Hari S. Sharma · Dafin F. Muresanu
Aruna Sharma *Editors*

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Preface

Recent advances in nanotechnology suggest its involvement in health, environment, drug development, diagnostic purposes, industries affecting almost every aspect of household items, automobile engineering to body parts or tissue implants influencing every sphere of life. However, on the one hand nanotechnologies have advanced medical care, health improvement and drug delivery; on the other hand, their adverse effects on the environment, biological material, food and water intoxication, industrial waste, air pollution affecting human biospheres and other health factors cannot be ignored. Thus, use of nanotechnologies is still a matter of debate in relation to their beneficial vs. adverse effects on human health factors.

One of the important challenging tasks of nanotechnology is to deliver drugs to the central nervous system (CNS) for treating diseases afflicting brain. Since access of drugs to the CNS is very limited due to the presence of an effective blood–brain barrier (BBB), there is an urgent need to explore possible ways using nanotechnologies to deliver drugs or therapeutic agents across the BBB to induce neuroprotection and/or to enhance neuroregeneration.

This volume for the first time highlights nanodelivery of drugs and genes to the CNS for inducing neuroprotection and enhancing neuroregeneration in different diseases in a concise manner. The book is a reviewed collection of invited reviews from leading experts across the world. One of the key highlights of the book deals with enhanced capacity of nanodelivery of drugs in CNS pathology often associated with co-morbidity factors, e.g., hypertension, diabetes or heat exposure. Our military personnel are often exposed to various kinds of nanoparticles in the battlefield emanating from gun powder explosion, missile injury and other environmental nanomaterials, e.g., silica dust. All these factors could induce greater degree of brain pathology following any CNS insults. Under such situations, normal drug delivery may not be able to thwart brain pathology. However, experimental evidences indicate that nanodelivery of multimodal compounds may have better therapeutic efficacy. This suggests that nanodelivery of drugs and genes requires further exploration and explorations of our knowledge to provide effective therapeutic measures in future strategies to treat CNS injuries.

Another important aspect of nanodelivery of drugs is to choose suitable nanomaterials for effective delivery. This aspect is also discussed using various techniques of nanodelivery, e.g., nanowired drug delivery, viral vector gene delivery, or other nanocarriers for enhanced penetration into the CNS.

We feel that this book will open new vistas for study and research on the novel aspects of nanodelivery of drugs and genes into the CNS using various approaches for better therapeutic approaches in future. The book will be highly useful for policy makers, medical practitioners, health care givers, researchers, students, neuropharmacologists, neurologists, neurosurgeons and neuroscientists alike.

We hope that the book will be used as a novel basis for further research in the field for the benefit of mankind.

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Cluj-Napoca, Romania — Dafin F. Muresanu
Uppsala, Sweden — Aruna Sharma

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Chapter 1
Renewable Biomaterials as Nanocarriers for Drug and Gene Delivery

Shimiao Zhang, Liejiang Jin, Muhammad Arshad, and Aman Ullah

Abstract Recently, due to strong emphasis on environmental awareness worldwide, utilization of renewable feedstocks has been growing in the development of materials for various applications. Amphiphilic polymeric materials have been widely used as drug and gene delivery carriers. These materials can self-assemble into different nanostructures, such as micelles, nanospheres, hydrogels, nanocapsules and polymersomes, which can serve as reservoirs for various therapeutic agents. Among numerous materials that can be used to fabricate these systems, those from renewable resources are of particular interest, due to the increasing environmental concerns as well as their natural abundance and favorable properties including biocompatibility, biodegradability and non-toxicity. In this chapter, naturally occurring materials such as polysaccharides, vegetable oils, terpenes and proteins, and their applications as nano delivery systems are reviewed.

Keywords Renewable resources • Amphiphilic polymers • Nanocarriers • Drug delivery • Gene delivery

1 Introduction

In the past few decades, the biomedical applications of nanotechnology have attracted wide attention. Since its emergence in 1980s, the rapid development of nanotechnology, along with our improved understanding in diseases, have provided tremendous promise in the prevention, diagnosis, monitoring and therapy of current intractable

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diseases such as cancer [1–3]. One of the most significant impacts of this parallel advancement is the development of various nanoparticulate pharmaceutical carriers, including micelles, liposomes, dendrimers, niosomes, solid-lipid nanoparticles, quantum dots and so on [4, 5]. The use of these nanocarriers has an enormous influence in both of the current pharmaceutical research and clinical practice. Traditionally, the exploitation of drugs, in many cases, is associated with one or more of the following problems: (1) poor aqueous solubility. The applications of hydrophobic drugs, such as paclitaxel, are largely restricted by their poor solubility and thus limited bioavailability; (2) side effects. For example, drug compounds used in cancer therapy can kill tumour cells as well as normal cells. The clinical applications of many drugs are also limited due to other adverse side effects such as cardiac toxicity, acute nephrotoxicity and chronic neurotoxicity; (3) unfavorable pharmacokinetic characteristics. Drugs suffer from fast metabolism and phagocytic clearance, thus requiring high dose and/or continuous drug administration; (4) non-selective biodistribution. Insufficient accumulation of the administrated drugs in targeted pathological areas results in suboptimal therapeutic efficacy, while those distributed to normal tissues can cause side effects and thereby limit the dose of the drugs that can be infused; (5) drug inactivation. Drugs are deactivated under the effect of physiological environment such as local pH [2, 6, 7]. However, these problems can be solved or reconciled with the use of the above-mentioned nanoparticulate carriers. Depending on their types and characteristics, nanocarriers play different roles in drug delivery systems (DDS), in which they may physically encapsulate the drugs inside the core (e.g. micelles, liposomes) or conjugate with the drug molecules (e.g. dendrimer-doxorubicin conjugates) [8–12]. Much effort has been made so far to study these nanocarriers and their potentials as delivery vehicles, from which a variety of important properties have been shown. Designed surface (e.g. surface modification with polyethylene glycol (PEG)) improves longevity of nanocarriers in blood which provides more time for the drug-loaded nanoparticles to accumulate in targeted zones [13, 14]. The attachment of the various ligands and cell-penetrating molecules to the surface enables specific targeting to disease sites and improved intracellular delivery [15–20]. The stimuli-sensitivity (e.g. pH-responsiveness and temperature-responsiveness) allows controlled drug release from the nanocarriers with the stimuli of local physiological environment [4, 21–23]. Multifunctional nanocarriers (i.e. a combination of several functions/properties in one particle) are also being studied, which are expected to be capable of further enhancing the therapeutic efficacy of many drugs [5, 24, 25].

Among various materials for delivery applications, amphiphilic compounds (e.g. block copolymers, surfactants) have been extensively used as carrier-forming materials. They can be organized into a range of nanoparticulate structures such as micelles, hydrogels, polymersomes, nanospheres and nanocapsules (Fig. 1.1) [26, 27]. Polymeric micelles, typically formed from amphiphilic block copolymers, have been recognized as one of the most promising carriers for water-insoluble therapeutic agents [28–31]. They belong to a family of colloidal dispersions and are formed from spontaneous association of amphiphilic molecules above a certain concentration. At a low concentration, such molecules disperse in the aqueous medium separately. However, as the concentration increases and reaches a critical value called critical micelle concentration (CMC), the unimers start to aggregate and form micelles [8].

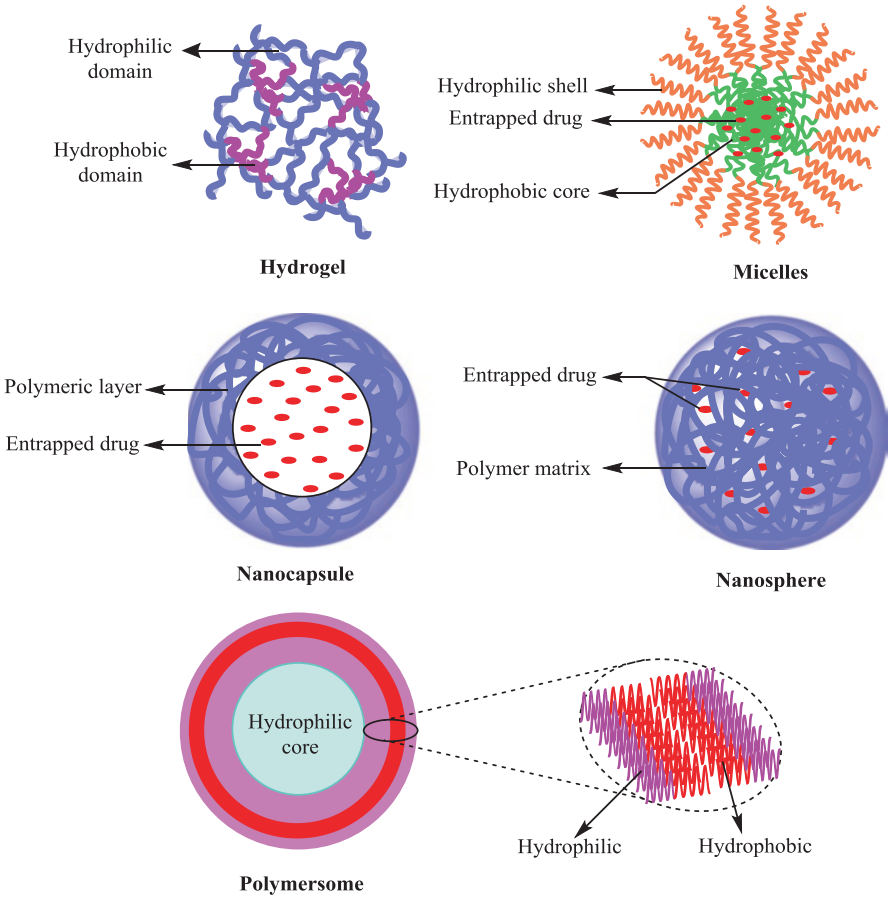

Fig. 1.1 Schematic illustrations of nanoparticulate carriers formed by amphiphilic copolymers

Such association process is driven by the free energy reduction of the system, as the interactions between hydrophobic segments and water molecules are replaced by hydrogen bond among water and hydrophobic interaction among hydrophobic fragments [32]. The formed micelles have a core-shell structure, in which the core comprises hydrophobic blocks and is stabilized by the shell formed from hydrophilic parts of the amphiphilic molecules [33]. When used as drug carriers, the water-insoluble drugs can be incorporated into the hydrophobic micellar core. Therefore, the solubility of drugs in aqueous medium can be significantly increased. Meanwhile, drugs are well protected by the hydrophilic corona as well [8]. The advantages of the polymeric micelles as nanocarriers are also shown by their small size (usually 10–200 nm) and narrow size distribution, stability due to the low CMC and capacity of passive targeting to tumor through the enhanced permeability and retention (EPR) effect, *etc* [7, 8, 34–36]. Moreover, the outer surface of polymeric micelles can be modified (e.g. end-functionalization of the micelle-forming block copolymers) to enable multiple functions (e.g. stimuli-sensitive, specific targeting) [37–39].

While sharing a similar architecture with polymeric micelles, polymeric nanospheres formed from amphiphilic copolymers have a phase-separate structure, in which the particle core is 'frozen' or 'solid-like' [26, 40]. They can be considered as matrix-type nanocarriers in which the drugs are entrapped and dispersed in the solid polymer matrix (particle core) [41]. However, it is not always possible to clearly distinguish polymeric nanospheres from micelles. For example, block copolymers can self-assemble into nanoparticles with 'solid-like' core when the molecular weight of the hydrophobic segments is high. They may also produce micelles with shorter core-forming blocks. Other factors such as preparation methods also play an important role in the formation of nanoparticles [26]. Unlike polymeric nanospheres, nanocapsules and polymersomes are reservoir-type, vesicular systems in which the drugs are incorporated inside the nanoparticles and surrounded by polymer membrane [41]. An essential difference between these two nanocarriers is that nanocapsules are composed of an oily core and a single polymer layer [42, 43], whereas the core of polymersomes is in an aqueous state and the surrounding polymer membrane is a bilayer [44–46]. Due to the presence of hydrophilic core and hydrophobic layer, polymersomes can be employed to deliver both water-soluble and poorly soluble molecules [46].

A variety of amphiphilic block copolymers can serve as nanocarrier-forming materials and typically they can be manufactured from fossil and petroleum resources. However, the rapid depletion of these resources and the increased awareness of environmental protection have resulted in a growing interest in materials derived from renewable resources such as wood, plant seeds and many other biomass materials [47–50]. Such renewable materials include, but not limited to, polysaccharides (e.g. cellulose, chitosan, starch and dextran), vegetable oil, terpenes and rosin [51–55]. Although these materials are not always naturally present as polymers (e.g. vegetable oil), they are precursors that can be used to synthesize a number of polymers such as polyurethanes, polyesters and polyethers [55, 56]. The biomass-based polymers then can be further modified before serving as drug delivery carriers. The studies of these materials have become a field of intensive interest due to the universally available resources, low price and generally-believed better biocompatibility and biodegradability than their synthetic polymer counterparts from fossil resources [57–60]. This chapter attempts to provide an overview of the research on the amphiphilic polymeric nanocarriers based on renewable materials. However, due to the sheer size of this field and limited space of this chapter, a complete coverage of all the materials is not possible. Therefore, the emphasis will be placed on some of the most important domains such as polysaccharides and vegetable oil. Other less common materials will be also briefly introduced.

2 Polysaccharides

Polysaccharides have abundant resources in nature (e.g. crustacea, insects, microbes, algae and many other plants). This class of materials includes a wide range of natural macromolecules such as cellulose, chitosan, pectin, hyaluronic acid, pullulan

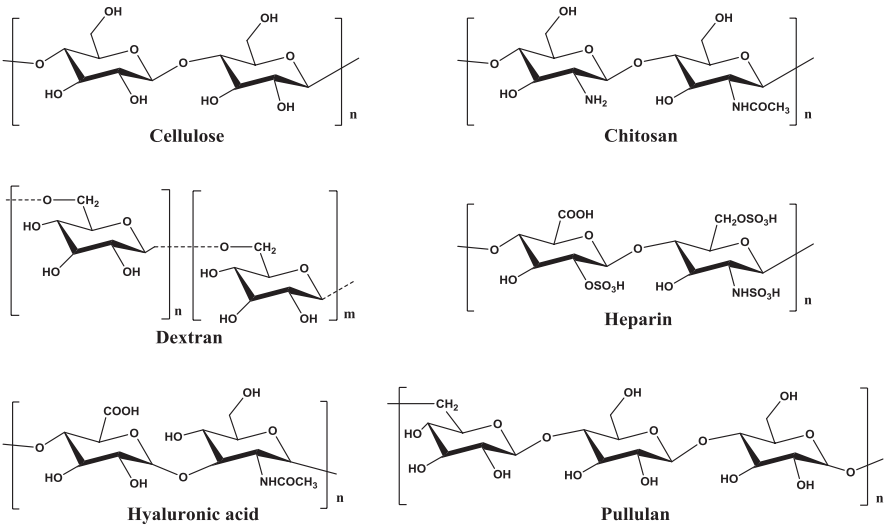

Fig. 1.2 Chemical structures of polysaccharides

and dextran [61–63]. As natural polymers, polysaccharides have shown advantages in stability, safety, biodegradability as well as low processing cost [62]. They can be easily modified due to the presence of many derivable groups in polymer chains and therefore various polysaccharides derivatives can be processed. All of these characteristics suggest their use as biomaterials. In recent years, a number of studies have been carried out to investigate the potential applications of this class of naturally occurring materials as nanocarriers for drug and gene delivery [64–68]. Because of the hydrophilic nature of these polymers, in many cases they are hydrophobically modified and used to form self-assembling nanoparticles. In the following subsections, polysaccharides are classified by chemical structures (Fig. 1.2) and their applications as amphiphilic polymeric nanocarriers are introduced.

2.1 Cellulose

Cellulose is the most abundant natural polymer available worldwide [61, 69]. It is an essential structural component of the cell walls of green plants, fungi, bacteria and many algae. Cellulose is a polymer of glucose units with β-glycosidic linkages and has a highly crystalline and fibrous structure, which restricts this polymer from dissolving in many organic and aqueous solvents [69, 70]. On the other hand, as a renewable polymer, the use of cellulose in pharmaceutical and biomedical field are attractive due to its biocompatibility, biodegradability, protein rejecting ability and safety [71]. To overcome the limitations of solubility and processability, many cellulose derivatives have been developed, such as hydroxypropylmethylcellulose

(HPMeC), hydroxypropylcellulose (HPC), hydroxyethylcellulose (HEC) and carboxymethylcellulose (CMeC), to name a few. These water-soluble derivatives of cellulose can be further modified for desired properties and/or functions. Compared with the extensive research on applications of cellulose and its derivatives in paper and pulp products and fibers, utilization of cellulose-based materials as amphiphilic nanocarriers is less frequent. However, several strategies have been proposed during the last few decades to develop cellulose-based amphiphiles for drug and gene delivery.

Amphiphilic cellulose-based polymers can be synthesized by hydrophobic modification of water-soluble cellulose derivatives with long chain alkyl or alkyl halides groups, alkyl epoxy compounds and cholesteryl groups [72–78]. These modified cellulose derivatives have potential applications as drug nanocarriers. Yang et al. [74] synthesized amphiphilic cholesteryl-bearing carboxymethylcellulose derivatives (CCMCs) and characterized the microstructures of the CCMC self-aggregates. The same group also used the prepared CCMC micelles as nanocarriers for a hydrophobic drug, indomethacin [79]. In the study, indomethacin could be loaded into the CCMC nanoparticles, which had a loading capacity (defined by the weight ratio of loaded drug and carrier) of more than 50%. The drug-loaded nanoparticles showed a slow and steady drug release behavior. Moreover, the CCMC micelles also showed pH-responsive capability during the drug encapsulation and release processes. Hydrophobically modified carboxymethylcellulose can also be prepared by esterification of this water-soluble cellulose derivative [75]. Graft copolymer of cellulose is a focus of recent research and various hydrophobic polymers have been used to modify cellulose, such as poly(poly(ethylene glycol) methyl ether methacrylate) (PPEGMA) [76], poly(acrylic acid) (PAA) [80] and poly(2-hydroxyethyl methacrylate) (PHEMA) [81], in which amphiphilic ethyl cellulose (EC)-based copolymers are synthesized by atom transfer radical polymerization (ATRP) technique. Wang et al. [82] synthesized pH-responsive ethyl cellulose graft poly(2-(diethylamino) ethyl methacrylate) (EC-g-PDEAEMA) via ATRP and the obtained amphiphilic graft copolymers could form micelles in acidic aqueous media. It was found that an increase in the graft length and graft density would result in a decreased CMC of the copolymers. The EC-g-PDEAEMA micelles were pH-sensitive, and the observed decrease in micellar hydrodynamic radius at pH 6–6.9 could be attributed to the collapse of shell-forming PDEAEMA chains. The study also used a model drug, rifampicin (RIF), to investigate the drug loading and release behavior of the micelles. The cumulative amount of RIF released from drug-loaded micelles in the buffer solution at pH 6.6 was found to be higher than that at pH 7.4. Other polymerization methods have also been used. Dong et al. [83] utilized ring opening graft polymerization to modify cellulose with poly(L-lactide) (PLLA) and the synthesized cellulose-g-PLLA copolymer exhibited good biocompatibility, as indicated by low cytotoxicity, and potential applicability as drug carriers.

Hydroxypropylcellulose (HPC) is a non-ionic polymer and can be dissolved in water and a number of organic solvents [62]. Stimuli-responsive systems based on modified HPC polymers have been studied, such as thermo- and pH-sensitive graft

copolymers HPC-graft-poly(4-vinyl pyridine) (HPC-g-P4VP) and HPC-graft-poly(*N*,*N*-dimethylaminoethyl methacrylate) (HPC-g-PDMAEMA) [84, 85]. HPC-based copolymers have also been used as drug and gene delivery carriers. Francis et al. [86] prepared hydroxypropylcellulose-g-polyoxyethylene alkylether (HPC-g-$(POE)_y$-C_n) polymeric micelles, aiming at improving the oral bioavailability of hydrophobic drugs. The cytotoxicity of the synthesized HPC-g-$(POE)_y$-C_n copolymers was evaluated by incubation with human colon adenocarcinoma (Caco-2 cells) for 24 h and compared to unmodified HPC and free $(POE)_y$-C_n molecules. While free $(POE)_y$-C_n could inhibit cell growth, no significant cytotoxicity was observed for HPC or HPC-g-$(POE)_y$-C_n copolymers. Cyclosporin A (CsA), a water-insoluble immunosuppressant, was chosen as a model drug in the study. The results showed that the HPC-g-$(POE)_y$-C_n copolymers exhibited improved capacity of drug incorporation compared to unmodified HPC, which could be further enhanced by increasing the number of $(POE)_y$-C_n units per HPC chain in the grafted polymers. Xu et al. [87] focused on comb-shaped cationic copolymers HPC-g-PDMAEMA (HPDs) composed of long HPC backbones and short PDMAEMA side chains, which were synthesized by ATRP. Quaternary ammonium HPDs (QHPDs) could be prepared by partial quaternization of the PDMAEMA side chains in HPDs. Both of the HPDs and QHPDs were evaluated *in vitro* as gene vectors. It was found that compared to PDMAEMA homopolymers of high molecular weight, HPDs showed much lower cytotoxicity and higher gene transfection yield. Meanwhile, due to the presence of more cationic charges on surface, QHPDs exhibited an enhanced ability to complex plasmid DNA (pDNA). Based on these results, the study indicated a versatile strategy for designing novel gene carriers by means of structural tailoring of functional comb-shaped cationic copolymers. Some other cellulose-based amphiphilic polymers have also been reported, such as 2,3-epoxypropylphenylether-modified HEC [88], cellulose-pyrene conjugates [89] and O-(2-hydroxy-3-butoxypropyl) cellulose (HBPC) [90]. The potential application of some of these materials as nanocarriers have been evaluated as well [91].

2.2 Chitin and Chitosan

Chitin is the second most abundant polysaccharides, only after cellulose [61]. It has a structure very similar to that of cellulose and the only difference is in the replacement of the hydroxyl group by an acetamido group. This material is highly insoluble and its chemical reactivity is low [92]. Chitosan is an *N*-deacetylated derivative of chitin and is only soluble in dilute acidic solutions, which restricts it from widespread use [93]. This can be attributed to the presence of numerous intra and intermolecular hydrogen bonds and the formation of semicrystalline structure when chitosan is in the solid state [92, 94]. However, chitosan has many water-soluble derivatives, such as glycol chitosan [95, 96], carboxymethyl chitosan [97] and *N*-trimethyl chitosan [98, 99], which have been widely used in chitosan-based drug delivery systems. Many hydrophobic molecules have been used to modify chitosan,

such as various fatty acids (e.g. linoleic acid, oleic acid and stearic acid), bile acids (e.g. deoxycholic acid and 5β-cholanic acid) and polyesters (e.g. poly(ε-caprolactone)). The introduction of hydrophilic or hydrophobic substitutes to chitosan molecular chains may weaken the hydrogen bonding and destroy the semicrystalline structure, thereby increasing its solubility [93]. Meanwhile, other factors, such as degree of deacetylation, distribution of acetyl groups and degree of polymerization, also influence the solubility of chitosan [100]. For example, chitosan with a low molecular weight, such as chitosan oligosaccharide, can be used to overcome the limitation of insolubility issue [101].

Many hydrophobic acids (e.g. linoleic acid, bile acids) are indispensable for human body and play an important role in the biological processes, such as digestion and absorption of fats and lipophilic vitamins [102]. These acids can be used to modify chitosan to facilitate self-assembling process because of their amphiphilicity. They can also enhance biocompatibility of the modified materials. Chen et al. [103] used linoleic acid (LA) to modify chitosan via a 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC)-mediated reaction. In this study, a dilute acetic acid solution was used to dissolve chitosan and prepare LA-chitosan nanoparticles. The self-assembling properties of the amphiphilic LA-modified chitosan polymer, the influence of oil/water (O/W) emulsification and sodium chloride on the nanoparticle formation process, and the encapsulation capacity of the formed polymeric micelles were systematically investigated. It was found that the LA-modified chitosan micelles had a size ranging from 200 to 600 nm and an encapsulation efficiency of 50% when lipid-soluble retinal acetate was used as hydrophobic model drug. The formation of nanoparticles could be enhanced by O/W emulsification with methylene chloride which served as an oil phase. Meanwhile, the addition of 1 M sodium chloride could also promote self-assembly of the LA-modified chitosan into micelles with or without employing emulsification. Polymeric micelle based on LA-modified chitosan is also a focus in many other studies [104–106]. Using the same synthesis methodology (EDC-mediated coupling reaction), oleic acid-grafted chitosan was synthesized and used to prepare self-assembling nanoparticles. The oleic acid-modified chitosan nanoparticles showed limited cytotoxicity and had a low hemolysis rate, and were successfully used to encapsulate an anti-cancer drug (doxorubicin) [107, 108]. Hu et al. [109] used stearic acid (SA) to modify chitosan oligosaccharide (CSO), while varying the degree of amino substitution (SD). The micellar shell was cross-linked by glutaraldehyde to increase the stability of the nanoparticles and control drug release. In this study, paclitaxel was used as a model drug and the effects of drug feeding amount, SD, cross-linking degree on the micellar size, drug entrapment efficiency and in vitro drug release behavior were investigated. It was found that the prepared CSO-SA micelles maintained high drug entrapment efficiency (>94%) in all cases (i.e. different drug feeding, SD and cross-linking degree). In addition to the fatty acids introduced above, many other amphiphilic acid-modified chitosan self-assembling systems, such as linolenic acid [110], deoxycholic acid [111–114], palmitic anhydride [115] and 5β-cholanic acid [95, 116–119], have also been extensively studied.

Cholesterol is an important lipid molecule for all animals as it is not only a structural component of animal cell membranes, but also a raw material for the synthesis

of vitamin D and bile acids [120]. As a hydrophobic molecule, cholesterol has also been used to modify chitosan and the synthesized amphiphilic copolymer can form self-aggregated nanoparticles in aqueous solutions. Chen et al. [121] synthesized 6-O-Cholesterol modified chitosan (O-CHCS) conjugates via a protection-graft-deprotection method. The O-CHCS conjugates could self-assemble into micelles in 0.1 M acetic acid solution, with a size ranging from 100 to 240 nm. All-trans retinoic acid (ATRA), used as a model drug in the study, could be successfully entrapped into the formed nanoparticles. The drug-loaded micelles showed a sustained drug release behavior for more than 72 h. The interactions between O-CHCS nanoparticles and bovine serum albumin (BSA) was investigated in another study [122]. The results showed that the O-CHCS nanoparticles could lead to a decrease in α-helical content and an increase in β-strand content of BSA. Cell tests indicated that the nanoparticles were non-cytotoxic and biocompatible up to a concentration of 200 μg/ml. The same group also synthesized a cholesterol-modified chitosan conjugate with succinyl linkages (CHCS) as a drug carrier for Epirubicin (EPB) [123]. Other similar systems (e.g. cholesterol-modified glycol chitosan (CHGC) conjugates) have also been studied as nanocarriers for hydrophobic drugs such as doxorubicin [124], indomethacin [125] and cyclosporine A [126]. More cholesterol-polymer conjugates used for drug and gene delivery will be introduced later.

Due to the excellent mechanical strength, biocompatibility, biodegradability and non-toxicity, synthetic polyesters such as poly(e-caprolactone) (PCL) have been widely used for drug delivery applications [127–129]. It is promising to use these biodegradable polyesters in combination with chitosan to prepare amphiphilic polymeric nanocarriers. There are many studies in which PCL-modified chitosan copolymers are used as carrier-forming materials. Duan et al. [130] synthesized cationic chitosan-graft-polycaprolactone (CS-g-PCL) copolymers via ring-opening polymerization, with different grafting content of PCL. By using dialysis method, the obtained amphiphilic brush-like polycations could form micelles with spherical shape and narrow size distribution (47–113 nm). Authors reported that a water-insoluble anticancer drug, 7-ethyl-10-hydroxy-camptothecin (SN-38), could be easily incorporated into the CS-g-PCL polymeric micelles. Furthermore, compared to free SN-38, drug-loaded nanoparticles showed decreased cytotoxicity, while drug-free CS-g-PCL nanoparticles were almost non-toxic. Synthesis of the PLC-modified chitosan conjugates and their use as nanocarriers can be found in many other studies [131–133].

In addition to hydrophobic modification of chitosan using various fatty acids, bile acids, cholesterol and polyesters as discussed above, chitosan can also be hydrophilically modified and used as nanocarriers for drug and gene delivery. Among many choices, poly(ethylene glycol) (PEG) is one of the most important polymers that can be employed for this purpose. PEG has shown many favorable characteristics including ease of chemical modification, non-toxicity, non-immunogenicity, non-antigenicity and high solubility in water [134–136]. Due to these unique properties, PEG-based materials have been extensively employed in pharmaceutical and biomedical fields. In recent years, the applications of PEG-modified chitosan in drug delivery have been studied by many research groups. Jeong et al. [137] developed polyion complex micelles based on synthesized

methoxy poly(ethylene glycol) (mPEG)-grafted chitosan (CP) with different molecular weight ratio of mPEG and chitosan segments in the polymer chains. The study showed that the self-assembled nanoparticles were spherical and had a size of about 50–200 nm. Authors reported that ATRA could be encapsulated into the polyion complex micelles with encapsulation efficiency higher than 80% in all formulations. It was found that the ATRA-loaded nanoparticles were more effective in inhibiting *in vitro* migration of tumor cells compared to free ATRA. Prego et al. [138] focused on Chitosan-PEG-based nanocapsules. Authors reported that by grafting PEG to chitosan, the stability of the nanocapsules in gastrointestinal fluids was improved. Meanwhile, the chitosan-PEG nanoparticles showed reduced cytotoxicity compared with chitosan without PEGylation. Apart from improved *in vitro* stability and decreased cytotoxicity, it was also observed that the intestinal absorption of salmon calcitonin, a model peptide, by nanocapsules was enhanced if chitosan was PEGylated. Such in vivo performance was influenced by PEGylation degree, which could be modulated to optimize the properties of the nanocapsules. Nanospheres formed from PEG-modified chitosan have also been studied. Yoksan et al. [139] prepared nanospheres using *N*-Phthaloylchitosan-grafted poly(ethylene glycol) methyl ether (PLC-g-mPEG) while controlling hydrophobic/hydrophilic degree of the polymer chains. This PEG-modified chitosan was used in another study to prepare self-assembly micelles [140], but with different molecular weight ratio of chitosan and mPEG. Drug encapsulation and release behaviors of PEG-modified chitosan nanoparticles have been investigated in many studies using various model drugs and polymer formulations [141–146].

Instead of physical loading of hydrophobic drug into nanocarriers, the drug molecules can also be covalently bound to polymeric carriers. In this case, polymer-drug conjugates are formed [11, 12, 147, 148]. This strategy has been widely exploited to improve the efficacy of therapeutic agents and polymer-drug conjugates have demonstrated enhanced stability, prolonged half-life, and can be used for specific targeting to tissues and cells [12]. In addition, unintended release of chemically conjugated drugs from nanoparticles are less frequent than that of physical incorporation cases [149, 150]. Hydrophobic molecules, such as anticancer drugs (e.g. doxorubicin) and photosensitizers used for photodynamic therapy, have been used to modify chitosan and the formed amphiphilic conjugates can self-aggregate in aqueous media. Kwon's group synthesized fluorescein isothiocyanate-glycol chitosan conjugate (FTC-GC) and doxorubicin-glycol chitosan conjugate (GC-DOX) and the polymeric amphiphiles were used to prepare self-assembled nanoparticles. Biodistribution of these self-aggregates in tumor-bearing mice were studied. The results showed that only a negligible amount of the nanoparticles was distributed to heart and lungs while majority was observed to be accumulated in tumor with the increase in blood circulation time. The self-aggregates maintained a high concentration in blood even after 3 days of intravenous injection (>8% of the dose) [151–153]. Cho et al. [154] prepared various self-assembled nanoparticles by combining different hydrophilic polymers with hydrophobic moieties, including FTC-GC conjugates. The study employed radionuclide imaging to investigate the in vivo distributions of these nanoparticles in tumor-bearing mice, based on which

the underlying mechanism of tumor targeting properties of the nanoparticles was proposed. It was found that several key factors played significant roles in determining the magnitude and pattern of the nanoparticle distribution in tumor, including in vivo colloidal stability, particle size, intracellular uptake of nanoparticles and tumor angiogenesis. In a more recent study, Lee et al. [155] compared *in vitro* and *in vivo* performance of two systems: nanoparticles formed from glycol chitosan-Ce6 (a photosensitizer) conjugates (GC-Ce6) and nanoparticles formed from glycol chitosan-5β-cholanic acid conjugates with Ce6 encapsulated physically inside the core (HGC-Ce6). Both of the nanoparticles had a close size, similar *in vitro* generation efficacy of singlet oxygen and a fast cellular uptake profile. However, HGC-Ce6 showed a burst drug release *in vitro* while GC-Ce6 shown a more sustained release. Besides, GC-Ce6 nanoparticles had a prolonged circulation profile and displayed an efficient tumor accumulation behavior, which were not observed in the case of HGC-Ce6.

2.3 Other Polysaccharides

2.3.1 Dextran

Hydrophobically modified dextran with bile acids (e.g. cholic and deoxycholic acids) [156], poly(methyl methacrylate) (PMMA) [157] and PCL [158–160] have been used to prepare polymeric nanocarriers for proteins (e.g. BSA, lectin), anticancer drugs (e.g. DOX) and other hydrophobic compounds (e.g. *N*-vinyl carbazole, fluorescent probes). In the study carried out by Sun et al. [160], a thiol-disulfide exchange reaction was exploited to synthesize disulfide-linked dextran-b-poly(ε-caprolactone) diblock copolymer (Dex-SS-PCL), which could form micelles in phosphate buffer solutions (50 mM, pH 7.4) with an average size of around 60 nm. The study showed that these Dex-SS-PCL micelles were reduction-responsive. The addition of dithiothreitol (DTT) could destabilize Dex-SS-PCL micelles, resulting in a rapid formation of large aggregates. This might be attributed to reductive cleavage of the intermediate disulfide bonds in the polymer chains by DTT, which led to shedding of the dextran shells and subsequent micellar aggregation. A model drug, DOX, could be encapsulated into the micelles with an encapsulation efficiency of about 70%. *In vitro* drug release study showed that the DOX-loaded Dex-SS-PCL micelles rapidly released almost all the incorporated drug (within 10 h) under a reductive environment, while less than 30% of the drug was released from reduction-insensitive Dex-PCL micelles under the same conditions or from Dex-SS-PCL micelles under nonreductive conditions. The rapid drug release to cytoplasm and cell nucleus was also observed due to their reductive character. In some other studies, dextran-based amphiphilic copolymers are prepared by both hydrophobic and hydrophilic modifications. Such candidates include methoxy-polyethylene glycol/poly(ε-caprolactone) (mPEG/PCL), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine/polylactide (DPPE/PLA), *etc* [161, 162]. Polymersomes prepared by polystyrene-modified dextran have also been reported [163].