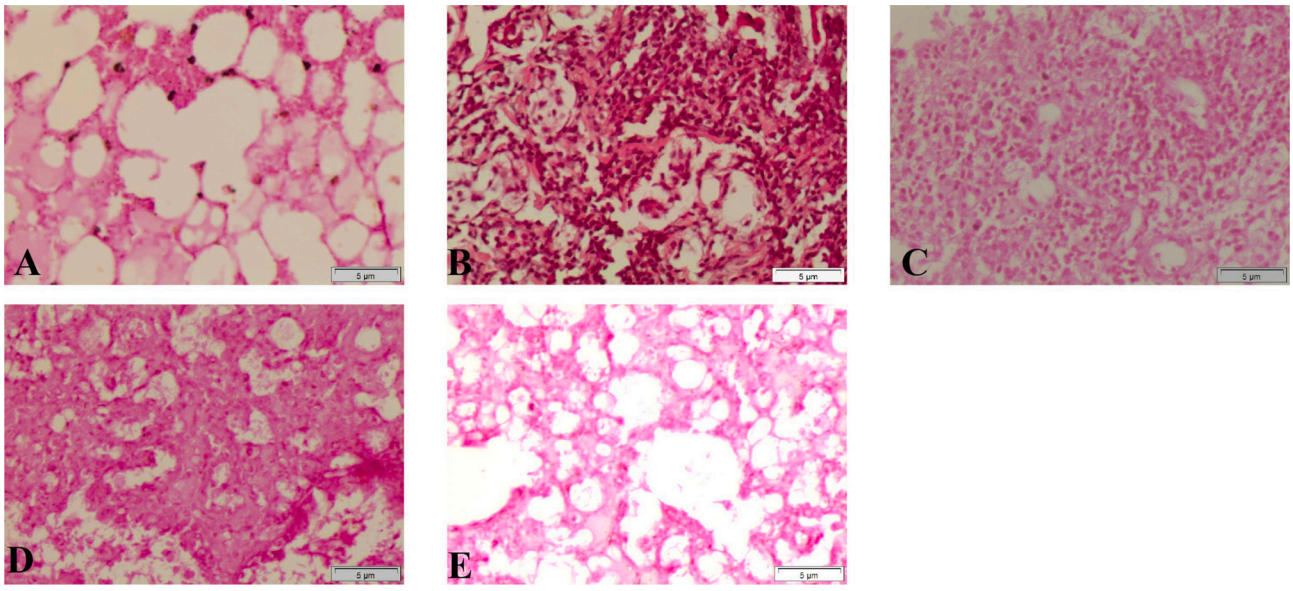

Fig. 15. Photomicrograph of a lung section showing (A) the histological appearance and architecture of alveolar cells with thin interalveolar septa composed of simple squamous epithelial cells in control mice. (B) Mice treated with urethane showed invasion islands of alveolar adenoma in solid structures. The cells were round with stained cytoplasm, loosely defined cell boundaries, and moderately differentiated squamous cell carcinoma was noticed. (C) Animals that were given urethane displayed alveolar adenomas with a solid pattern, which were demarcated from the surrounding parenchyma. The adjacent alveoli showed collapse and compression. A mass of inflammatory cells infiltrated the lung and caused serious damage. (D) Animals that were given urethane and then treated with BBR showed a partial alleviation of lung structure degradation, along with mild aggregations and areas of inflammatory cellular infiltration in the alveolar spaces. (E) After being administered urethane, animals treated with BBR/CSNPs showed significant improvement in lung structure degradation (Mahmoud et al., 2022).

Formulations of CSNPs that have longer PEG chains or higher PEG surface density exhibited drug release that is more sustainable compared to the control formulations (Papadimitriou et al., 2012). A recent study examined the impact of different levels of mPEG (methoxy polyethylene glycol) surface densities and chain lengths on mPEG-coated CSNPs. These NPs can avoid being eliminated by the RES and can therefore remain in the bloodstream for a more extended period. The study used TPP as a cross-linking agent and MTX as an anticancer drug model to create the mPEG-coated CSNPs *via* an ionic gelation method. The researchers discovered that as the mPEG MW and mPEG surface density increased, the AUC of MTX also increased while MTX accumulation in the liver, spleen, and lung decreased. These findings indicate that the

PEG MW and mPEG surface density are crucial factors in enhancing blood circulation time and improving biodistribution (Ait Bachir et al., 2018).

PLGA NPs have frequently been modified using CS. PEGylated CS and PLGA were co-assembled to form pH-responsive nanovehicles. DOX-loaded hybrid NPs were prepared by the single-step nanoprecipitation method. The stability of the formulation in the serum-containing water phase was maintained while also achieving faster drug release in response to pH changes. The results of *in vitro* cellular uptake and cytotoxicity studies showed that, compared to non-pH-sensitive formulation, the pH-responsive NPs rapidly released the drug after being endocytosed by TRAMP-C1 cells. The preliminary *in vivo* findings showed that the DOX/PEG CS + PLGA NPs had no adverse reaction to normal tissues (Huang et al., 2022b).

Studies on the application of CS-sodium TPP NPs produced through ionotropic gelation as drug carriers have revealed potential instability under simulated physiological conditions with a pH range of 7.2–7.4 (Echeverri-Cuartas et al., 2020). Li et al. have achieved an exceptional feat by developing an innovative delivery system that combines DOX and nano selenium, utilizing a PEGylated CS-based co-delivery technique. The PEGylated NPs formed through a one-pot co-precipitation process have been proven to be highly stable in a physiological environment. This breakthrough discovery implies that the DDS in the bloodstream could be significantly more stable, resulting in a potential reduction of side effects and improved bioavailability of DOX. The co-delivery system demonstrated rapid drug release at pH 5.0, and PEGylation enhanced this process by increasing the solubility of CS and improving the hydrophilicity of DDS. *In vitro* cell experiments confirmed the co-delivery system's anti-cancer efficacy and intracellular delivery capability. These findings showcase the revolutionary potential of this co-delivery system in cancer treatment (Li et al., 2023b), and we eagerly anticipate further progress in this field.

For even more advanced PEGylated CSNPs see Section 5.4 Ligand-conjugated/decorated CSNPs.

5.4. Ligand-conjugated/decorated CSNPs

Active targeting reduces the non-specific effects of passive targeting by binding specifically to overexpressed receptors on cancer cells (Bae and Park, 2011). The study of cancer biology has led to the advancement of chemotherapy, which now involves targeting growth factor receptors instead of identifying new toxic agents. One cost-effective and simple method involves decorating CSNPs with folic acid (Esfandiarpour-Boroujeni et al., 2017). Tumor cells have a significantly higher level of folate receptor (FR) expression, approximately 100–300 times more than normal cells (Ross et al., 1994). Geethakumari et al. designed cytarabine (CTR)-loaded, FA-conjugated CSNPs (CTR/FA*CSNPs) for targeted delivery of CTR in breast adenocarcinoma cell lines by making use of the overexpressed folate receptors on the surface of human breast adenocarcinoma cell lines (MCF-7). Folate was conjugated to CS using carbodiimide. Cytotoxicity studies showed a significantly decreased cell viability by CTR/FA*CSNPs treatment than free cytarabine. They confirm that folate-conjugated CSNPs improved the cytotoxicity of CTR in MCF-7 (Geethakumari et al., 2022).

Liang et al. have discovered that HA can target CD44, making it an effective coating for CSNPs. In a recent study, a gene-drug delivery nanosystem was developed using a targeted approach. To avoid cytotoxicity from additional crosslinker, hyaluronic acid dialdehyde (HAD) was directly bound to CS's amine groups to attach HA to the NP surface. The resulting nanosystem had good stability, high siRNA encapsulation ability, low cytotoxicity, and good blood compatibility due to loading a novel siRNA sequence and CD44-targeting (Liang et al., 2021).

In lung cancers, FR and epidermal growth factor receptors (EGFR) are present at high levels. They can be effectively targeted with dual receptor-targeted nanomedicine to provide better anti-cancer treatment. Vikas et al. developed dual receptor-targeted CS NPs for effective lung cancer therapy (*e.g.*, pre-conjugation for folic acid targeting and post-conjugation for cetuximab targeting). The IC50 value of DTX-loaded, FA, and cetuximab-conjugated CSNPs (DTX/FA + CTXmab*CSNPs) was found to be 34 times lesser than that of DTX control (Vikas et al., 2021). The combination of Na-TPP and Na-alginate was used as the crosslinking agents to prepare CS + ALNPs. The polyelectrolyte complex of the CS + ALNPs overcomes the shortcomings of CS, including poor mechanical strength and stability at lower pH. The dual-receptor NPs were again developed using the preconjugation technique described in the previous study. The cellular-uptake study performed on A-549 cells demonstrated a significant enhancement in the uptake of cabazitaxel-loaded, FA, and cetuximab-conjugated CS + ALNPs than non-targeted and single-receptor targeted CS + ALNPs (Vikas et al., 2022).

To ensure that DOX is released in a targeted and consistent manner in the breast TME, modified CSNPs were created using PEG to improve their circulation in the bloodstream. Schematic illustration of the final mAb functionalized DOX-loaded PEGylated CSNPs are illustrated in Fig. 16. To ensure that the drug is highly selective, the CSNPs were customized with two types of breast cancer-specific monoclonal antibodies (mAbs): anti-human mammaglobin (Anti-hMAM) and anti-human epidermal growth factor receptor (Anti-HER2). The study demonstrated that using monoclonal antibodies to functionalize nano-formulated DOX can increase its cytotoxic effect in MCF-7 by three times compared to free DOX. Additionally, the normal L-929 cells showed higher viability rates (Helmi et al., 2021).

Sathiyaseelan et al. studied ROS-responsive cerium oxide (CeO_2) NPs decorated 5-fluorouracil (5FU) loaded chitosan (CS) nanoparticles (5-FU/CeO_2 NPs-CSNPs). The aim was to enhance the anticancer activity in hepatocellular carcinoma, specifically targeting HepG2 cells. The CeO_2 NPs-based DDS act as both oxidation and reduction catalysts, with variable oxidation states of Ce^{4+} and Ce^{3+} depending on the environment. *In vitro* drug release studies revealed that the 5-FU/CeO_2 NPs-CSNPs release the 5-FU in a pH-responsive manner. This delivery system enhanced apoptosis in HepG2 cells by delivering the anticancer molecules (5-FU) and antioxidants (CeO_2 NPs) (Sathiyaseelan et al., 2022).

One way to achieve photo-controlled DDS is by using chlorin e6 (Ce6) to decorate NPs. Ce6 is a type of photosensitizer that is particularly effective in tumor cytotoxicity and has stronger absorption at longer wavelengths, resulting in fewer side effects. Interestingly, when irradiated DOX/Ce6-CS NPs (CSNPs loaded with DOX and decorated with Ce6) were used for treatment, there was a significant increase in cell death compared to the control group. This suggests that the DOX was released from the NPs after irradiation. However, when non-irradiated DOX/Ce6-CSNPs were used for treatment, there was very little cell death compared to the control group because DOX was not efficiently released from the NPs (Bhatta et al., 2019).

Mobil Composition of Matter No. 41 MCM-41, a type of mesoporous silica, is an excellent carrier for targeting cancer, drug delivery, and imaging applications.(Cheng et al., 2020). Esmaeili et al. have prepared CUR-loaded CSNPs conjugated with mesoporous silica conjugated with the conjugate of gold NPs and aptamer (CUR/CSNPs*MCM*(AuApt). By utilizing pH-sensitive CSNPs, researchers could deliver a higher concentration of CUR to the intended cells. Additionally, when the aptamer binds to the MUC-1 receptor, the double strands separate under low pH conditions, prompting the release of the drug and activating fluorescence ("On" state). The toxicity tests revealed that this nanosystem was more toxic to MUC-1-positive tumor cells than MUC-1-negative cells, demonstrating its targeted selectivity (Esmaeili et al., 2022).

5.5. CS-coated NPs

Many research groups have used CS to modify the physical and chemical properties of pre-existing NPs. The coating of NPs with CS has also been found to offer new biological properties. This coating exposes electro-charged amino groups on the surface of the NPs (Del Prado-

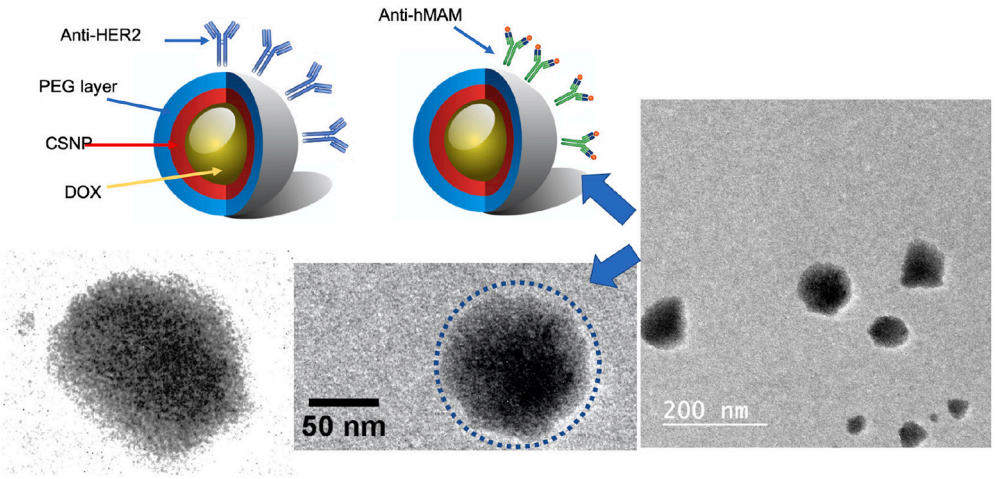

Fig. 16. Schematic illustration of the final mAb functionalized DOX-loaded PEGylated CSNPs (Helmi et al., 2021).

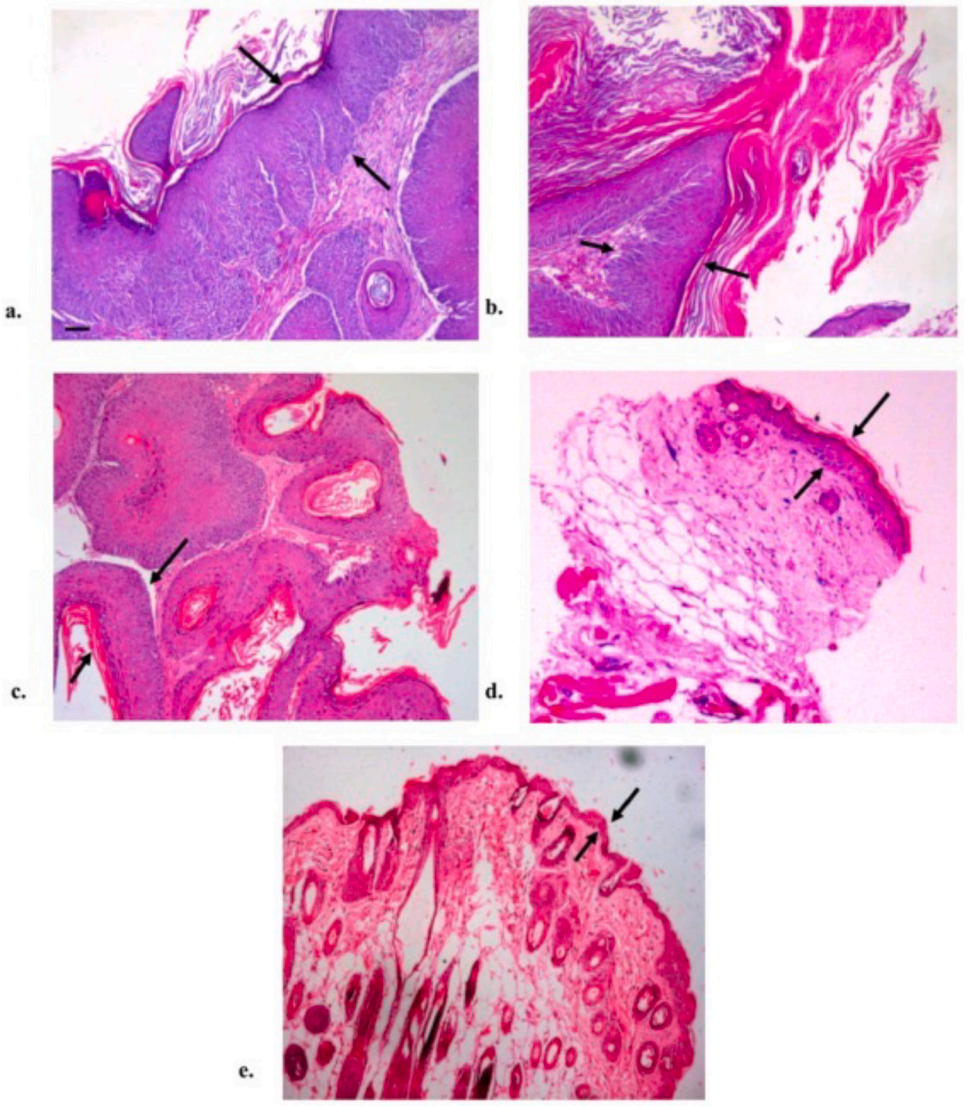

Fig. 17. Histopathological analysis of skin tumors. a. Untreated tumor b. CS@AgNPs (chitosan-coated silver nanoparticles) treated tumor c. NS*CS@AgNPs (nisin-conjugated chitosan-coated silver nanoparticles) treated tumor d. 5-FU/CS@AgNPs (5-fluorouracil-loaded, chitosan-coated silver nanoparticles) treated tumor e. 5-FU/+NS*CS@AgNPs (5-fluorouracil-loaded and nisin-conjugated chitosan-coated silver nanoparticles) treated tumor (Rana et al., 2022).

Audelo et al., 2020). CS can loosen tight junctions of epithelial cells, making it easier for drugs to be delivered across epithelial barriers. The presence of CS on the surface not only improves the paracellular transport of NPs but also increases their cellular uptake (Elkomy et al., 2022). There are two methods to coat NPs with CS: covalent adhesion and adsorption. Adsorption involves depositing chains of the polymeric material onto the surface of the material to be decorated. Through this process, NPs are immersed in a CS dispersion until equilibrium is reached. CS's polyelectrolyte behavior promotes the adsorption of polymeric chains at the surfaces, primarily through an electrostatic mechanism known as electrosorption (Del Prado-Audelo et al., 2020).

Rana et al. developed and characterized a nanoconstruct comprising of CS oligomer-coated AgNPs (silver NPs), which could provide a single platform for loading a chemo drug (5-FU) and an anticancer peptide (nisin) simultaneously and to further evaluate its anticancer therapeutic potential in a murine skin cancer model. CS is figured as an oligomer with which either covalently (nisin) or non-covalently (5-FU) interacted with the therapeutics used. When oligomeric CS was applied as a coating to NPs, the resulting NPs had a mostly spherical shape. Histopathological studies (see Fig. 17) revealed a considerable anticancer therapeutic potential of nanoconstruct in terms of decreased focal epidermal thickness, presence of degenerative sites, and decreased extent of hyperkeratosis in 5-FU/+NS*CS@Ag NPs (5-FU-loaded and nisin-conjugated CS-coated silver NPs) treated tumor group as compared to untreated tumor group. It is important not to overlook the potential anticancer benefits of CS. In fact, it may have indirectly contributed to more significant anticancer effects at lower doses. This could be due to the increased efficacy of nanoconstructs in targeting cancerous cells. As a result, it is possible to achieve stronger anticancer effects with lower doses of the drugs (Rana et al., 2022).

It is essential to have a suitable surface coating to avoid the aggregation of NPs and to improve drug loading and targeting. Coating magnetic NPs with a natural biodegradable polymer like CS can effectively prevent the formation of clusters in the bloodstream. This ensures that the NPs remain separate and do not agglomerate, providing an efficient and safe solution. Delivering hydrophobic drugs safely to the tumor site remains a significant challenge for the scientific community. Manjusha et al. have developed a novel PTX/CS@IONPs~METAC (PTX-loaded, CS-coated iron oxide NPs, cross-linked by (methacryloyloxy) ethyl] trimethyl ammonium chloride polymer) to improve the *in vivo* efficacy of hydrophobic drugs by avoiding solubility concerns. The recently developed polymeric material is highly effective in delivering PTX in a controlled and targeted manner. This material has demonstrated remarkable anticancer properties, good hemocompatibility, and a favorable pharmacokinetics profile (Manjusha et al., 2023).

Functional groups on an NP can form covalent bonds with CS molecules through various reactions, either before or after NP formation. The specific method of synthesis and chemical properties of the materials used will determine the optimal reaction pathway (Del Prado-Audelo et al., 2020). A new technique called the "grafting-from" method has been developed to coat inorganic nanomaterials with polymer chains through covalent bonding. This approach involves applying surface-initiated atom transfer radical polymerization on Fe_3O_4 NPs. With this method, researchers were able to create smart magnetic core-shell NPs that have a magnetite (Fe_3O_4) core and a covalently stabilized polymeric shell. These NPs were designed for targeted drug delivery and included a layer of special stimuli-responsive polymeric material. Moradi et al. developed a novel triple-responsive nanocarrier for anticancer drugs, including pH, temperature, and magnetic field as stimuli. It has been scientifically established that cancerous cells display a higher temperature (while in thermotherapy) and lower pH values than healthy cells. This critical information has been utilized to target drugs more accurately towards the specific body region where cancerous cells are present, with the aid of a magnetic field gradient. They prepared MTX-loaded, CS-grafted, poly (N-isopropyl acryl amide)-coated iron oxide NPs (MTX/CS#PNIPA Am@ Fe_3O_4 NPs). CS has been used here mainly because of its pH-dependent properties. The cytotoxicity of MTX, a negatively charged anticancer drug, has been significantly enhanced against MCF7 and A549 cell lines by loading it onto a prepared nanocarrier, which showed the highest release under cancer cell conditions (Moradi et al., 2022).

Solid tumors can release acidic substances interacting well with NPs coated with CS. This interaction results in better adhesion to the tumor cell membrane and an extended lifespan in the bloodstream. CS coating makes the NPs more stealthy, which further enhances their effectiveness (Del Prado-Audelo et al., 2020).

5.6. Chitosan derivatives nanoparticles

In Section 4, we explored different ways of creating CS derivatives. As these derivatives offer numerous benefits, they can be effectively applied in producing NPs for administering chemotherapeutic drugs. Recently prepared NPs made of CS derivatives loaded with anticancer drugs and studied as innovative anticancer DDS are listed in Table 3 and discussed in Subsections 5.6.1–5.6.4.

5.6.1. Thiolated CSNPs

GSH is crucial in creating and breaking down disulfide bridges and is a universal reducing agent for cells. Tumor cells, such as those found in bone marrow, breast, colon, larynx, and lung cancers, have increased levels of GSH to reduce damage caused by oxidative stress (Grosso and De-Paz, 2021). There are some drawbacks to using anticancer drugs, but these have been addressed through the use of TCS. This substance has excellent mucoadhesivity, can enhance membrane permeation, and offers improved inhibition for P-glycoprotein (Negm et al., 2020). TCS-based NPs do not disintegrate because of the formation of disulfide bonds within the polymeric network, stabilizing the microparticles strongly and providing controlled drug release. Thiol groups on CS are immobilized, and adding multivalent anionic compounds to TCS-based NPs significantly improves their mucoadhesion properties compared to regular CS (Divyesh H Shastri, 2017). Research shows that TCS coatings are more advantageous for the oral delivery of taxane-loaded NPs than CS-based coatings. The CS coating can function as a chelating agent for metallic bivalent ions that are necessary for certain intestinal enzymes to perform their functions, including aminopeptidase N and chymotrypsin. These enzymes may be involved in deactivating therapeutic molecules. Blocking the degrading enzymes can improve the intestinal absorption of taxanes, leading to increased half-life when taken orally. Thiomers are effective in opening tight junctions between epithelial cells through their thiol groups that can hinder the protein tyrosine phosphatase responsible for closing these junctions. As a result, these materials help promote the entry of drugs into the bloodstream, leading to better absorption of the therapeutic agent. They are commonly called permeation enhancers (Grosso and De-Paz, 2021).

5.6.1.1. Ligand-decorated TCSNPs. By modifying CS with FA and thiol moieties, NPs that are loaded with DTX and have exceptional efficiency in targeting breast cancer cells have been developed. These NPs have enhanced cellular internalization, mucoadhesion, and the ability to inhibit the P-glycoprotein (P-gp) efflux pump. *In vitro* cytotoxicity studies show significant improvement against folate receptor-positive MDA-MB-231 (MD Anderson-Metastatic Breast-231 Cells) cancerous cells, with an IC50 of 0.58 μg/mL, significantly lower than unmodified DTX. These polymeric NPs were found to be excellent vehicles for delivering hydrophobic cytotoxic drugs to specific sites, making them promising candidates for further research (Sajjad et al., 2019).

5.6.1.2. Ligand-conjugated TCS' coated NCPs. Core-shell nanocapsules (NCPs) were prepared, with hydrophobic DTX in the core and Ag NCPs embedded TCS in the shell. The DTX/FA*TCS@Ag NCPs (DTX-loaded, FA-conjugated, with TCS-coated silver NCPs) demonstrated superior

potential for oral drug delivery compared to DTX suspension. The blood levels remained constant for 24 h, exhibiting a half-life that was six times greater and a bioavailability that was nine times greater. Furthermore, their combined usage exhibited improved efficacy in eliminating MDA-MB-231 breast cancer cells. The DTX/FA*TCS@Ag NCPs are an efficient and versatile carrier with tremendous potential for oral delivery of DTX, with increased activity against breast cancer (Sohail et al., 2018).

5.6.1.3. Coated TCSNPs. Samprasit et al. proposed an innovative method to deliver drugs to the colon effectively and safely. Dual-drugs-loaded mucoadhesive TCS-based NPs were coated with Eudragit® S100 (S) to guarantee that Alpha-mangostin (M) and resveratrol (R) are released in the colon and not in the upper gastrointestinal tract. This coating allows for a higher drug load, significantly increasing activity against colon cancer cells. The mucoadhesion of the formulation in the colon ensures that the drugs are retained in that area. This new method has led to a decrease in the half-maximal inhibitory concentration values of the NPs, indicating a higher level of activity against colon cancer cells (Samprasit et al., 2022).

5.6.1.4. Ligand-decorated TCSNPs. Kousar et al. aimed to create a green synthesis approach to develop HA-decorated, TCS nanocarriers that can release cisplatin in a controlled manner and target CD44 in cervical cancer cells. Their results showed that the Cis/HA-TCSNPs (cisplatin-loaded, HA-decorated TCSNPs) nanoformulation was more effective in killing cancer cells in HeLa, while pure cisplatin was more toxic in HCK1T. Based on these findings, it appears that the formulation could be a highly effective DDS for specifically targeting cancer cells through the CD44 receptor (Kousar et al., 2023).

5.6.1.5. Single polymer TCSNPs. In other work, authors created DOX + CUR (curcumin)-loaded, CS and stearic acid-crosslinked NPs (DOX + CUR/CS + SANPs) by combining thiolated CS and thiolated stearic acid and using air oxidation. They then loaded the NPs with hydrophilic DOX hydrochloride and hydrophobic CUR. The drugs remained stable under normal conditions, with only small amounts released. However, under GSH-reducing conditions, almost all of the drugs were released after 136 h. The NPs were tested for cytotoxicity against HCT116 cells and were found to be more effective than either drug used alone (Sood et al., 2022).

5.6.2. Carboxyalkylated CSNPs

Self-assembled NPs made from amphiphilic CS derivatives are promising drug carriers due to their hydrophobic regions that can store insoluble drugs. When ionic liquids are added, these NPs can offer a higher number of adsorption groups, including -OH, -COOH, and -NH-, which increases their adsorption capability. Studies have demonstrated that CMCSNPs can adjust to various pH levels thanks to amino protonation and dissociation of the carboxylic group.

5.6.2.1. Ligand decorated CMCSNPs. Zhu et al. developed novel T7 peptide-modified NPs based on CM CS, which is capable of targeted binding to the transferrin receptor (TfR) expressed on lung cancer cells and precisely regulating drug release according to the pH value and ROS level. The DTX + CUR/T7 + BAPE-CMCSNPs (DTX and CUR-loaded, T7 peptide and polyoxyethylene polyoxypropylene amine-decorated CM CS NPs) complexes exhibited better *in vitro* and *in vivo* anti-tumor effects than DTX monotherapy and other nanocarriers loaded with DTX and CUR alone. In addition, it has been found that the complex can improve the immune-suppressive micro-environment to suppress the growth of tumors (Zhu et al., 2021).

5.6.2.2. Single polymer CMCSNPs. OXE/CMCSNPs (oxaliplatin-loaded, CMCSNPs) and RES/CMCSNPs (resveratrol-loaded, carboxymethyl CSNPs) were prepared by ion crosslinking and emulsification cross-linking, respectively. The treatment with both types of NPs combined exhibited more significant anti-colon cancer activity than the free drugs or either type of NP alone. The *in vivo* and *in vitro* cytotoxicity experiments demonstrated the excellent biocompatibility of NPs. CMCS may have these desirable properties because of its good water solubility, biocompatibility, biodegradability, and low immunogenicity (Wang et al., 2021a).

Single polymer CMCSNPs. Lu et al. prepared CMCS-based hypoxia-responsive NPs loaded with DOX and Tanshinone IIA (TSIIA) to treat breast cancer. The study showed that the use of hypoxia-responsive nanoparticles led to better delivery of drugs and improved the effectiveness of DOX in comparison to free DOX. The nanoparticles exhibited hypoxia-responsive behavior in laboratory testing, while their synergistic efficacy was significantly demonstrated in live testing. The tumor inhibitory rate was found to be 85.87 % (Lu et al., 2023).

5.6.3. Quaternized CSNPs

By introducing a quaternary ammonium group, CS can transform into amphoteric polymers to achieve specific goals. This allows them to exhibit responsiveness to factors such as acidity, temperature, and reduction, among others. Trimethylated CS is highly superior to other carriers in terms of both structure and performance, making it an ideal candidate for use as a gene vector (Zhang et al., 2021). Lately, the medical community has been using chemotherapy and gene therapy to treat tumors. This approach can boost the effectiveness of antitumor treatments by addressing the limitations of single-target therapy. Survivin, a protein that inhibits cell death, tends to be overproduced in cancerous tumors.

5.6.3.1. Ligand-conjugated TMCSNPs. Li et al. developed FA and DPA (2-(Diisopropylamino) ethyl methacrylate) double-grafted amphiphilic polymers, which self-assembled into NPs in an aqueous solution with a hydrophobic core and a hydrophilic shell. TM ammonium CS was reacted with DPA *via* free radical graft copolymerization. Then FA and DPA grafted TM ammonium CS was synthesized by attaching the carboxyl groups of FA onto the amino groups of DPA grafted TM ammonium CS *via* the amide bonds. The NPs were created through self-assembly to efficiently co-deliver DOX and Survivin CRISPR/Cas9-expressing plasmid or DOX and Survivin shRNA-expressing plasmid. These NPs showed improved effectiveness against tumors both *in vitro* and *in vivo*, compared to single-delivery NPs, due to a cooperative effect. This formulation also demonstrated good structural stability, pH-responsive drug release, and better targeting of tumor cells (Li et al., 2022).

5.6.3.2. Ligand-conjugated TMCSNPs. Zhang and his colleagues produced a new type of NP by combining FA and carboxymethyl-β-cyclodextrin (CMβC)-grafted TM CS. They used ionic gelation to create the NPs and tested their potential as carriers for both DOX and siRNA. The NPs could hold a significant amount of the drugs and release them in a controlled manner depending on the pH level. The researchers found that the drug-loaded FA and CMβC-grafted TMCSNPs effectively improved the effectiveness of antitumor drugs, according to their cytotoxicity study (Zhang et al., 2021).

5.6.3.3. Coated TMCSNPs. PEG-modified TMCSNPs with pH and redox responsiveness were designed to co-deliver DOX and QUE. The PEG coating of the formulation protected it from plasma protein adsorption during neutral blood circulation and was removed in the acidic TME as pH and GSH levels changed. Compared to free DOX, QUE, and single-loaded NPs, the formulation showed superior antitumor efficacy. The photographs of tumors from the mice excised on day 12 are illustrated in Fig. 18. These findings suggest that the NPs may be a promising platform for delivering multiple hydrophobic antitumor components

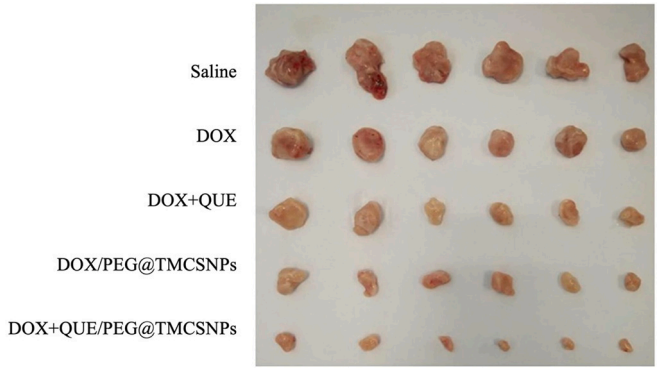

Fig. 18. The photograph of tumors from the mice (six reproducibles) excised on day 12 (Saline as a control; DOX; DOX + QUE; DOX/PEG@TMCSNPs (doxorubicin-loaded, polyethylene glycol-coated trimethyl ammonium chitosan nanoparticles); DOX + QUE/PEG@TMCSNPs (doxorubicin and quercetin-loaded, polyethylene glycol-coated trimethyl ammonium chitosan nanoparticles)) (Liu et al., 2021).

simultaneously (Liu et al., 2021).

5.6.4. Glycol CSNPs

This modification mainly applies to shifting the surface of CSNPs with ethylene glycol groups. Adding ethylene glycol groups to nanocarriers can provide various benefits, including improved stability and solubility in the serum of the human body, as well as increased effectiveness in delivering CSNPs to target cells. Additionally, this modification can help sustain therapeutic delivery and prevent MDR (Khalaf et al., 2023). Recent reports suggest that high molecular weight GCSNPs have better pharmacokinetic properties than low molecular weight ones, allowing them to remain in the bloodstream for more extended periods. Furthermore, studies have confirmed the safety of GCS-based nanogels when administered systemically. Interestingly, GCS-based NPs have a pH-dependent surface charge, which enables them to circulate at physiological pH levels without interacting with serum proteins (Sahiner et al., 2023). GCSNPs are a class of promising drug carriers for hydrophobic chemodrugs. Their hydrophobic cores are suitable for encapsulating hydrophobic drugs, while the hydrophilic outer shells can ensure the stability of the nanoagents during blood circulation until they arrive at the target site (Lin et al., 2019).

5.6.4.1. Ligand-conjugated, multiple polymer GCSNPs. Breast cancer tissues tend to show an increased expression of estrogen receptors (ER) compared to normal mammary glands. By using estrone (ES) as a ligand molecule, chemotherapeutic agents can be targeted towards breast cancer cells. To improve delivery efficiency, ES-modified GCSNPs were created. These NPs use active targeting with ES conjugation and pH-dependent drug release with PDPA (poly(2-(diisopropylamino)ethyl methacrylate)) segments. PDPA is hydrophobic at pH 7,4 but becomes hydrophilic at pH values below 6,3 due to the protonation of diisopropyl tertiary amines. Researchers used PTX as a model drug and put it inside NPs. These NPs, called PTX/ES*GCS + PDPANPs (which stands for PTX-loaded, estrone-conjugated GCS and (poly(2-(diisopropylamino)ethyl methacrylate)) NPs), were found to be effective in targeting breast cancer cells. They showed increased cellular uptake, improved tumor distribution, and better antitumor efficacy than PTX solution and unmodified GCS NP. The PTX/ES*GCS + PDPANPs also had intracellular pH-responsive properties that prevented drug leaks in circulation and allowed for timely payload release upon uptake in targeted cells. *In vivo* studies showed that PTX/ES*GCSNPs did not cause any histological, hematological, or hepatic toxicity (Yang et al., 2018).

5.6.4.2. Ligand-conjugated single polymer GCSNPs. Lee and colleagues created NPs that target the CD44 receptor and are decorated with HA. These NPs were linked to the drug DOX and loaded with celecoxib (CXB). *In vitro*, it was found that combining DOX and CXB had a synergistic effect both in their free drug form and in the NP formulation. *In vivo*, it was observed that CXB/HA + DOX*GCSNPs (celecoxib-loaded, hyaluronic acid and doxorubicin-conjugated glycol chitosan nanoparticles) had a significant tumor suppressive effect and inhibited tumor growth more effectively than formulation with just DOX. Additionally, it suppressed inflammation and genes and proteins associated with metastasis in tumor tissues (Lee et al., 2020).

5.6.4.3. Ligand-conjugated single polymer GCSNPs. Song et al. proposed a new all-in-one nanoparticle system comprising anti-PD-L1 peptide-conjugated (PP) and DOX-loaded GCSNPs for synergistic immunotherapy. Safe and more effective synergistic immunotherapy using DOX/PP*GCSNPs (doxorubicin-loaded, anti-PD-L1 peptide conjugated glycol chitosan nanoparticles) was assessed in the colon and metastatic lung tumor models. Passive and active targeting of nanoparticle-delivered PP and DOX led to high rates of complete tumor regression (60 % CR) through an effective antitumor immune response (Song et al., 2023).

6. Assessment of associated clinical research and other implementations of CSNPs

6.1. Consideration of CS for biomedical and dietary use

CS faces restrictions due to the strict standards for purity and characterization set by regulatory authorities. Although the FDA (Food and Drug Administration) has sanctioned CS as a biomaterial, its application in pharmaceuticals is constrained by concerns about its source, purity, and potential to trigger immune responses. Notably, CS is authorized as a dietary supplement to address obesity in several countries, including the USA, Finland, Italy, and Japan (Kantak and Bharate, 2022). CSNPs and their advantages seem promising in *in vitro* or *in vivo* studies, but only some of these formulations have been used in clinical settings (Dilnawaz et al., 2024). A comprehensive analysis has identified over 100 clinical studies involving CS, where it has been utilized as a dietary supplement, biomaterial, therapeutic device, and for non-therapeutic purposes. The therapeutic potential of CS spans various applications, with ongoing investigations into its role as an immunoadjuvant in cancer therapy being particularly noteworthy (Kantak and Bharate, 2022). There have been reports of Rylomine™, a nasal formulation of morphine-loaded CSNPs, being approved for Phase 2 clinical trials in the UK and EU and Phase 3 clinical trials in the USA. This development is an encouraging sign for the future of these NPs (Dilnawaz et al., 2024). However, despite the enormous efforts of the scientific community, it is important to note that no CSNPs have yet entered practice due to several shortcomings and hurdles that make their clinical translation difficult, which we discuss below.

6.2. Shortcomings and hurdles in the clinical translation of chitosan nanoparticles

6.2.1. Synthesis protocols and reproducibility issues

As this polymer originates from natural sources, it is imperative to investigate the biological variability of CS thoroughly. This essential task for the future will involve implementing well-defined methods and robust quality control measures to minimize the influence of biological variability (Lunawat et al., 2024). The major challenge of CSNPs is the need for more understanding of their synthesis protocols, leading to poor reproducibility of particle characteristics (Radha et al., 2023). Various innovative methods have been employed in developing CS-based vehicle systems, including ionic gelation, covalent cross-linking, and reverse micellar techniques. Among these, ionic gelation has attracted significant attention due to its cost-effective control over parameters, practicality, non-toxic properties, and elimination of organic

solvent usage (Radha et al., 2023). Although this method is the most widely used, even a tiny change in the procedure, *e.g.*, speed of mixing the solutions, speed of dropping the crosslinking agent into the CS solution, changes in the pH of the solutions, *etc.*, will significantly affect the properties of the NPs. Producing CSNPs with consistent size, shape, and properties on a large scale is technically challenging. To improve scalability, an ion gelation method procedure using microfluidic technology was initiated (Farahani et al., 2021). Microfluidic technologies process or manipulate fluids using micro-channels (Zhong et al., 2021). The microfluidic approach offers practical features such as rapid mixing, ease of use, reduced material consumption, and precise production control (Farahani et al., 2021). However, microfluidic systems significantly increase the preparation cost of NPs, and the amount of the product resulting from one run is limited, which has practical consequences. In addition, preparing NPs by combining polymers, modifying NPs with ligands such as PEG to prolong systemic circulation or FA for active targeting, and so on require more complex methods and preparation procedures, often using more unsafe chemicals (Huang et al., 2022a). However, many ongoing clinical trials involving surface-modified NPs for drug delivery may face challenges in advancing beyond Phase II for a variety of reasons, such as rapid elimination by the immune system, unsatisfactory drug release and targeting results, or inadequate *in vitro* and *in vivo* translatability (Shreffler et al., 2019).

6.2.2. Toxicity evaluation and assessment of drug-loaded chitosan nanoparticles

The evidence strongly supports the idea that CS is an ideal natural carrier due to its low toxicity and impressive biodegradability (Sun et al., 2017). The mechanism of CS's *in vivo* biodegradability still needs to be understood. However, several studies have suggested the degradation of CS and its micro/nanoparticles by lysozyme (Huang et al., 2022b). Chemical modifications of this biopolymer have significantly enhanced its solubility in aqueous media, elevating its biological activity and broadening its range of applications (Ajalli et al., 2022). The CS modifications can alter its toxicity, so residual reactants should be removed carefully (Kean and Thanou, 2010). Thoroughly investigating potential toxic effects in preclinical studies is crucial to establishing the nontoxicity and biocompatibility of CSNPs (Sun et al., 2017). The toxicological profile of CSNPs is currently under thorough investigation to ensure a comprehensive understanding of their potential impact (Dilnawaz et al., 2024). The exact toxic effects of these NPs on humans have not been documented yet in details. There are stil inconsistent findings regarding the impact of these NPs on different organs and cell lines. Furthermore, the methods used to test the toxicity of each NP on various cell lines and organs differ in terms of the reagents employed and the criteria for determining toxicity (Zoe et al., 2023). It is crucial to include testing of NP interactions with proteins and various cell types as an integral part of the toxicology assay (Ajalli et al., 2022). The positively charged NPs attract proteins during blood circulation, causing rapid elimination from the body and potential tissue toxicity. This may not be advantageous for accumulation within tumors (Huang et al., 2022b), despite recent studies indicating the safety of formulated CSNPs in healthy tissues.

6.2.3. Challenges in targeted drug delivery

One of the most crucial problems in targeted drug delivery is the binding of nanocarriers to the proper site in the body (Rostami, 2020). Design and testing are essential for CSNPs to target specific tissues or cells without impacting other areas of the body effectively (Shreffler et al., 2019). Predicting response rate variability and therapeutic efficacy in patients can be more challenging, especially for molecularly targeted therapeutics. Scientists are cautious about giving too much weight to positive preclinical study results. Many drugs that showed promise in animal studies have ultimately performed less well in patients, particularly in the field of oncology (Metselaar and Lammers, 2020). Studies often fail to consider the immune system's influence on NP progress towards targeted drug delivery, resulting in low clinical translation rates (Shreffler et al., 2019). To deal effectively with these problems, it is advisable to employ *in vitro* complement binding and cell interaction assays. It is recommended to conduct preclinical safety studies in larger animals, notably in pigs, to gain comprehensive insights (Metselaar and Lammers, 2020).

6.3. Quality control and practical considerations

6.3.1. Quality control in nanomedicine

Quality control is crucial for any medicinal product, including nanomedicine. Factors such as particle size, surface morphology, drug loading, and release require thorough quality control testing and standard checks. It is vital to consider critical quality attributes early in the formulation design, such as particle size, size distribution, charge, morphology, drug encapsulation, and release. To ensure optimal performance, it is essential to define narrow specifications for the formulations (Metselaar and Lammers, 2020). Surface chemistry and functionalization are crucial in determining NP absorption, distribution, metabolism, and excretion (ADME) (Shreffler et al., 2019). It is essential to consider that the interaction of CS with a drug can lead to changes in the drug's biodistribution and pharmacokinetics. When using a nanoparticulate formulation, the size and charge of the NPs will dictate the distribution and elimination processes rather than CS (Kean and Thanou, 2010). As it is obvious from many studies, to control and keep required optimum parameters of CSNPs, especially when implementing into complex and variable biological systems, is still challenging and demanding to overcome at least one of the above discussed shortcomings and hurdles affecting their attributes.

6.3.2. Economic and practical considerations

Developing, testing, and producing CSNPs at scale can be expensive. While the academic community rightly prioritizes research goals, it is essential to consider the initial investment risk *versus* the potential return at an early stage (Metselaar and Lammers, 2020). The unique properties of nanomedicines, such as their small size and large surface area, present stability challenges, including susceptibility to aggregation, contamination, and degradation. While specific storage conditions can be costly, efforts to develop more affordable and accessible storage solutions for growing and low-income countries are essential. Additionally, there is potential to explore alternative administration routes beyond injection to enhance patient convenience and adherence (Younis et al., 2022).

As discussed herein, these are main issues considered in the clinical translation of CSNPs, prolonging their full implementation into clinical practice and still requiring further research and analytical data. Nevertheless, all these reached scientific findings and outputs shift CS nanoparticulate systems closer to a true drug delivery being acceptable in oncological research and therapy.

7. Conclusions

Chemotherapeutic drugs used to treat cancer have been in clinical practice for many decades. However, their use is associated with significant side effects, primarily due to their lack of selectivity. This not only causes damage to rapidly dividing cancer cells but also to other cells with a high division rate. With the development of innovative dosage forms, much research is focusing on using nanoparticles as transport systems for these anticancer drugs. It has been found that such systems could transport the drug more selectively to the tumor site, which would protect other cells in the body from the toxic effects of anticancer drugs. Targeting the tumor site is possible due to gaps in the endothelium of the new blood vessels that nourish the tumor and also due to inadequate lymphatic drainage. As a result, nanoparticles can pass from the blood vessels to the tumor area and accumulate there. However, there are significant differences between the characteristics of

different tumors and also differences between individuals, therefore it will still take some time to accomplish all necessary issues before such transport systems can be brought into clinical practice. So far, the most promising approach is the cancer-specific microenvironment. Depending on the type of cancer, this is characterized by different physicochemical properties compared to healthy tissue. A true active targeting lies in labeling nanoparticles with onco specific ligands (related to receptors overexpressed on the surface of cancer cells). Therefore, much emphasis is devoted to the investigation of low as well as high molecular weight ligands and their incorporation in/on nanoparticulate systems. Overall success in targeted biodistribution is further potentiated *via* proper stabilization of drug-loaded nanoparticles, preservation from destructive chemical and biochemical conditions in the body, and establishing of a controlled responsivity to the stimuli. These strategies are reflected in the research works discussed in this review.

In inorganic onco-specific nanoparticulate DDS dominate iron oxide nanoparticles, gold nanoparticles, carbon quantum dots, carbon nanotubes, fullerenes, and silica nanoparticles. They offer advantages such as unique electrical, magnetic, and optical properties, variability in size, structure, and geometry, and easier tracking compared to their organic counterparts. These approaches, which rely on either an internal or external stimulus, also have their drawbacks (toxicity, biodegradability and solubility limitations), and it is therefore important to continue to gather knowledge on innovative drug carriers.

In organic onco-specific nanoparticulate DDS dominate lipid-based nanostructures (liposomes, solid lipid nanoparticles, nanostructured lipid carriers, nanoemulsions, self-nanoemulsifying DDS and lipid nanodiscs) and polymeric nanostructures (polymeric nanospheres, nanocapsules, dendrimers, polymeric micelles, polymerosomes, polymer-drug conjugates and nanogels). They offer advantages such as excellent biocompatibility, precise control of particle characteristics, and possible interaction with specific cell receptors. Known disadvantages are batch-to-batch variability and poor traceability.

Within polymeric systems, a special attention is paid to chitosan-based onco-specific nanoparticulate DDS due to the biocompatibility and biodegradability of chitosan materials. Hence, they can be used safely in biological systems and will eventually decompose into non-toxic components. Chitosan itself and also some of its derivatives exhibit anticancer effects by disrupting cell membranes and inducing apoptosis. It effectively interacts with negatively charged tumor cells and endothelial cells. As reported in the literature, ion gelation is the most commonly used method for the preparation of chitosan nanoparticles. Types of chitosan nanoparticles intended for the administration of anticancer drugs include single polymer chitosan nanoparticles, multiple polymer chitosan nanoparticles, PEGylated chitosan nanoparticles, or conjugated/decorated chitosan nanoparticles. Chitosan nanoparticles have been found to have stronger anticancer effects in both *in vitro* and *in vivo* models as compared to the use of the drug alone. Moreover, these nanoparticles do not seem to harm healthy cells, which is a significant advantage of using them. Chitosan also was demonstrated to be an excellent stimuli-responsive matter recognizing cancerous and healthy tissue environments. This was utilized as a stimulus for drug release in many works showing chitosan nanoparticles holding the drug at the normal pH of the body but releasing it at the more acidic pH characteristic of the tumor. It is also advantageous to coat other nanoparticles with chitosan, either by covalent adhesion or by adsorption of chitosan. This enables more efficient disruption of tight junctions between epithelial cells, facilitating drug transport across barriers.

Chitosan derivatives significantly spread potentialities of onco-specific chitosan-based DDS. The use of thiolated chitosans to form nanoparticles may enhance their mucoadhesion. This improvement can be exploited in the colon, for example, ensuring that drugs are retained in this area for longer periods. Some types of thiolated chitosan nanoparticles are single polymer thiolated chitosan nanoparticles, ligand-decorated thiolated chitosan nanoparticles, or coated TCSNPs. Carboxymethyl chitosan, in turn, can effectively influence the properties of nanoparticles in a pH-dependent manner. Carboxymethyl chitosan nanoparticles can be either single polymer or ligand-decorated. Trimethyl ammonium chitosan nanoparticles are very common in the literature as carriers for co-delivery of chemotherapeutics and gene therapy. Types of trimethyl ammonium chitosan nanoparticles include, for example, ligand-conjugated or coated trimethyl ammonium chitosan nanoparticles. It has been reported that glycol chitosan has an exceptional ability to target tumor cells. There are different types of glycol chitosan nanoparticles, including ligand-conjugated single polymer and ligand-conjugated multiple polymer glycol chitosan nanoparticles.

When comparing CS NPs with other polymeric or organic and inorganic nanoparticulate systems, following observations can be summarised from the reviewed works:

- CS NPs are unique among polymers by their cationogenic character and, due to the reactivity of the amino group, the relatively wide scale of their derivatives tailored for a given purpose, *i.e.*, drug and its target. However, structurally variable natural polymers providing variable activity (structure-activity relationship), such as CS, need to be structurally proven and standardized before their full consideration in preclinical and clinical implementations.
- The polymeric character of CS is advantageous among the lipid-building units of nanoparticulate systems due to its higher stability in biological fluids and its ability to provide better control over the release of the encapsulated drug. In contrast, liposome systems typically display improved biocompatibility because their phospholipid bilayer composition resembles the plasma membrane of human cells. Lipid nanocarriers can encapsulate hydrophobic drugs, increasing their solubility and bioavailability.
- Due to their natural origin, CSNPs exhibit low toxicity, high biodegradability and biocompatibility compared to inorganic nanocomposites. In contrast, inorganic nanoparticles have unparalleled chemical stability, can have a large, scalable surface area, and can exhibit specific electrical or magnetic properties, making them indispensable nanosystems for drug delivery.
- To utilize the benefits of both chitosan and other building units of nanoparticulate systems, their combinations represent an attractive advanced solution. Polymeric lipid hybrid nanoparticles are becoming more versatile carriers for a wider range of drugs (hydrophilic, hydrophobic, proteins, DNA, RNA), are more physically stable, biocompatible, and allow better control of drug release. The body's immune response to the presence of inorganic nanocomposites could be bypassed by coating them with a CS coating. However, such combined nanoparticulate systems are naturally more challenging for their preparation and validation, and therefore, cost that also affects potentialities for their practical implementations.

In summary, further investigation into the properties of nanoparticles, focusing on their toxicity and elimination from the organism (especially inorganic ones), and their physical-chemical standardization (especially biopolymeric derivatives) are necessary in the future for their widespread implementation. Despite promising attributes of biodegradability, challenges like standardization of chitosan derivatives production processes and ensuring cost-effectiveness of their preparation need to be addressed to shift *in-vitro* or *in-vivo* applications to further preclinical and clinical studies and therapeutical practice. Nevertheless, the beneficial properties of NPs, and especially those made from chitosan- and chitosan-derivatives, position them as an innovative solution for onco-specific drug delivery systems worthy of further investigation and, perspectively, medicinal implementation.

CRediT authorship contribution statement

Prieložná Jarmila: Writing – original draft, Visualization. **Mikušová Veronika:** Writing – review & editing, Writing – original

draft, Conceptualization. **Mikuš Peter:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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