Chitosan Mediated Targeted Drug Delivery System: A Review

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ABSTRACT - Chitosan has prompted the continuous movement for the development of safe and effective drug delivery systems because of its unique physicochemical and biological characteristics. The primary hydroxyl and amine groups located on the backbone of chitosan allow for chemical modification to control its physical properties. When the hydrophobic moiety is conjugated to a chitosan molecule, the resulting amphiphile may form self-assembled nanoparticles that can encapsulate a quantity of drugs and deliver them to a specific site of action. Chemical attachment of the drug to the chitosan throughout the functional linker may produce useful prodrugs, exhibiting the appropriate biological activity at the target site. Mucoadhesive and absorption enhancement properties of chitosan increase the in vivo residence time of the dosage form in the gastrointestinal tract and improve the bioavailability of various drugs. The main objective of this review is to provide an insight into various target-specific carriers, based on chitosan and its derivatives. The first part of the review is concerned with the organ-specific delivery system using chitosan and its derivatives. The subsequent section considers the recent development of drug delivery carriers for cancer therapy with special focus on various targeting strategies.

INTRODUCTION

Drug discovery and development involve highly challenging, laborious, and expensive processes. Most of the drugs in the clinical phase, however, fail to achieve favorable clinical outcomes because they do not have the ability to reach the target site of action. A significant amount of the administrated drug is distributed over the normal tissues or organs that are not involved in the pathological process, often leading to severe side effects. An effective approach to overcome this critical issue is the development of targeted drug delivery systems that release the drugs or bioactive agents at the desired site of action. This could increase patient compliance and therapeutic efficacy of pharmaceutical agents through improved pharmacokinetics and biodistribution [1–4].

The idea of developing a drug that selectively destroy disease cells without damaging healthy cells was proposed by Paul Ehrlich, almost a century ago; he called his hypothetical drug the “magic bullet” [5]. Thereafter, over the past several decades, many scientists have focused their attention on the development of ideal drugs that specifically target the site of action. Although little progress has been made in this field, the advent of nanomedicine and our understanding of cellular and molecular biology have opened new avenues to transform the Ehrlich's concept into clinical reality [6]. The targeted drug delivery system is comprised of three components: a therapeutic agent, a targeting moiety, and a carrier system. The drug can be either incorporated by passive absorption or chemical conjugation into the carrier system. The choice of the carrier molecule is of high importance because it significantly affects the pharmacokinetics and pharmacodynamics of the drugs.

A wide range of materials, such as natural or synthetic polymers, lipids, surfactants and dendrimers, have been employed as drug carriers [7–10]. Among these, polysaccharides have received increasing attention because of their outstanding physical and biological properties [11]. Chitosan, a linear aminopolysaccharide composed of randomly distributed (1→4) linked D-glucosamine and N-acetyl-D-glucosamine units, is obtained by the deacetylation of chitin, a widespread natural polysaccharide found in the exoskeleton of crustaceans such as crab and shrimp [12].

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This cationic polysaccharide has drawn increasing attentionwithin pharmaceutical and biomedical applications, owing to its abundant availability, unique mucoadhesivity, inherent pharmacological properties, and other beneficial biological properties such as biocompatibility, biodegradability, non-toxicity and low-immunogenicity [12–14]. The physicochemical and biological properties of chitosan are greatly influenced by its molecular weight and degree of deacetylation. Detailed characteristics of chitosan for biomedical applications arewell described in several comprehensive reviews [12–14]. The presence of reactive functional groups in chitosan offers great opportunity for chemical modification, which affords a wide range of derivatives such as quaternized chitosan (N,N,N-trimethyl chitosan; TMC), carboxyalkyl chitosan, thiolated chitosan, sugar-bearing chitosan, bile acid-modified chitosan and cyclodextrin-linked chitosan [15–20]. Various synthetic strategies for the modification of the chitosan have been extensively reviewed elsewhere [12,21,22]. These chitosan derivatives have been designed to improve specific properties of native chitosan. For example, thiolation of chitosan remarkably improves its mucoadhesive properties because of the formation of disulfide bonds with cysteine-rich subdomains of mucus glycoproteins [23]. The chemical modification of chitosan imparts amphiphilicity, which is an important characteristic for the formation of self-assembled nanoparticles, potentially suited for drug delivery applications. The hydrophobic cores of the nanoparticles could act as reservoirs or microcontainers for various bioactive substances. Because of their small size, nanoparticles can be administrated via the intravenous injection for targeted drug delivery. Conjugation of the targeting moieties to the surface of drug-loaded nanoparticles may improve therapeutic efficiency of the drug [24]. Chitosan has been widely utilized as drug delivery systems for low molecular drugs, peptides and genes [16, 25, 26]. Despite the recent emergence of biomacromolecular drugs, the majority of therapeutic drugs that are being developed and marketed are primarily low molecular weight drugs. Recent comprehensive surveys also disclosed that many molecular targets have been explored for therapeutic interventions, and most of the drugs approved for these targets are from small molecules [27]. Hence, the successful delivery of low molecular drugs to their respective targets is still of prime important in therapeutics.

The primary focus of this review is to provide an insight into various target-specific drug carriers based on chitosan and its derivatives. The first part of the review deals with organspecific delivery using chitosan and its derivatives. In subsequent sections we discuss the recent progress in chitosan-based drug carriers for cancer therapy with special focus on various targeting strategies.

Organ-specific drug delivery using chitosan and its derivatives

Colon- specific drug delivery systems have gained increasing attention for the treatment of diseases such as Crohn's disease, ulcerative colitis, and irritable bowel syndrome [28,29]. Colon targeting has proven useful for systemic delivery of protein/peptide drugs because of the relatively low proteolytic activities in the colon and even for other nonpeptide drugs such as cardiovascular and antiasthmatic agents. Several strategies are currently pursued for colon-targeted delivery, including the use of prodrugs that becomeactive at the colon, drug-eluting system responding to the pH, andmicro.ora-activatable drug delivery systems. The major obstacles in delivering drugs to the colon are the absorption and degradation pathways in the upper gastrointestinal tract. Hence, all the above strategies have attempted to prevent loss of the drug at the stomach and the small intestine, thereby facilitating quantitative drug delivery to the colon.

Chitosan-based delivery systems have been widely studied for colonic drug targeting since this system can protect therapeutic agents from the hostile conditions of the upper gastrointestinal tract and release the entrapped agents speci.cally at the colon through degradation of the glycosidic linkages of chitosan by colonic micro.ora [30,31]. Yamamoto et al. investigated the use of chitosan capsules for colon-specificdelivery of 5-aminosalicylic acid (5-ASA) [32]. The surface of the chitosan capsules containing 5-ASA was coated with hydropropyl methylcellulose phthalate as an enteric coating material. The experimental results demonstrated that the capsules were able to reach the large intestine 3.5 h after oral administration into 2,4,6-trinitrobenzenesulfonic acid-induced ulcerative rats. The release of 5-ASA from the capsule was markedly increased in the presence of

rat cecal contents. Chitosan capsule-based formulations showed better therapeutic effect than a carboxymethylcellulose suspension in vivo. Chitosan capsules can also be used as carriers for colon-specificdelivery of absorption enhancers. Oral delivery of the absorption enhancer along with poorly absorbable drugs using chitosan capsules could improve the absorption characteristics of the drugs [33]. Varshosaz et al. reported chitosan microspheres coated with cellulose acetate butyrate, prepared by the emulsion-solvent evaporation technique, for delivery of 5-ASA into the colon [34]. The authors found that decreasing the coat content and increasing the molecular weight of chitosan increased its bioadhesion significantly. Chitosan-Ca-alginate microparticles have also been used for colon specific delivery of 5-ASA [35]. The microparticles were prepared using the spray drying method, followed by ionotropic gelation/ polyelectrolyte complexation. In vitro drug-release experiments carried out under conditions similar to colon exhibited controlled release behavior of the drug. Biodistribution studies of chitosan-Ca-alginate microparticles loaded with 131I-ASA showed localization of 5-ASA in the colon with low systemic bioavailability. Recently, Jain et al. prepared hydrogel microspheres of chitosan grafted with vinyl polymers for the controlled and targeted delivery of 5-ASA to the colon, which exhibited better therapeutic effects [36].

Chitosan has often been limited in colonic targeting of drugs because of its high solubility in gastric fluids, sometimes resulting in burst release of the drug at the stomach. Although chitosan can be insoluble in acidic .uids through chemical cross-linking of the microsphere with aldehydes, it is not effective in preventing the release of the encapsulated drugs. To alleviate this problem, Alonso et al. developed microencapsulated chitosan microspheres coated with enteric coating materials [37]. The potential of this microsphere was evaluated using sodium diclofenac (SD), an anti-in.ammatory drug. SD was entrapped into the chitosan cores by the spray drying method, after which the chitosan cores were microencapsulated into Eudragit® L-100 and Eudragit® S-100 using an oil-in-oil solvent evaporation method. The in vitro release studies revealed that no SD was released at the gastric pH; however, when the microsphere reached the colonic environment, a continuous release was observed for a variable time (8–12 h). In a similar study, Onishi et al. prepared Eudragit®-coated microspheres composed of chitosan–succinylprednisolone conjugates (Ch–SP) using the sonication method [38]. These authors demonstrated that Eudragit®-coated microspheres protected the Ch–SP microspheres from morphological changes at pH 1.2, and regenerated them at pH 6.8 and 7.4. The release of prednisolone was suppressed at pH 1.2, whereas gradual release of the drug was observed at pH 6.8, indicating the potential of the coated microspheres for specificdelivery systems of the drug to the colon. Jain et al. developed chitosan hydrogel beads, exhibiting pH-sensitive properties and specificbiodegradability for colon targeted delivery of satranidazole [39]. The chitosan beads were prepared by the chemical cross-linking, followed by enteric coating with Eudragit® S-100. The results exhibited that Eudragit S100 coating on the chitosan beads prevented the premature drug release in simulated upper gastrointestinal conditions. As a consequence, most of the loaded drugs were released in the colon, an environment rich in bacterial enzymes that degrade the chitosan. Chourasia et al. prepared a similar multiparticulate system by coating cross-linked chitosan microspheres with Eudragit® L-100 and S-100 as pH-sensitive polymers, for targeted delivery of metronidazole, a broad-spectrum antibacterial agent [40]. In vitro drug-release studies were performed in conditions simulating stomach-to-colon transit in the presence and absence of rat cecal contents. The results showed a pH-dependent release of the drug attributable to the presence of the Eudragit® coating. Moreover, the release of drug was found to be higher in the presence of rat cecal contents, indicating the susceptibility of the chitosan matrix to colonic enzymes. Similar nanoparticular systems for colon-specificdelivery of metronidazole were reported by Elzatahry and Eldin [41]. Hyaluronic acid-coupled chitosan nanoparticles bearing 5-.uorouracil (5-FU) were also prepared by an ionotropic gelation method for the effective delivery of the drug to the colon tumors [42]. These nanoparticles showed enhanced cellular uptake by HT-29 colon cancer cells compared to the uncoupled nanoparticles. The cytotoxicity of 5-FU incorporated in nanoparticles was higher compared to the free 5-FU solution.

Liver-targeted drug delivery

The liver is a critical target tissue for drug delivery because many fatal conditions including chronic hepatitis, enzyme de.ciency, and hepatoma occur in hepatocytes. In general, liver-targeting systems employ passive trapping of microparticles by reticuloendothelium or active targeting based on recognition between hepatic receptor and ligand-bearing particulates [43]. Machida et al. evaluated the potential of lactosaminated N-succinyl-chitosan (Lac-Suc), synthesized by reductive amination between N-succinyl-chitosan and lactose in the presence of sodium cyanoborohydride, as a liverspeci .c drug carrier [44]. When Lac-Suc labeled with FITC was intravenously injected into mice, it initially underwent fast hepatic clearance and showed maximum liver localization at 8 h. The specific binding of Lac-Suc to the asialoglycoprotein receptors, which are found at the liver parenchymal cells, was also examined using competitive binding studies with asialofetuin in vivo. The results revealed that the liver uptake of Lac-Suc was inhibited by asialofetuin, and it was suggested that the liver distribution of Lac-Suc should be concerned with the asialoglycoprotein receptor. In another study, the authors demonstrated the targeting ability of Lac-Suc to the early metastatic stage of liver cancer [45].

Recently, Liu et al. prepared polyion complex micelles (PIC micelles) based on methoxy poly (ethylene glycol) (PEG)-grafted chitosan and lactose-conjugated PEG-graft-chitosan for liver-targeted delivery of diammonium glycyrrhizinate (DG) [46]. DG has been used in the treatment of chronic hepatitis and immunode.ciency virus infection. Pharmacokinetic experiments carried out using rats showed that the area under the curve (AUC) values of DG for PIC micelles were higher than that for DG injection. The lactose-conjugated PIC (Lac-PIC) micelles delivered more DG to the liver than conventional PIC micelles, indicating that Lac-PIC micelles were promising livertargeted nanocarriers for DG. Ping et al. conjugated glycyrrhizin (GL) to the surface of chitosan nanoparticles (CS-NP)s, prepared by an ionic gelation process [47]. These nanoparticles were developed for a drug delivery system targeting the liver through a specific interaction between GL and hepatocytes. In this study, adriamycin, chosen as the model drug, was encapsulated into the nanoparticles. The loading ef.ciencies of the drug for CS-NPs and GL-modi.ed nanoparticles (GLCS- NPs) were 65.5% and 91.7%, respectively. The higher loading ef.ciency of GL-CS-NPs was attributed to the ionic interaction between adriamycin and oxidized GL. Flow cytometry and confocal laser microscopy studies exhibited preferential accumulation of GLCS- NPs in hepatocytes. The cellular uptake of GL-CS-NPs was dependent on incubation time and dose of nanoparticles, suggesting that internalization of these nanoparticles into hepatocytes was mostly mediated by a ligand–receptor interaction.

Kidney and lung targeted delivery

Kidney-targeted drug delivery is critical when attempting to reduce extra-renal toxicity of the drug and to improve its therapeutic ef.ciency for diseases occurring at the kidney. It may be particularly bene.cial for drugs such as non-steroidal anti-in.ammatory drugs (NSAIDS) [48]. The mesangial cells of the glomerulus, the proximaltubular cells, and the interstitial .broblasts are principal targets for renal drug delivery since they play a pivotal role in many disease processes in the kidney. Several strategies have been proposed for drug targeting to the kidney in the form of drug-carrier conjugates [49–51]. However, these systems often suffer from renal toxicity, cardiovascular side effects, and poor biocompatibility [52,53]. Therefore, researchers have devoted their efforts in developing highly safe carrier systems for the drugs. Recently, Zhang et al. reported that randomly 50% N-acetylated low molecular weight chitosan (LMWC) selectively accumulated in the kidneys, especially in the renal tubes after intravenous injection into mice [54]. In an attempt to develop drug delivery system for renal targeting, the authors conjugated prednisolone to LMWC (19 kDa) through a succinic acid spacer. The distribution of the conjugates in the kidney was found to be 13 fold higher than that of prednisolone alone. It was concluded that LMWC with a proper molecular weight could be applied as a promising carrier for renal targeting. In an additional study, the site-specific uptake of LMWC was found to be mediated by the megalin receptor whose ligand shares a similar glucosamine unit level with LMWC [55]. To elucidate the exact mechanism behind the selective accumulation of LMWC in the kidney, the renal uptake process of LMWC was investigated. A megalin-shedding animal model along with the competitive inhibition assay con.rmed that after selective accumulation of LMWC in the kidney,

LMWC was speci.cally taken up by renal tubular cells, where the megalin receptor mediated its binding and uptake.

Lung cancer is one of the most prevalent cancers and is the leading cause of cancer mortality in the developed world [56]. In particular, non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancers. Delivering drugs to the lungs has many advantages over others because lungs have a large alveolar surface area, thin epithelial barrier, extensive vascularization and relatively low enzymatic metabolic activity [57]. Intravenous injection of microspheres and inhalation are the possible administration routes for targeting drugs to the lungs. However, some studies have shown that microspheres with a particle diameter greater than 5 ìm could block blood capillaries and induce chronic obstructive pulmonary emphysema [58]. On the other hand, frequent inhalation may induce lung .brosis [59]. Hence, designing a proper carrier system is essential for successful delivery of the drug to the lung. Paclitaxel has shown signi.cant activity in advanced NSCLC. Recently, Shim et al. prepared chitosan-modi.ed poly(lactic-coglycolic acid) nanoparticles containing paclitaxel (C-NPs-paclitaxel) with a mean diameter of 200–300 nm by a solvent evaporation method [60]. The study demonstrated that the in vitro uptake of the nanoparticles by a lung cancer cell line (A549) was significantly increased by chitosan modi.cation. In particular, a lung-specific increase in the distribution index of paclitaxel (i.e., AUC(lung)/AUC (plasma)) was observed for C-NPs-paclitaxel, when administered to lung-metastasized mice via the tail vein at a paclitaxel dose of 10 mg/kg. Transient formation of nanoparticular aggregates in the bloodstream, followed by enhanced trapping in the lung capillaries, was proposed as the mechanism of lung tumor-specific distribution of C-NPs-paclitaxel. Also, the authors showed that under acidic tumor conditions, C-NPs became more positive and interacted strongly with the negatively charged tumor cells [61]. The enhanced interaction between C-NPs and tumor cells at the acidic microenvironment might be the underlying mechanism of lung tumor-specific accumulation of paclitaxel from C-NPs-paclitaxel.

Cancer-targeted drug delivery using chitosan and its derivatives

The critical bottleneck of conventional cancer chemotherapeutics includes high toxicity of most anticancer drugs, due to indiscriminate distribution of drugs towards disease and healthy cells following systemic administration. In addition, anticancer drugs often suffer from poor solubility in water and thus need to use organic solvents or detergents for clinical applications, resulting in undesirable side effects such as venous irritation and respiratory distress [62]. Therefore, designing a distinct carrier system that encapsulates a large quantity of drugs and speci.cally targets tumor cells is indispensable for successful cancer therapy.

Passive targeting — enhanced permeability and retention (EPR) effect

The origin of the EPR concept dates back to the late 19[illegible], when Maeda et al. discovered the selective accumulation of macromolecular drugs in tumor tissues [63,64]. The specificpassive accumulation of macromolecules was attributed to defective tumor vasculature with disorganized endothelium at the tumor site and a poor lymphatic drainage system. Since then, researchers have capitalized this concept for the delivery of various drugs by conjugating them with polymers or encapsulating within nanoparticles. Nowadays, it is evident that long circulating macromolecules (polymer–drug conjugates) and nano-sized particulates (such as micelles and liposomes) accumulate passively at the tumors due to the EPR effect [7].

Chitosan–drug conjugates

In 1975, Ringsdorf .rst proposed the concept of polymer–drug conjugates for delivering hydrophobic small molecular drugs to their site of action [65]. The polymer–drug conjugates are composed of a water-soluble polymer that is chemically conjugated to a drug via a biodegradable spacer. The spacer is usually stable in the bloodstream but cleaved at the target site by hydrolysis or enzymatic degradation. Such drug conjugates can be selectively accumulated at the tumor site by the EPR effects, followed by release of the drug by cleavage of the spacer. Based on this concept, several polymer–drug conjugates have recently entered into phase I/II clinical trials. The representative example is N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-based drug

conjugates such as HPMA copolymer –doxorubicin conjugate (PK1) and HPMA copolymer–doxorubicin conjugate containing galactosamine as a targeting moiety (PK2), developed for the treatment of primary or secondary liver cancer [66].

In recent years, chitosan–anticancer drug conjugates have also been investigated, as shown in Fig. 1. For example, doxorubicin-conjugated glycol chitosan (DOX–GC) with a cis-aconityl spacer was synthesized by chemical attachment of N-cis-aconityl DOX to GC using carbodiimide chemistry [67]. DOX–GC conjugates containing 2–5 wt.% DOX formed self-assembled nanoparticles in an aqueous condition, but those that contained DOX above 5.5 wt.% precipitated because of increased hydrophobicity. It is of interest to note that the hydrophobic nature of DOX within the conjugate allowed for its physical entrapment inside the nanoparticles. The loading contents of DOX in the nanoparticles increased up to 38.9 wt.%. The release rate of DOX from the nanoparticles was signi.cantly dependent on the pH of the media because the cis-aconityl spacer is readily cleavable at a low pH. When the DOX–GC nanoparticles were systemically administrated into the mice, they preferentially accumulated into the tumor tissue, ascribed to the EPR effect [67].

Low molecular weight chitosan conjugated with paclitaxel (LMWC-PTX) was also synthesized by chemical conjugation of LMWC and PTX through a succinate linker, which can be cleaved at physiological conditions (Fig. 1) [68]. This conjugate was evaluated as a carrier for the oral delivery of paclitaxel. LMWC (MW<10 kDa) exhibited more favorable characteristics than high molecular weight chitosan, such as lower toxicity and higher water solubility.Moreover, LMWC could quickly and reversibly open the tight junctions between human epithelial colorectal adenocarcinoma cells (Caco-2). This is a highly useful characteristic for a carrier of drug molecules, especially for oral delivery. LMWC-PTX was absorbed in the small intestine after oral administration and remained in its intact conjugate form until it reached the bloodstream. An advantage of LMWC-PTX for oral delivery of PTX is that LMWC-PTX has the ability to bypass the P-gpmediated barrier (ef.ux pump) in the gastrointestinal tract and CYP450-dependent metabolism in the intestine and liver [68]. N-succinyl-chitosan derivatives were conjugated with mitomycin C (MMC) using carbodiimide chemistry [69]. Owing to the hydrophilicity of N-succinyl-chitosan, the conjugate is water-soluble when the MMC content in the conjugate is less than 12%. The N-succinylchitosan conjugates exhibited good antitumor activities against various tumors such as murine leukaemias (L1210 and P388), B16 melanoma, Sarcoma 180 solid tumor, a murine liver metastatic tumor (M5076), and a murine hepatic cell carcinoma (MH134) [70].

Cross-linked chitosan nanoparticles

Chitosan and its derivatives can be covalently cross-linked to prepare nanosized particles as the drug carriers [71]. Fig. 2 shows the chemical reactions between chitosan and bifunctional cross-linkers. The cross-linking process involves formation of the covalent bonds between the chitosan chains and functional cross-linking agents. The representative chemical cross-linkers that have been widely used for chitosan include bi-functional agents such as PEG dicarboxylic acid, glutaraldehyde or monofunctional agents such as epichlorohydrin [72,73].

For the preparation of chitosan particles, several techniques are available such as emulsion, ionotropic gelation, reverse micellar, solvent evaporation, spray drying, coacervation, and sieving methods [74–76]. A variety of hydrophilic and hydrophobic drugs can be loaded into the chitosan nanoparticles during the preparation of the nanoparticles, in which the loading ef.ciency of the drug may depend on its physicochemical characteristics and the preparation method. The detailed methods for preparation of chitosan micro- and nanoparticulate systems have been extensively reviewed elsewhere [71,76]. For cancer therapy, a hydrophilic 5-.uorouracil was successfully loaded into chitosan nanoparticles (250–300 nm in diameter) using the water-in-oil emulsion method, followed by chemical crosslinking of the chitosan in the presence of glutaraldehydes [74]. Mitra et al. encapsulated doxorubicin conjugates into cross-linked chitosan nanoparticles using the reverse micellar method [77]. The antitumor effect of the resulting nanoparticles was evaluated in J774A.1 macrophage tumor cells implanted subcutaneously in Balb/c mice.

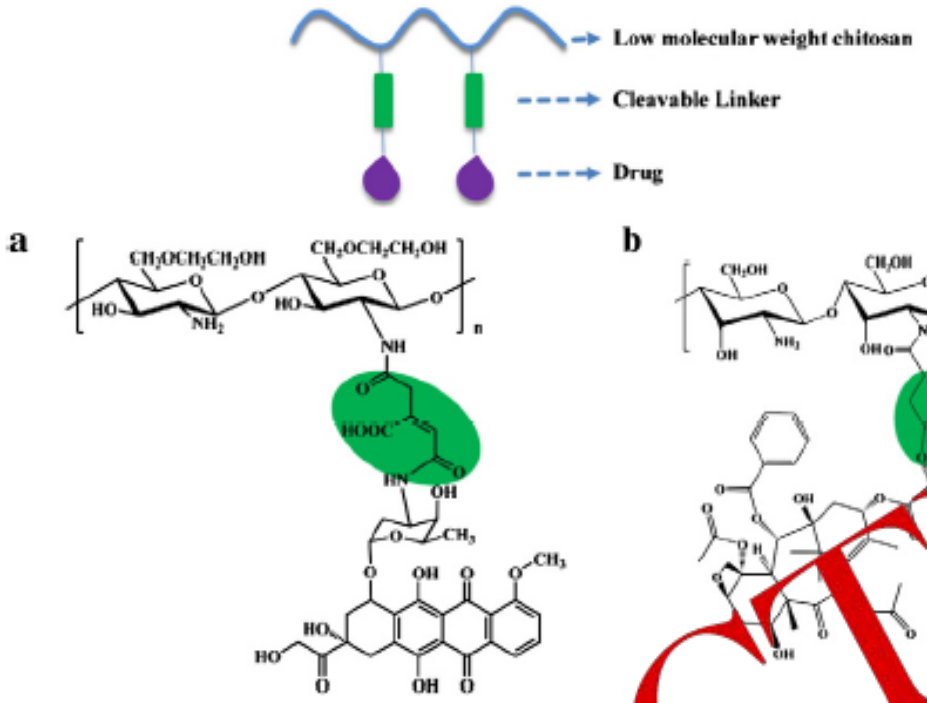

Figure 1. Schematic representation of the chitosan–drug conjugate bearing the cleavable linker. Chemical structure of (a) glycol chitosan–doxorubicin conjugate with the cis-aconityl linkage and (b) chitosan–paclitaxel conjugate with the succinate linkage.

The drug conjugate-encapsulated nanoparticles exhibited enhanced tumor regression than the drug conjugates itself, and the nanoparticular formulation showed better performance in relation to life expectancy.

The ionically cross-linked nanoparticles have often been prepared using the chitosan and its derivatives by exploiting their cationic nature, in which the amino groups of the chitosan backbone can interact with salts such as sodium sulfate, tripolyphosphate (TPP), or other multiple-charged anionic molecules [77]. The ionic cross-linking of chitosan is advantageous since the process is simple and often carried out under mild conditions without using organic solvents. Ionotropic gelation of chitosan using TPP for the encapsulation of drugs was .rst demonstrated by Bodmeier et al. [78] who intended to the preparation of chitosan beads. Later, Alonso et al. developed the preparation technique of chitosan nanoparticles, immediately formed through ionic interactions between the negatively charged phosphates of TPP and positively charged amino groups of chitosan [75]. Thereafter, TPP-cross-linked chitosan nanoparticles have been widely employed to deliver various small molecular drugs and biomacromolecular therapeutics. For example, Janes et al. effectively entrapped DOX into the chitosan nanoparticles during ionotropic gelation of the chitosan with TPP [79]. The cytotoxicity results of DOX-loaded nanoparticles in human melanoma A375 cells and C26 murine colorectal carcinoma cells indicated that nanoparticular formulations containing dextran sulfate were able to maintain cytostatic activity relative to free DOX. In addition, the confocal microscopy studies supported that DOX-loaded nanoparticles are internalized by cells and degraded intracellularly to release the drug.

Chitosan-based polyelectrolyte complex (PEC) nanoparticles

PECs, prepared by electrostatic interactions between oppositely charged polyions, have received considerable attention as carrier systems for drug and gene delivery [80–82]. The complex formation and the physical properties of PECs are in.uenced by many factors such as degree of ionization of the chitosan and anionic counterparts, chain .exibility, charge distribution over the polymer chain, pH,

temperature, time of interaction, ionic strength, and concentration of the polymeric solutions [83]. The preparation of PEC nanoparticles is quite simple and can be easily performed under mild conditions without the use of toxic organic reagents. It has been demonstrated that chitosan can form PEC nanoparticles with various polyanions such as hyaluronic acid, chondroitin sulfates, alginate, carboxymethyl cellulose, carrageenan, heparin, and poly(acrylic acid) [84–86]. Recently, chitosan has been investigated as the carrier of a hydrophilic 5-FU by forming PEC nanoparticles with polyaspartic acid sodium salt [87]. The drug-loaded nanoparticles showed sustained release of 5-FU both at the in vitro and in vivo conditions, compared to the pure 5FU solution. From the in vivo animal test, it was found that the tumor inhibition rate of PEC nanoparticles is much higher than that of 5-FU alone [88]. Cafaggi et al. prepared and evaluated the potential of PEC nanoparticles formed between anionic alginate and cationic chitosan or N-trimethyl chitosan as a particulate formulation for cisplatin [89]. The particle size of the nanoparticles was in the range of 180–350 nm, and the surface can be tuned to be negative or positive depending on the polyelectrolyte weight ratios. The resulting nanoparticles released cisplatin in a sustained manner in a PBS (pH=7.4). Cheng et al. have investigated the potential of DNA/chitosan nanocomplexes as a carrier of DOX [90]. They assessed in vivo biodistribution of FITC-chitosan and DNA/FITC-chitosan nanocomplexes after intravenous injection to the mice. After 24 h post-injection, the DNA/FITC-chitosan nanocomplexes were accumulated into the liver and kidney and remained at a relatively high stable level in blood, while the .uorescence intensity of free FITCchitosan decreased rapidly within 4 h post-injection. From in vitro cytotoxicity test, it was con.rmed that DNA/chitosan–DOX conjugate exhibited cytotoxic effects on HeLa, HepG2, QGY-7703, and L02 cells. Hu et al. prepared hollow nanosphere based on chitosan–poly (acrylic acid) (CS–PAA) as a carrier of DOX [91]. The in vitro cytotoxicity of DOX-loaded CS-PAA hollow nanospheres against HpG2 cells was comparable to the free DOX. The potential of folateconjugated PEC nanoparticles as targeted drug carrier was estimated by the cellular uptake behavior of the complex, formed between folate-conjugated poly-ã-glutamic acid (ã-PGA-FA) and FITC-labeled chitosan (CS-FITC), using A2780/AD ovarian cancer cells which overexpress folate receptors [92]. The confocal microscopic images revealed that the folate-bearing nanoparticles were readily taken up by the cells within 60 min.

Self-assembled chitosan nanoparticles

Polymeric amphiphiles can form self-assembled nanoparticles (SNPs) in an aqueous environment via hydrophobic interactions between the hydrophobic parts, primarily to minimize interfacial free energy. Since chitosan is a hydrophilic and cationic polysaccharide, chitosan-based SNPs can be readily obtained by chemically attaching the hydrophobic moiety to the backbone of chitosan and its derivatives, as shown in Fig. 3. These SNPs can circulate in the bloodstream for a relatively long time without recognition by phagocytes and can easily accumulate at the leaky vasculature throughout the EPR effect [93,94]. Enhanced accumulation at the tumor site can be achieved by conjugating the targetingmoiety to the SNPs (Fig.). Owing to the insoluble nature of chitosan (pKa=6.4) in water, the SNPs from chitosan amphiphiles are rapidly precipitated in a biological solution (pH 7.4).

Therefore, water-soluble chitosan derivatives have often been applied for development of SNPs in drug delivery systems [25,95–97]. For chemical conjugation of the hydrophobic moiety, the primary hydroxyl and amine groups of chitosan have been utilized using various synthetic routes. Numerous hydrophobic moieties have been used for development of amphiphilic chitosan derivatives such as bile acids (e.g., 5â- cholanic acid, cholic acid and deoxycholic acid) and fatty acids (e.g., palmitoyl acid, stearic acid, oleic acid) (Table 1) [20,95,98–102]. By varying the degree of substitution of the hydrophobic moiety, it is easy to control the particle size and zeta potentials of the nanoparticles which are important parameters affecting biodistribution of nanoparticles in vivo. As described earlier, chitosan-based SNPs can encapsulate a quantity of hydrophobic drugs inside the nanoparticles. Studies using chitosan nanoparticles have been carried out for various anticancer drugs [25,67,95–97]. In general, the results have demonstrated that chitosan nanoparticles are stable in a physiological solution without signi.cant change in the particle size for a long period of time. The cancer cells ef.ciently take

them up in vitro because the positively charged surface allows for strong interaction with themembrane of the cancer cells, facilitating endocytosis. When chitosan-based SNPs are systemically administrated into tumor-bearing mice, the nanoparticles are circulated in the bloodstream for at least 1 day, thereby increasing the probability of the nanoparticles reaching the target site [25]. The drug-loaded SNPs could release the biologically active agent in a sustainedmanner, in which the release rate of the drug is dependent on the type of hydrophobic moiety, its degree of substitution, and the physicochemical properties of the drugs. It should be emphasized that a signi.cant amount of chitosan-based SNPs have been reported to be selectively accumulated into the tumor site, primarily owing to the EPR effect [96,97]. As a consequence, drug-loaded nanoparticles have shown better therapeutic ef.cacy in vivo than the free drug. A few examples of SNPs for drug delivery, published in recent years, are as follows.

Kwon et al. developed hydrophobically modi.ed glycol chitosans (HGCs) by covalent conjugation of bile acid (5â-cholanic acid or deoxycholic acid) to the backbone of glycol chitosan using carbodiimide chemistry [20,103]. They controlled the degree of substitution, de.ned as the number of bile acids per 100 sugar units, by varying the feed ratio of the bile acid to glycol chitosan. The amphiphilicity, which is the hydrophobic–hydrophilic balance, was shown to greatly in.uence characteristics of nanoparticles such as their size, zeta potential, and morphology. In the aqueous state, SNPs were stable in biological conditions for at least 1 week. The critical aggregation concentration of HGCs was lower than those of low molecular weight surfactants. Animal experiments showed that HGCs prolonged blood circulation and exhibited high tumor speci.city for delivery of diverse anticancer drugs such as doxorubicin, paclitaxel, docetaxel, camptothecin and cisplatin [25, 67, 95–97].

Zhang et al. synthesized a series of chitosan derivatives carrying long alkyl chains (n=8, 10, 12) as hydrophobicmoieties and sulfated groups as hydrophilic moieties [104,105]. Alkylation was performed at the C-2 position and sulfonylation at the C-6 position of the chitosan. The resulting chitosan amphiphiles exhibited no intravenous stimulation, injection anaphylaxis, hemolysis, and cytotoxicity [106]. The authors suggested that the alkylated sulfate chitosans possessed a promising potential as the carrier of PTX. Wang et al. synthesized a cholesterol- modi.ed chitosan conjugatewith a succinyl linkage [107]. The potential of the conjugate as a drug carrier was evaluated using epirubicin. The drug loading capacity of the nanoparticles was found to be 7.97–14.0%. The drug was slowly released in vitro at phosphate-buffered saline (PBS, pH 7.4), bywhich the total amount of the drug released was 1.9% in 48 h. You et al. synthesized stearate-grafted chitosan oligosaccharide (CSSA) by reacting the carboxyl group of stearic acid with the amine group of chitosan [108]. CSSA exhibited a glycolipid-like structure because of the formation of multiple hydrophobic microdomains near the surface of the nanoparticles. This special spatial structure facilitated the effective internalization of the nanoparticles within the cancer cells (A549 cells). In addition, PTX-loaded nanoparticles were able to effectively deliver the drug into the cytoplasmof cancer cells. Thiswas due to protonation of the amine group of chitosan under acidic intracellular conditions, which exerts electrostatic repulsion between the molecules of the nanoparticle and increases the particle size. Recently, the authors also demonstrated that CSSA nanoparticles can effectively deliver doxorubicin into the nuclei of cancer cells [109,110].

Recently, carboxymethyl chitosan has been modi.ed with linoleic acid and evaluated as carrier for adriamycin [111]. The SNPs released adriamycin in a sustained manner, in which the drug-release rate was dependent on the linoleic acid degree of substitution on hydrophilic carboxymethyl chitosan. The in vitro antitumor activity of the drugloaded nanoparticles against HeLa cells was comparable to that of free adriamycin. Zhao et al. synthesized linoleic acid and poly(â-malic acid) double grafted chitosan (LMC) derivatives, which could selfassemble in the aqueous condition with a particle size of 190–350 nm [112]. The surface charge of the particles was negative in the physiological pH due to the presence of the ionized carboxyl groups of the poly(â-malic acid). PTX-loaded LMC nanoparticles exhibited signi.cant tumor inhibition ef.cacy relative to that of PTX in Sarcoma 180-bearing mice. Hemolysis and acute toxicity assessment indicated that the LMC nanoparticles could be safe drug carriers for intravenous administration.

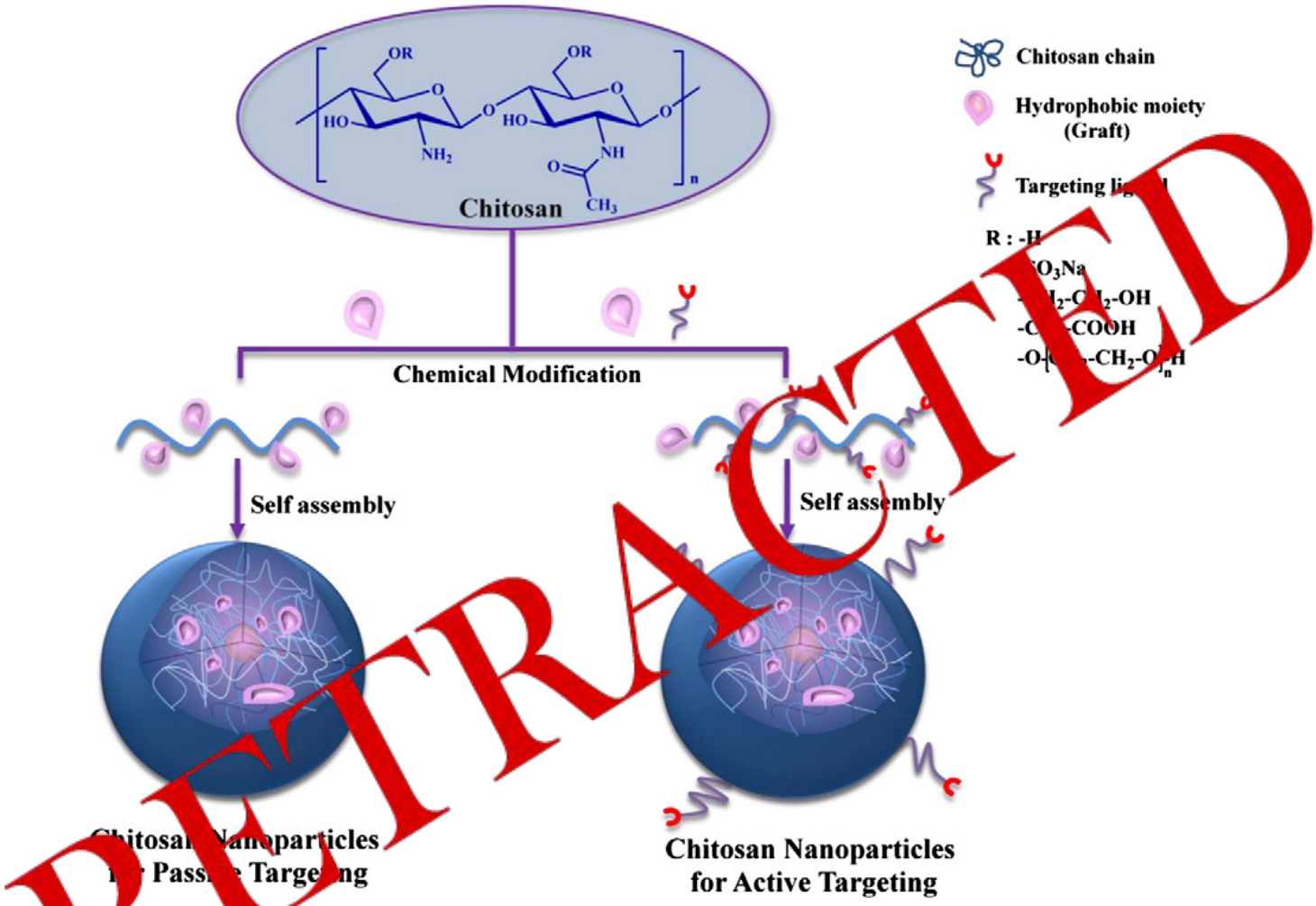

Figure 3. Self-assembled chitosan nanoparticles for passive or active cancer targeting.

PEGylated chitosan nanoparticles

Engineering the surface of the chitosan nanoparticles with PEG has attracted increasing attention because of its great potential in the therapeutic applications [113]. There are numerous publications that reviewed the importance and advantages of PEGylated nanoparticles for biological and pharmaceutical applications [114,115]. PEGylation of chitosan nanoparticles can increase their physical stability and prolong their circulation time in blood by reducing the removal by the reticuloendothelial system [113]. In addition, modi.cation of chitosan with PEG can decrease the positive charge of the particle surface.

PEGylated chitosan nanoparticles have been investigated as carriers for diverse small molecular drugs such as paclitaxel, camptothecin, methotrexate, and all-trans retinoic acid (ATRA) [116–120]. Recently, the effect of PEG conjugation on PTX-loaded N-octyl-sulfate chitosan nanoparticles was investigated by Qu et al. [116]. They found that PEG conjugated particles were phagocytized less than unconjugated nanoparticles by the reticuloendothelial system. The area under the curve of PEG-conjugated nanoparticles was much higher than the unconjugated one. All-trans retinoic acid (ATRA), a compound from retinoid class, is an effective drug for the treatment of epithelial and hematological malignancies but it can readily undergo degradation when exposed to light. This could be surmounted by incorporation of ATRA into N-phthalolylchitosan-g-mPEG (PLC-g-

mPEG) nanoparticles [117]. The photostability of ATRA in the nanoparticle was signi.cantly improved, when compared to ATRA in ethanol solution. Recently, Jeong et al. found that ATRA can be effectively incorporated into the methoxy poly(ethylene glycol)-grafted chitosan nanoparticles through ionic complexation [118].

Active targeting — receptor-mediated endocytosis (RME)

The accumulation of drugs in tumor tissue does not always guarantee successful therapy if the drug does not reach the target site of the tumor cell such as the cell membrane, cytosol, or nucleus. Therefore, a more effective mechanism should be employed such that the therapeutic agents are able to reach their molecular targets. Cancer cells often over-express some specific antigens or receptors on their surfaces, which can be utilized as targets in modern nanomedicine. Active targeting can be achieved by chemical alteration of nanosized drug carriers with targeting components that precisely recognize and specifically interact with receptors on the targeted tissue [121– 123]. In its early stage, researchers attempted direct conjugation of the targeting moiety to drugs. However, most clinical studies conducted for targeted drug conjugates failed to demonstrate their improved therapeutic effects on cancer treatment. This was due to a decrease in the biological activity of the drugs, compromised by conjugation of the targeting moiety. In addition, conjugation negatively affected the targeting molecule by disrupting receptor/ligand recognition [124]. To circumvent this problem, researchers developed an efficient drug delivery system comprised of (a) active chemotherapeutic drug, (b) targeting moiety, and (c) a nano-sized carrier made up of polymers or lipids. In this system, the therapeutic agents are physically entrapped in the carrier. This ternary system is very attractive over the ligand– drug conjugates for the following reasons: (i) the physically entrapped drugs can preserve its activity, (ii) a relatively large payload of drugs can be loaded into the hydrophobic cores of the carriers exceeding their intrinsic water solubility, (iii) the targeting moieties on the surface of the carriers can be precisely tuned to increase the probability of binding to the target cells, and (iv) owing to the small size of the carrier system, it can effectively infiltrate across the inflamed leaky disease vasculature but not at the normal vasculature [122]. For successful active targeting, the specific receptors should be expressed exclusively on the cancer cells but not on the normal cells. Several targeting moieties or ligands have been identified and successfully utilized for chitosan-based drug delivery systems.

Folic acid, a low molecular weight (441 Da) vitamin, has a high affinity for folate receptors (FRs), which are frequently over-expressed in many types of human cancerous cells, particularly those found in the epithelial tumors of various organs such as colon, lung, prostate and ovaries. Therefore, folate-conjugated drugs or carriers can be rapidly internalized into cancer cells via receptor-mediated endocytosis. You et al. synthesized folate-conjugated stearic acid-grafted chitosan oligosaccharides (Fa-CSOSA) by reacting CSOSA with folic acid in the presence of carbodiimide coupling agents [125]. The cellular uptake of Fa-CSOSA nanoparticles bearing PTX (4.8% (w/w)) via receptor-mediated endocytosis was tested. The authors demonstrated that HeLa cells expressing a large amount of FRs on the cell membrane rapidly took up the Fa-CSOSA nanoparticles, in comparison to A549 cells, an FR-de.cient cell line. Transferrin (Tf), an 80-kDa glycoprotein, is found abundantly in the blood. The main function of Tf is to transport iron to cells with the transferrin receptors (TfRs). Since TfRs are over-expressed in malignant tissues, Tf can be used as a ligand for tumor targeting. It has been con.rmed that Tf-mediated drug delivery systems can overcome drug resistance because they can be internalized by avoiding the membrane-associated drug resistance proteins such as p-glycoprotein [126]. Dufes et al. prepared Tf-decorated palmitoylated glycol chitosan (GCP) nanoparticles which encapsulated a quantity of DOX [24]. The results showed that A431 cells effectively assimilated the Tf-GCP nanoparticles in comparison to the nontargeted nanoparticles. All nanoparticular formulations using GCP showed a superior in vivo safety pro.le, compared to the free drug. As described earlier, a chemical compound containing the galactose moiety can be recognized speci.cally by the asialoglycoprotein receptors found in liver parenchymal cells. Therefore, galactosylated chitosan provides an opportunity for the development of imaging agents and drug carriers for liver-related diseases [127].

Ping et al. prepared galactosylated chitosan-coated BSA nanoparticles containing 5-FU for the treatment of liver cancer [128]. In this study, 5-FU was physically encapsulated into BSA nanoparticles, followed by surface coating with N-galactosylated chitosan by electrostatic interactions. Compared to the uncoated nanoparticles, coated nanoparticles showed a sustained release of 5-FU without the signi.cant initial burst in vitro. In general, successful and active drug targeting depends on various parameters, such as the choice of targeting ligands, the conjugation method of the ligands to carriers, and the ligand density on the carrier surface. For example, coupling of ligand to carrier can entrap ligands in the particle interior, which may not be available for receptor-binding [122,129]. The reactive amino group of chitosan allows the facile conjugation of the targeting moieties. The schematic illustration for syntheses of FA-conjugated and galactosylated chitosans is shown in Fig. 4. The carboxylic acid of the folic acid has often been reacted with the amino group of the chitosan and its derivatives in the presence of [1-ethyl-3- (dimethylamino)propyl]carbodiimide hydrochloride (EDC) [125]. The galactosylated chitosan was synthesized by the reaction of chitosan with lactobionic acid in the presence of N,N'-dicyclohexylcarbodiimide (DCC) and N,N,N',N'-tetramethylethylenediamine (TEMED) [128].

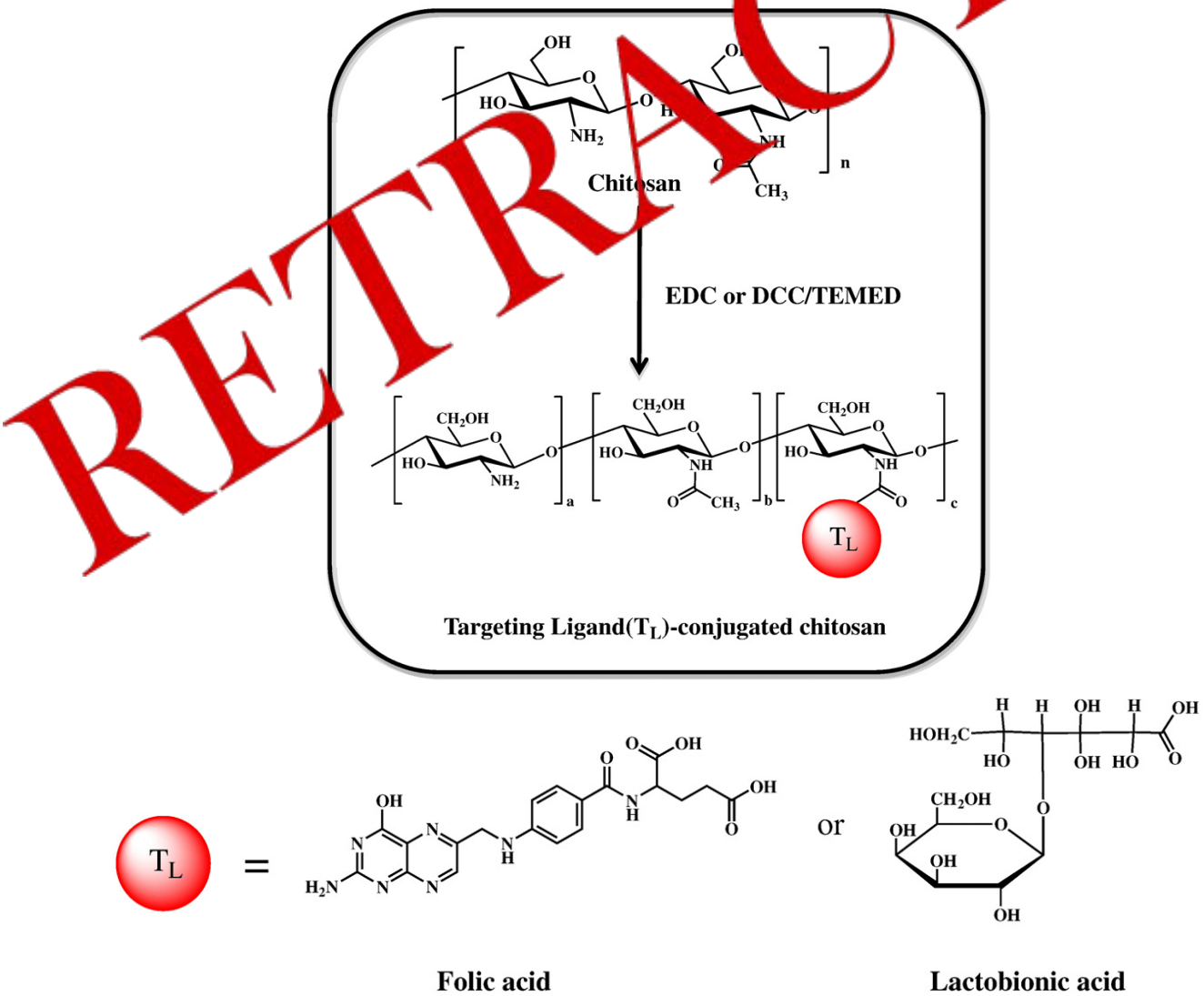

Figure 4. Representative synthetic route for chitosan derivatives containing targeting moieties.

Physical targeting

Chitosan-based stimuli-sensitive formulations Increasing efforts have been made to exploit physiological signals such as pH, temperature, ionic strength, and metabolites for targeted drug delivery applications [130–133]. Of the various stimuli, pH and temperature have been widely investigated for the treatment of solid tumors.Numerous reports havedemonstrated that in.amed or neoplastic tissues could exhibit a lower pH value (acidosis) or a higher temperature (hyperthermia) than healthy tissue [131,132]. Therefore, drug targeting to solid tumors can be achieved by designing stimuli-sensitive drug carriers, which disintegrate and release the entrapped drugs in response to a lower pH or higher temperature speci.cally at the tumor site. The interstitial pH of the tumor plays a prominent role in cancer therapy. In a healthy human, the extracellular pH of the body tissue and blood is maintained around 7.4. In contrast, the tumor tissue exhibits substantially lower pH values varying from5.7 to 7.8, depending on the tumor histology and volume [134,135]. The decrease in extracellular pH values in the tumor tissue is primarily due to poor organization of the vasculature in the tumor resulting in slowblood pressure, local hypoxia, and accumulation of acidic metabolites. This difference in pH between tumors and normal tissue has stimulated many investigators to design novel pH-sensitive carriers [136,137]. For example, Yan et al. prepared a camptothecin-loaded poly(N-isopropylacrylamide) poly(NIPAAm) chitosan nanoparticle and evaluated its potential as a pH-sensitive carrier in tumor targeting [138]. The nanoparticles encapsulated 8.4% of the drug with a loading ef.ciency of 73.7%. The in vitro cytotoxicity of the drug-loaded nanoparticles was compared with free camptothecin against SW480 cells at pH values of 6.8 and 7.4. The drug-loaded nanoparticles significantly enhanced cytotoxicity at pH 6.8 but displayed minimal cytotoxicity at pH 7.4. This distinction was ascribed to pH-sensitive drug-release behavior of the carrier system. In particular, when the mass ratio between the NIPAAm and chitosan was 4:1, the drug-loaded nanoparticles were more sensitive to tumor pH [138].

For anticancer drugswhose targetmolecules arewithin the cells, the drugs have to penetrate the cellular membrane and escape from the endosome before exhibiting their biological effects. In the case of paclitaxel, whose primary site of action is the microtubule, its intracellular concentration is critical for its pharmacological effect. Therefore, ef.cient intracellular delivery of such drugs is essential to eradicate cancer cells. Recently, N-acetyl histidine conjugated glycol chitosan (NAcHis–GC), where histidine (with imidazole group, pKa value of 6.5) acts as pH-responsive fusogen, was developed for the ef.cient intracytoplasmic delivery of paclitaxel [139]. TheNAcHis–GC conjugate formed self-assembled nanoparticles, with mean diameters of 150–250 nm, at neutral pH due to the hydrophobic nature of the NAcHis group. However, under slightly acidic conditions (similar to endosomal), theimidazole group ofNAcHis gets protonated. Thismay induce the in.ux of water and ions into endosomes when the nanoparticles are taken up by the cells, causing disruption of endosomal membranes (Fig. 5). As a consequence, the disassembled nanoparticles could release the encapsulated paclitaxel into the cytosol.

Kumacheva et al. prepared pH-responsive chitosan-based microgels (<200 nm diameter) by ionically cross-linking N-[(2-hydroxy- 3-trimethylammonium)propyl]chitosan chloride in the presence of tripolyphosphate [140]. These microgels were loaded with methotrexate and conjugated to apo-transferrin. The authors demonstrated that the conjugated microgels exhibited a signi.cant increase in cell mortality of HeLa cells, compared to non-conjugated microgels. This was ascribed not only to receptor-mediated endocytosis of the conjugated microgels, but also to pH-mediated release of methotrexate from the microgels by their swelling at the intracellular level.

Chitosan-based magnetic nanoparticles

Magnetic targeting, an attractive physical targeting technique, is garnering substantial attention for drug delivery applications. Here, the therapeutic agents to be delivered are either immobilized on the surface or encapsulated into the magnetic micro- or nanoparticulate carriers. These magnetic carriers, upon intravenous administration, concentrate at the specificsite of interest (tumor site) using an external high-gradient magnetic .eld (Fig. 6) [141]. After accumulation of the magnetic carrier at the target tumor site in vivo, drugs are released from the magnetic carrier and effectively taken up by the tumor cells.

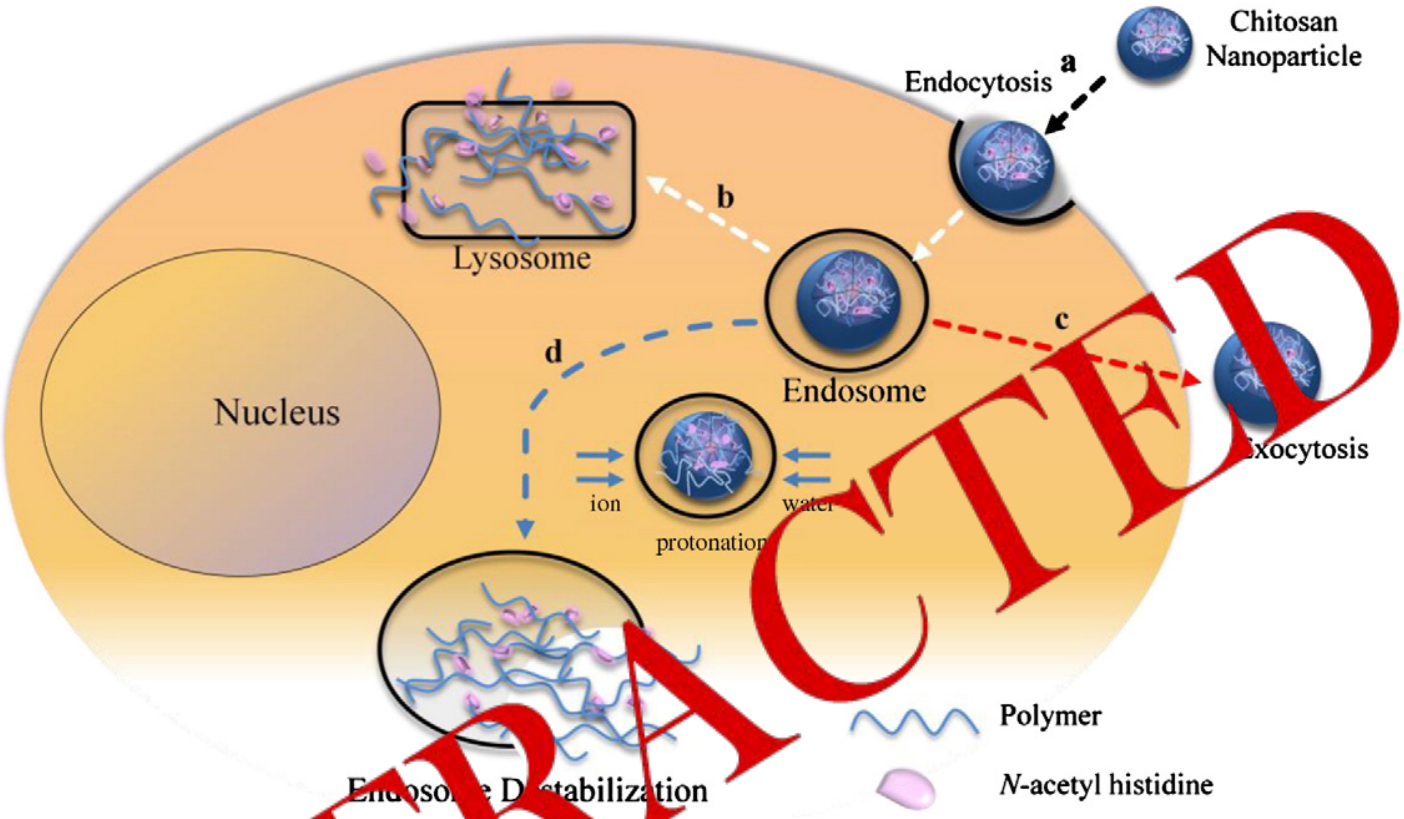

Figure 5. Schematic representation of a proposed model for the cellular internalization and drug release of NAcHis–GC nanoparticles. (a) Internalization of NAcHis–GC nanoparticles is initiated by nonspecific interactions between nanoparticles and cell membranes. (b) A part of the nanoparticles is exocytosed. (c)Without a specific mechanism for endosomal escape, drug-loaded nanoparticles are trafficked to lysosomes,where a high level of lysosomal enzymes is present. Drugs sensitive to these enzymes are degraded and lose their activity. (d) Under slightly acidic environments in endosomes, the imidazole group of histidine is protonated, causing the disruption of endosomal membranes and simultaneous delivery of drugs into the cytosol. Modified with permission from Ref. [64].

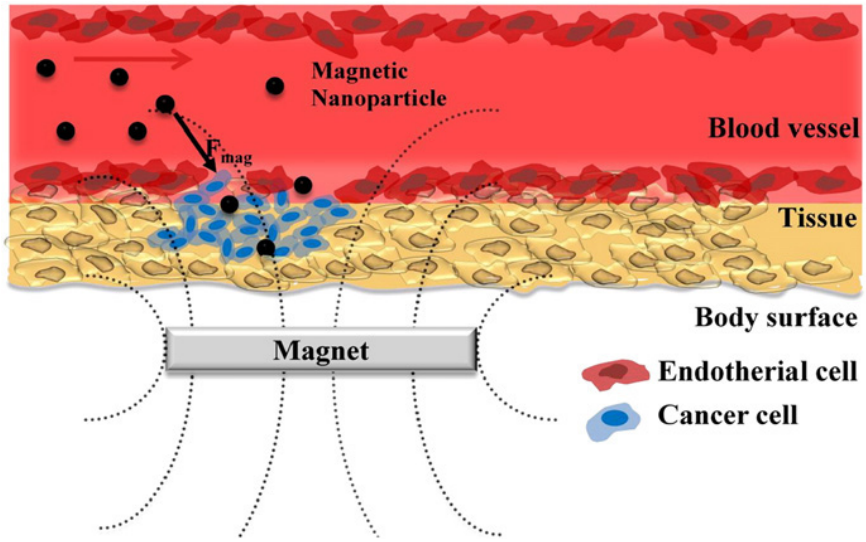

Figure 6. Schematic representation of magnetic nanoparticle-based drug delivery system.

The ef.ciency of the carrier accumulation depends on various parameters that include intensity of the magnetic .eld, rate of blood .ow, and surface characteristics of carriers.

Targeted delivery of therapeutic agents to the brain has enormous potential for the treatment of several neurological disorders such as Alzheimer's disease and brain tumor. However, the blood–brain barrier (BBB) signi.cantly impedes the entry of drug molecules into the brain from the bloodstream. Drug-loaded magnetic particulates represent a promising alternative strategy in overcoming the BBB. Gallo et al. developed magnetic chitosan microspheres containing oxantrazole (MCM-OX), an anticancer drug, for the treatment of brain tumors [142]. The authors monitored the levels of OX in the brain after administering intraarterial injections of MCM-OX to male Fischer 344 rats under a magnetic .eld of 6000G for 30 min. Compared to OX in solution, there was at least a 100 fold increase in OX concentrations in the brain after administration of MCM-OX. Interestingly, even in the absence of an external magnetic .eld, the OX concentrations were similar at 120 min and 30 min after MCM-OX treatment. This was attributed to the cationic–anionic interactions of MCM-OX with the blood brain barrier. More recently, Sheng et al. developed chitosan coated magnetic nanoparticles containing 5-FU (CS-5-FU MNPs) through a reverse microemulsion method, as a potential drug delivery system [143]. The resulting nanoparticles released their drugs in a sustained manner under in vitro conditions. The FITC-labeled CS-5-FU MNPs effectively gained entry into the SPC-A-1 cancer cells and induced cell apoptosis. In a similar study, Chen et al. prepared chitosan-bound magnetic nanoparticles loaded with epirubicin, an anthracyline drug used for cancer chemotherapy [144]. The magnetic nanoparticles were stable at pH 3–7, and approximately 80% of the drug was released after 150–300 min in a biological buffer. The in vitro anticancer ef.cacy of the drug-loaded magnetic nanoparticles was comparable to that of the free drug. Misra et al. encapsulated doxorubicin-conjugated magnetite nanoparticles into a thermosensitive polymer, chitosan-g-poly(N-isopropylacrylamideco- N,N-dimethylacrylamide) [145]. The thermosensitive polymer exhibited a low critical solution temperature of ~38°C. Since doxorubicin was conjugated to the magnetite via acid-labile hydroazone- bond, the nanoparticles released the drugs in response to a change in external temperature or pH. This particular system is expected to have potential applications in magnetic field-assisted drug delivery.

CONCLUSIONS

Targeted delivery of drugs is critical in improving therapeutic efficacy and minimizing side effects. Since Paul Ehrlich suggested the concept of a "magic bullet", many research scientists have attempted to develop drugs that selectively destroy disease cells but are not harmful to healthy cells. Many approaches are currently available to deliver the drugs to the specific site of action. The drug conjugate can be designed by covalently attaching the targeting moiety to the drug. Otherwise, the drug can be physically encapsulated into nano-sized particles that have the ability to reach the target site. It is also possible to design prodrugs that are not biologically active until they meet the target molecules. For the development of such targeted delivery systems, chitosan and its derivatives possess various advantages such as biocompatibility, biodegradability, mucoadhesivity, and other unique biological properties. Over the last decade, increasing attention has been paid to the development of systems to deliver drugs for long periods at controlled rates. Some of these systems can deliver drugs continuously for over one year. However, little effort has been given to developing systems for the controlled release of nucleic acids. Recently, a novel gene transfer method which allows prolonged release and expression of plasmid DNA in vivo in normal adult animals was established. In this system, a biocompatible natural polymer such as collagen or its derivatives acts as the carrier for the delivery of DNA vectors. The biomaterial carrying the plasmid DNA was administered into animals and, once introduced, gradually released plasmid DNA in vivo. A single injection of plasmid DNA biomaterial produced physiologically significant levels of gene-encoding proteins in the local and systemic circulation of animals and resulted in prolonged biological effects. These results suggest that the biomaterials carrying plasmid DNA may enhance the clinical potency of plasmid-based gene transfer, facilitating a more effective and long-term use of naked plasmid vectors for gene therapy. Furthermore, the

biomaterials can be removed surgically, minimizing the effect of gene products if some unexpected side effects should be observed after application. The application of these systems to expand the bioavailability of molecular medicine, including antisense oligonucleotides and adenovirus vectors, and to aid in stem cell transplantation in the context of DNA-based tissue engineering will be discussed. Chitosan has been the subject of interest for its use as a polymeric drug carrier material in dosage form design due to its appealing properties such as biocompatibility, biodegradability, low toxicity and relatively low production cost from abundant natural sources. However, one drawback of using this natural polysaccharide in modified release dosage forms for oral administration is its fast dissolution rate in the stomach. Since chitosan is positively charged at low pH values (below its pK_a value), it spontaneously associates with negatively charged polyions in solution to form polyelectrolyte complexes. These chitosan based polyelectrolyte complexes exhibit favourable physicochemical properties with preservation of chitosan's biocompatible characteristics. These complexes are therefore good candidate excipient materials for the design of different types of dosage forms. It is the aim of this review to describe complexation of chitosan with selected natural and synthetic polyanions and to indicate some of the factors that influence the formation and stability of these polyelectrolyte complexes. Furthermore, recent investigations into the use of these complexes as excipients in drug delivery systems such as nano- and microparticles, beads, fibers, sponges and matrix type tablets are briefly described. The properties of chitosan are greatly influenced by its molecular weight and degree of deacetylation. The presence of reactive functional groups in chitosan provides great opportunity for chemical modification, which affords a wide range of derivatives possessing unique properties. Overall, it is evident that chitosan and its derivatives are useful carriers for low molecular drugs requiring targeted delivery. The scope of polymers used in dosage form design can be increased by several approaches such as modification of their chemical structure, by combining different polymers in physical mixtures or by formation of polymer-polymer associations such as polyelectrolyte complexes. Polyelectrolyte complexes combine unique physicochemical properties of different polymers with the advantage of retaining high biocompatibility. It is therefore not surprising that polyelectrolyte complexes are gaining importance in modern pharmaceutical technology. From the *in vitro* studies conducted on chitosan-based polyelectrolyte complexes it is clear that they are valuable excipients with specific properties for efficient dosage form design, which may be valuable in the development of modified drug delivery systems. Unfortunately, the literature lacks *in vivo* data in terms of drug delivery from these dosage forms which makes it difficult to be conclusive in terms of their effectiveness as drug carriers at this stage. Since some work has been done on *in vitro-in vivo* correlations with chemically cross-linked chitosan hydrogels with successful sustained drug delivery in animals, it is anticipated that optimized chitosan based polyelectrolyte complexes may also perform up to expectation for *in vivo* drug delivery.

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