Amphiphilic Polysaccharide-Hydrophobicized Graft Polymeric Micelles for Drug Delivery Nanosystems

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Abstract: Self-assembled amphiphilic graft copolymers in aqueous solution to form polymeric micelles, have received growing scientific attention over the years. Among the polymeric micelles, hydrophobicized polysaccharides have currently become one of the hottest researches in the field of drug delivery nanosystems. It is attributable to such appealing properties as small particle size and narrow size distribution, distinctive core-shell structure, high solubilization capacity and structural stability, tumor passive localization by enhanced permeability and retention (EPR) effect, active targeting ability *via* tailored targeting promoiety, long-circulation property and facile preparation. The polymeric micelles self-assembled by hydrophobicized polysaccharides can be employed as targeted drug delivery nanosystem by including thermo- or pH-sensitive components or by attaching specific targeted moieties to the outer hydrophilic surface. Beside encapsulation of water-insoluble drugs, hydrophobicized polysaccharide polymeric micelles can complex with charged proteins or peptide drugs through electrostatic force or hydrogen bond, and serve as an effective non-viral vector for gene delivery. In the latter case, polymeric micelles can not only markedly protect these macromolecules from degradation by protease or ribozymes, but also increase the gene transfection efficiency. This review will highlight the state of the art self-assembled mechanism, characterization, preparation methods and surface modification of hydrophobicized polysaccharide polymeric micelles and their recent rapid applications as drug delivery nanosystems.

Keywords: Polymeric micelles, drug delivery system, amphiphilic graft copolymer, hydrophobicized polysaccharides, drug-polysaccharide conjugates, EPR effect, PEGylation, active drug targeting modification, preparation method, pharmaceutical application.

1. INTRODUCTION

Copolymeric micelles formed by amphiphilic macromolecules possess ordered hydrophobic and hydrophilic block domains, have recently emerged as a novel promising colloidal carrier for the delivery of drugs, proteins and genes. The copolymers usually employed include di-block, tri-block, or graft copolymers [1, 2]. The structure of copolymeric micelles formed by amphiphilic block or graft copolymers depends on whether the hydrophobic chain is randomly bound to the hydrophilic polymer (amphiphilic graft copolymers) or orderly grafted to one end of the hydrophilic chain (amphiphilic block copolymers) [3, 4].

Polymeric amphiphiles undergo intramolecular or intermolecular association promoted by hydrophobic segments in aqueous solution, and form nanoscopic supramolecular core-shell structure when above the critical micellar concentration (CMC). The core-shell structure is composed of hydrophobic segments as internal core and hydrophilic segments as surrounding shell in aqueous medium. The core creates a cargo space for loading hydrophobic drugs by means of physical interactions or covalent linkage, and the shell is responsible for providing the hydrophilic steric outer surface and protecting the incorporated drugs from premature degradation [3, 5, 6].

Amphiphilic block copolymers are characterized by a hydrophilic block chemically tethered to a hydrophobic block, and have been used extensively in pharmaceutical applications [7-9]. Micelle-forming block copolymers are usually di-block and tri-block copolymers, which frequently contain PEG or PEO chain. In di-block copolymers PEG chains are simply conjugated with various hydrophobic blocks, while in tri-block copolymers both termini of a hydrophilic or hydrophobic block may be coupled with the second component. In contrast, amphiphilic graft copolymers usually are hydrophobically modified water-soluble polymers, such as polysaccharides, albumin, etc. [10]. In the case of graft copolymers, multiple hydrophobic chains are distributed along the main chain composed of hydrophilic functional units. Fig. (**1**) compared the chemical structure, self-assembling and drug loading mechanisms of polymeric micelles from amphiphilic block and graft copolymers in aqueous solution. Synthesis of amphiphilic graft copolymers is usually much easier than that of the amphiphilic block copolymers [11]. Micelles formed by amphiphilic graft copolymers more readily form stable self-aggregates, since hydrophobic chains in such micelles are less mobile and more loosely packed than micelles formed by amphiphilic block copolymers [12]. Therefore, the development of self-assembled polymeric micelles formed by amphiphilic graft copolymers would be a promising nanocarrier for drug delivery.

During the past years, an increasing number of publications have appeared in the development of micelles formed by amphiphilic graft copolymers as drug delivery system (DDS). In particular, the intense attention has been focused on self-assembled polymeric micelles by hydrophobicized polysaccharides until now. Table **1** summarized some investigations of hydrophobicized polysaccharides used to deliver various drugs *via* polymeric micelles in the recent five years. The purpose of this review was to provide a concise description of the characteristics of amphiphilic graft copolymeric micelles as drug delivery nanocarriers. The emphasis would be placed on more recent developments in the area of polymeric micelles by hydrophobicized polysaccharides, including self-assembled mechanism, characterization, preparation methods, surface modification and pharmaceutical applications.

2. SYNTHESIS AND SURFACE MODIFICATION OF HYDROPHOBICIZED POLYSACCHARIDES

In biological system, biological macromolecules (such as polysaccharides, proteins, DNA, etc.) are well-controlled self-assembled nanoaggregates based on non-covalent interactions, including electrostatic interaction, hydrogen bonding and hydrophobic interactions. The concept of supramolecular self-assembly has led to new methodology for the design of functional nanostructures based on the tailored association of hydrophobically modified polymers in aqueous solution [46].

During the past years, a variety of amphiphilic copolymers have been synthesized by simulating the structural characteristics to biological macromolecules, including amphiphilic block copolymers and amphiphilic graft copolymers. Hydrophobically

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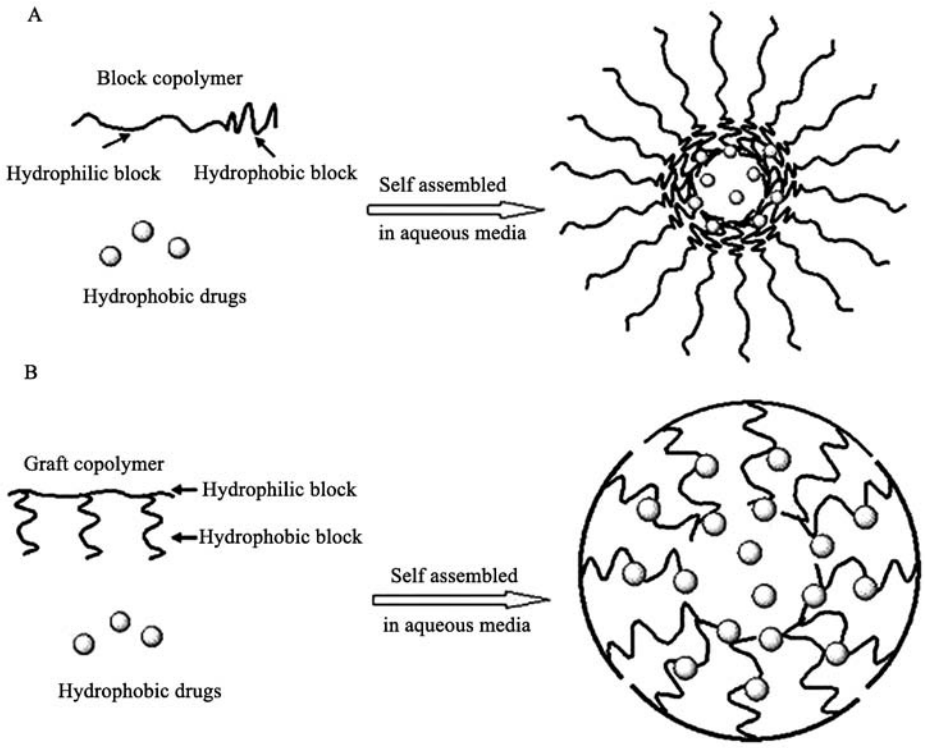

Fig. (1). The chemical structure, assembled and drug loading mechanisms of polymeric micelles from amphiphilic block (**A**) and graft (**B**) copolymers in aqueous media.

Table 1. Applications of Amphiphilic Graft Copolymeric Micelles to Targeted Drug Delivery

Graft copolymers	Drugs incorporated	Properties	References
CSO-SA	Paclitaxel	High drug entrapment, prolonged and controlled drug release, high cell cytotoxicity	[13, 14]
	Doxorubicin	Reduction the initial burst drug release, high cellular uptake and cytotoxicity	[15-17]
	Paclitaxel, doxorubicin	IC_{50} of paclitaxel and doxorubicin in micelles was lowered about 20-fold and 4–7-fold compared to that of Taxol and doxorubicin solution against drug sensitive cells, and was lowered more significantly against drug resistant cells.	[18]
	DNA	CSO–SA micelles could protect DNA from enzymatic degradation and exhibited high transfection efficiency. The optimal transfection efficiency of micelles in A549 cells was about 15%, which was higher than that of Lipofectamine™ 2000 (about 20%).	[19]
Linoleic acid-CS	Doxorubicin	High drug loading, controlled drug release	[20]
N-alkyl-N-trimethyl-CS	Hydroxycamptothecin	Improvement drug stability, controlled drug release	[21]
N-octyl-N,O-carboxymethyl-CS	Paclitaxel	High drug loading and entrapment, comparable cytotoxic effect to Taxol	[22]
N-succinyl-N'-octyl-CS	Doxorubicin	The doxorubicin-loaded micelles could significantly sustain drug release and exhibited high *in vitro* anti-tumor activity. The IC_{50} of doxorubicin-loaded micelles in HepG2 and A549 cells are three-fold more cytotoxic than free doxorubicin, while five-fold higher cytotoxicity compared to free doxorubicin in BGC and K562 cells.	[23]
Glycol-CS-adriamycin	Adriamycin	Micelles had higher drug loading of 38% and could obviously sustain drug release.	[24, 25]
N-octyl-N-(2-carboxylbenzoyl)-CS	Paclitaxel	The polymeric micelle possessed pH sensitivity, which was able to release paclitaxel in response to mild acidic environment (pH 5.0-6.0).	[26]
N-octyl-O-sulfate-CS	Gambogic acid	The micelles could significantly improve drug stability. The lyophilized powder could be stored at 4 °C for 2 months without the obvious change of gambogic acid content.	[27]
	Paclitaxel	Micelle had high drug loading capacity (69.9%) and entrapment efficiency (97.26%). The plasma AUC of paclitaxel in micelles was 3.6-fold lower than that of Taxol; but the V_d and CL were increased by 5.7 and 3.5-fold, respectively. Paclitaxel in micelles were mainly distributed in liver, kidney, spleen, and lung.	[28, 29]
PEGylated-SA-CSO	Mitomycin C	The uptake by macrophage of PEG-SA-CSO micelle was reduced to 17.7%, which was significant lower than that of SA-CSO micelle of 58.4%.	[30]

(Table 1). Contd…..

Graft copolymers	Drugs incorporated	Properties	References
N-phthaloyl-CS-g-mPEG	Camptothecin	High drug loading and stability, sustained drug release	[31, 32]
N-phthaloyl-CS-g-mPEG	All-trans retinoic acid	High drug loading and stability, controlled drug release	[33]
N-mPEG-N-octyl-O-sulfate-CS	Paclitaxel	Brain-targeting characteristics	[34]
Cholic acid-CS-g-mPEG	Camptothecin	High drug loading and stability, controlled drug release	[35]
Pluronic-CS	Indomethacin	Thermo-sensitivity, controlled drug release	[36]
P(CS–Ma–DMAEMA)	Coenzyme A	Copolymeric micelles exhibited temperature and pH-sensitivity. Coenzyme A was absorbed by copolymer at pH 7; while released from the micelle at pH 3.7. In addition, Coenzyme A was released from the micelle with the temperature increased from 35 to 55 ◦**C**.	[37]
Deoxycholic acid-CS	Plasmid DNA	High gene transfection efficiency, protection from enzymatic degradation	[38]
PLGA-HA	Doxorubicin	Micelle possessed tumor specific targeting, high cellular uptake and cytotoxicity. The IC_{50} value of doxorubicin (0.67 mg/mL) loaded PLGA-HA nanoparticles was about 5.2-fold lower than that of free doxorubicin (3.48mg/mL)	[39]
5β-cholanic acid-HA		Active tumor targeting	[40, 41]
HA-paclitaxel	Paclitaxel	High drug loading amount of 10.8%, tumor specific targeting and high cytotoxicity	[42]
HA-amino acid-paclitaxel	Paclitaxel	IC_{50} of paclitaxel (0.253 nM) in micelle was lowered about 3-fold than that of free paclitaxel (0.795 nM).	[43]
Heparin-folic acid-paclitaxel	Paclitaxel	Specific targeting delivery, high cytotoxicity	[44]
Folate-heparin-lithocholate		High antiangiogenic effect	[45]

CS, chitosan; CSO, chitosan oligosaccharide; SA, stearic acid; LA, linoleic acid; PLGA, poly[lactic-co-(glycolic acid)]; HA, hyaluronic acid; Ma: maleic anhydride; DMAEMA: 2-(dimethylamino)ethylmethacrylate.

modified water-soluble polymers usually consist of a water-soluble polymer backbone intermittently grafted with a number of short-chain hydrophobic groups. Various natural polymers and synthetic or semi-synthetic polymers have been used as the hydrophilic backbone of the polymer amphiphi1e [47, 48].

2.1. Hydrophobicized Polysaccharides Polymeric Micelles

In recent years, naturally derived biodegradable materials have attracted more attention for polymeric micelles. As an important class of natural biopolymers, polysaccharides have increased biotechnological and biomedical applications in view of their several advantages, including wide range of sources, biocompatibility, biodegradability, non-toxicity, non-immunogenicity, abundance of functional groups (-COOH, -NH_2, -OH) for surface modification or functionalization, and targeting ability through specific sugar moieties. More importantly, naturally biodegradable polysaccharides, belonging to polyhydroxy1 compounds, have the unique properties such as formation of polymeric micelles and distinct core-shell structure [10].

Grafting the hydrophobic and hydrophilic segments to polysaccharides backbone would give rise to the amphiphilic graft copolymers. Compared to the native polysaccharides, the hydrophobicized polysaccharides possess good self-assembly properties, while preserving original biocompatibility and biodegradability [49]. For example, physicochemical properties of water-soluble polysaccharides are drastically changed by partial modification with hydrophobic groups. When in contact with water, the hydrophobic substituents would self-associate to form hydrophobic microdomains with several transient cross-links connecting polymer chains, and the interactions among hydrophobic groups can control the conformation of polysaccharide polymers, ultimately affect their rheological properties in water [50]. Therefore, compared to synthetic polymers (PVA, PLGA) and other natural polymers (collagen, albumin), hydrophobically modified derivatives of polysaccharides (amphiphilic polysaccharide derivatives) are more suitable to serve as delivery carriers of hydrophobic drugs, proteins and genes in the formulation of polymeric micelles, because it is highly biodegradable, biocompatible, easy for chemical modification and so on [51].

Natural polysaccharides, such as chitosan [52-55], heparin [56, 57], hyaluronic acid (HA) [40, 41], pullulan [58], sodium alginate [59] and others [60-62], have been extensively or partially substituted with hydrophobic moieties, such as long alkyl chains [15, 28, 29, 63], cholesteryl groups [64], deoxycholic acid groups [65-67], PLGA [39, 68], PCL [69] and Pluronic [36], to prepare amphiphilic graft copolymers. Hydrophobic microdomains of the copolymers constituted by a number of polymer chains could form unique multi-core structures, and polysaccharide main chains would curl to form hydrophilic shell for enclosing hydrophobic microdomains to acquire the minimum energy state, ultimately self-assembling to polymeric micelles with multi-core structure [13, 16]. Fig. (**2**) illustrated nanoparticle microstructure of hydrophobic PLGA graft HA copolymeric micelles with doxorubicin encapsulation [39].

The size, intensity, zeta potential and number of cross-linking hydrophobic microdomains of these micelles and drug loading extent can be controlled effectively by tuning the configuration of hydrophobicized polysaccharides, length and number of branch chains, and modification sites of polysaccharides. Du Y.Z. *et al*. investigated the influence on particle size and zeta potential of different substitute degrees of linoleic acid chain in amphiphilic linoleic acid graft chitosan oligosaccharide micelles [20]. The results indicated that with the substitute degree of linoleic acid chain increasing from 3.3% to 6.1%, the micellar size changed from 150.7 to 196.8 nm and zeta potential ranged from 57.9 to 70.2 mV, respectively.

2.2. Polysaccharide-Drug Conjugated Micelles

The development of polymer-based drug delivery systems has made significant progress by covalently linking drugs to polymer. Polymer–drug conjugates usually consist of a hydrophilic polymer chain and hydrophobic drug moieties covalently linked in its side chains [70].

Micelles formed by polymer-drug conjugates have distinctive advantages over the conventional polymeric nanoparticles by demonstrating high drug content and good solubility in water, increased drug half-life and enhanced anti-tumor effects. Among all

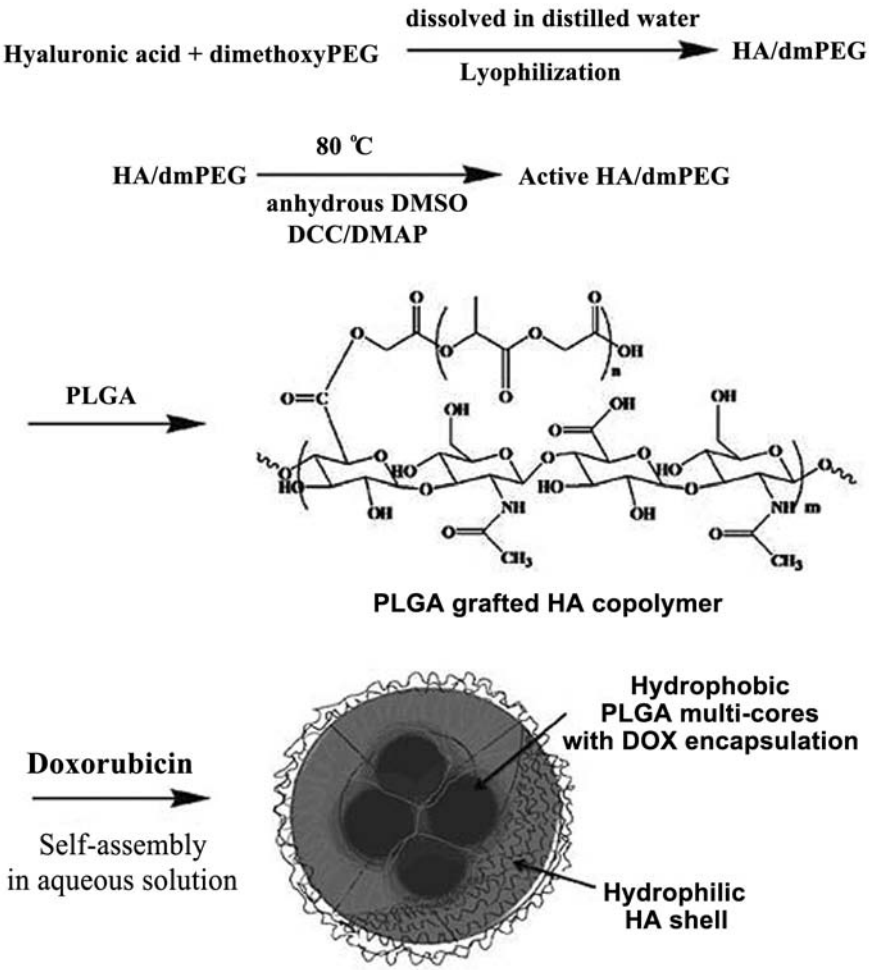

Fig. (2). Synthetic scheme for PLGA grafted HA copolymer and structure of PLGA grafted HA polymeric micelles [39].

the advantages, the most important feature of polymer–drug conjugates is to deliver high concentration of conjugate to tumor tissue *via* EPR (enhanced permeability and retention) effect, followed by sustained drug release from the conjugates. So far, various water-soluble synthetic polymers and the naturally occurring polymers have been widely exploited for conjugating with hydrophobic drugs [71]. Drug molecules are covalently coupled with the functional groups of the polysaccharides under certain conditions, and the synthesized polysaccharide-drug conjugate is designed to form polymeric micelles with controlled drug release pattern [42, 72].

Polysaccharide-drug conjugated micelles have been widely applied as a target specific drug nanocarrier and have shown the tremendous prospects in the pharmaceutical applications. Xin D.C. *et al*. synthesized HA-paclitaxel conjugates with different amino acids space linkers, including valine, leucine and phenylalanine [43]. The prepared HA-amino acid-paclitaxel conjugates containing 10-15% paclitaxel could self-assemble to form polymeric micelles in water, and demonstrated increased solubility and controlled release of paclitaxel. Furthermore, HA-leucyl-paclitaxel conjugate exhibited significantly enhanced cytotoxicity with a lower IC_{50} (50% inhibition of cellular growth) value of 0.253 nM, approximately 3-fold lower than that of free paclitaxel. Flow cytometry analysis showed that conjugate nanoparticles significantly enhanced the extent of apoptosis-induced MCF-7 cell death. Nanosized and self-assembled HA-amino acid-paclitaxel conjugate nanoparticles could be utilized as efficient carriers for its passive and active targeting delivery to cancer cells.

Park J.H. *et al*. conjugated adriamycin onto the backbone of glycol chitosan *via* an acid-labile *cis*-aconityl linkage to form nano-scale self-aggregates in aqueous medium with a drug loading as high as 38% [24, 25]. The release of adriamycin from self-aggregates was significantly dependent on the pH of the medium due to the *cis*-aconityl linkage, e.g., the release percentage of adriamycin for 4 days was 7.3 ± 0.3% at pH 7, whereas 29.3 ± 1.9% at pH 4. The pH-sensitive nanoaggregates exhibited a lower cytotoxicity with cell viability of 48.7 ± 5.8% than that of 7.3 ± 1.5% for adriamycin, which contributed to the sustained and pH-dependent release manner of adriamycin nanoaggregates.

3. SURFACE MODIFICATION OF HYDROPHOBICIZED POLYSACCHARIDES

Hydrophobicized polysaccharide polymeric micelles usually serve as the delivery nanocarrier for water-insoluble drugs and water-soluble biomacromolecules. They can increase drug efficacy by targeting specific cells or organs, while decrease its toxicity by the lowered accumulation in normal organs and sometimes allowing higher doses to be administrated, which would be hazardous under normal doses [2, 73, 74]. Accordingly, it is essential for micelles to target pathological organs or tissues in order to efficiently increase the accumulation of drugs in the

targeted area. There are several approaches capable of achieving this goal. Next we would discuss the surface-modification approaches to achieve passive, stimuli-sensitive and ligand-mediated targeted drugs delivery.

3.1. PEGylation of Hydrophobicized Polysaccharides for Passive Drug Targeting

The non-specific uptake of many drug nanocarriers by reticuloendothelial system (RES) is still a major barrier for clinical application, which may lead to the short circulation time of the nanocarrier in blood [20]. A number of strategies have been adopted to overcome the uptake of polymeric micelles by RES. Among these approaches, PEGylation is one of the most popular methods. Hydrophilic and flexible PEG chains create a highly water-bound barrier on the nanoparticle surface which blocks plasma opsonin adhesion, and subsequently avoids recognition and uptake by RES [9].

The water-soluble, biocompatible and nontoxic PEG polymer, which has been approved by the FDA for injection, has been widely used in biomedicine [36]. PEGylation of proteins [75], drugs [76, 77], liposomes [78], and nanoparticles [79] has been proven to be an effective approach for extending circulation time in the blood stream. Similarly, PEGylated polymers may improve the circulation of the drug delivery nanosystems in the blood by preventing opsonins absorption and uptake of RES. Moreover, PEG chains are usually between 1 and 15 kDa in molecular weight and some results indicated that the longer and the denser the chains are the greater the resulting "stealth" effect will be in the blood circulation [36, 80, 81].

At present, PEGylation is frequently used in chemical modification of hydrophobicized polysaccharides for biomedical applications. Stearic acid-grafted chitosan oligosaccharide (CSO-SA) micelles presenting a potential candidate for intracellular drug delivery nanocarrier was further modified by PEG [20]. About 58.4 ± 0.63% of CSO-SA micelles were taken up by macrophages in 24 h, but PEGylated CSO-SA (PEG-CSO-SA) micelles could significantly reduce the internalization of the micelles to only 17.7 ± 0.94%. On the other hand, the intracellular uptake percentage of PEG-CSO-SA micelles by normal cells and tumor cells was almost the same as those of CSO-SA micelles. *In vitro* anti-tumor activity tests indicated that the PEGylation of CSO-SA did not affect the *in vitro* anti-tumor activity of the drug-loaded micelles. Meanwhile, the PEG-CSO-SA increased the micellar size and decreased the zeta potential of the micelles, but did not affect the drug entrapment efficiency and *in vitro* drug-release behavior.

Other PEGylation of hydrophobically modified polysaccharides, such as *N*-phthaloyl chitosan [31-33], N-octyl-O-sulfate chitosan [34], and cholic acid chitosan [35], could also prevent drug-loaded micelles from recognition and phagocytosis by RES, decrease plasma opsonin adhesion and increase the circulation time of micelles in the blood.

3.2. Active Drug Targeting Modification of Hydrophobicized Polysaccharides

The EPR effect of hydrophobicized polysaccharide polymeric micelles in combination with PEGylation of hydrophobicized polysaccharide are usually considered as a passive targeting approach, but targeted delivery efficiency could be further increased through an active targeting strategy by introducing a sensitive polymer to environmental variation in temperature or pH or by chemically attaching target promoiety [82].

3.2.1. Stimuli-Sensitivity

The pH profile of pathological tissues, such as inflammation, infection, and cancer sites, is 1-2.5 pH units lower than that of the normal tissues. In addition, many pathological processes in various tissues and organs are accompanied with local temperature increase (by 2-5 ℃). So, the efficiency of the micellar nanocarriers in targeted drug delivery can be improved by making micelles capable of being readily disintegrated and then releasing drug locally due to decreased pH or increased temperature in the targeted pathological sites [3, 73]. Stimuli-sensitive nanosystems make it possible to well control the release behavior of drugs from the inner core of polymeric micelles, and then offer a promising opportunity for the targeted drug delivery.

Polysaccharides conjugated with pH- or temperature-sensitive polymers also can serve as stimuli-sensitive drug delivery system. For example, hydrophilic group chains of polysaccharides with -COOH [26, 83], $-NH_2$ [84] in the micellar surface, and the hydrazone bond in polysaccharide/drug conjugate are pH-sensitive [85]. In addition, hydrophilic chains formed by N-isopropylacrylamide monomer [48, 86] are sensitive to temperature. P(2-(dimethylamino) ethyl methacrylate) (PDMAEMA) [37] is also widely used as thermo- and pH-sensitive polymers. Li H.X. *et al.* developed a series of highly pH-sensitive graft copolymers such as N-octyl-N-(2-carboxylbenzoyl) chitosan derivatives for the delivery of anticancer drug paclitaxel [87]. The pH-sensitive polymeric micelle was able to release paclitaxel in response to mild acidic environment (pH 5.0-6.0) within the endosomal environment, while maintaining structural stability in the blood stream, thus improving the cytoplasmic delivery of paclitaxel after endocytosis.

Temperature-sensitivity is one of the most interesting characteristics in stimuli-sensitive polymeric nanocarriers and has been extensively investigated to exploit the hyperthermia condition for targeted drug delivery [88]. Park K.M. *et al*. prepared a thermo-sensitive chitosan-Pluronic copolymer by grafting mono-carboxyl Pluronic onto the chitosan, which showed sustained release behavior of indomethacin in comparison with the first-order kinetic release from Pluronic micelle [36].

A novel copolymer P(CS–Ma–DMAEMA) was synthesized with chitosan (CS), maleic anhydride (Ma) and functional monomer 2-(dimethylamino)ethyl methacrylate (DMAEMA) by grafting and copolymerization [37]. Polymeric micelle was prepared with this copolymer for encapsulating coenzyme A (CoA). It was found that the copolymer exhibited temperature and pH dual-sensitivity. When pH is 7.0, CoA was absorbed by copolymer greatly; while CoA was readily released from the copolymeric micelle at pH 3.7. In addition, CoA was largely released from the copolymeric micelle with the temperature increased from 35 to 55 ℃.

3.2.2. Receptor-Mediated Targeting

Receptor-mediated drug delivery system can deliver drugs to specific tissues and cells through receptor-mediated endocytosis, which takes advantage of specific receptors in some tissues and overexpressed receptors in tumor cells. Many target cells, especially cancer cells, overexpress certain receptors (such as transferring, epidermal growth factor and folate receptors) on their membrane surface. Additionally, there are a large number of functional groups (-COOH, $-NH_2$, -OH) of hydrophobicized polysaccharide available for chemical ligand modification. Therefore, hydrophobicized polysaccharide polymeric micelles could be further functionalized by conjugation with these cell specific ligands on their surface for achieving active targeting.

To our surprise, among various types of ligands used in the targeted drug delivery system, one of the most commonly used ligands is polysaccharide. As the shell of polymeric micelles, polysaccharides are a key component to the recognition by receptors on the cellular membrane, and play an important role in membrane transport of drug nanocarriers. For example, HA accounts for various functions within the extracellular matrix such as cell growth, differentiation, and migration. HA is also an important signal for activating kinase pathways and regulating

angiogenesis in tumor. It has a strong affinity with cell-specific surface markers such as glycoprotein CD44 and receptor for HA-mediated motility (RHAMM), which are abundantly overexpressed on the surface of many types of tumors [43, 89]. Thus, tumor cells exhibit enhanced binding and uptake of HA, and then HA and its derivatives have been popularly used as ligands for targeted drug delivery carriers [90, 91].

The folate receptor (FR) is a membrane-anchored protein which has high affinity towards folate or folate conjugated molecules and can mediate their physiological uptake by endocytosis. Most important, FR is overexpressed in several types of solid tumors, including ovarian, uterine, lung, breast and endometrial cancers [44, 45]. For example, FR expression was about 53% of these tumor samples; by contrast, normal bone marrow cells did not show any expression of FR. For the above reasons, the FR has been widely applied in the functionalization of nanocarriers to actively target tumor sites [92-94]. As a natural material, folic acid has several advantages as a potential targeting agent, including lower molecular weight and immunogenicity than most of antibodies, relatively high stability, and ease of synthesis. Fig. (**3**) illustrated the intracellular delivery of folate anchored nanocarrier through endocytosis process and release in response to internal stimuli [82]. Wang X. *et al*. synthesized a novel conjugate heparin-folic acid-paclitaxel (HFT), loaded with additional paclitaxel (HFT-T) [44]. The uptake percentage of HFT-T nanoparticles by tumor cells was 21.8 ± 2.9%, three times greater than that of unmodified heparin-paclitaxel conjugates loaded with paclitaxel (HT-T) nanoparticles (5.6 ± 1.8%). And the volume of tumor treated with HFT-T nanoparticles was 92.9 ± 78.2 mm^3, which was only 5% of that treated with free paclitaxel (1670.3 ± 286.1 mm^3) following intravenous administration. The FR-targeted micelle significantly enhanced the specific delivery paclitaxel into FR-expressing tumor cells and remarkably improved antitumor efficacy of paclitaxel. Another folate mediated targeted drug delivery system, folate-heparin-lithocholate (FHL) amphiphilic derivative, was also reported [45]. Cellular uptake test indicated FHL showed higher cellular uptake, especially in the nuclei, than those of heparin-lithocholate (HL). Tumor growth inhibition experiment showed FHL specifically induced apoptosis on KB cells highly expressing FR.

4. CHARACTERISTICS OF HYDROPHOBICIZED POLYSACCHARIDE POLYMERIC MICELLES

Polymeric micelles formed by hydrophobicized polysaccharides have various distinctive characteristics described as follows:

First, hydrophobicized polysaccharide polymeric micelles have unique core-shell structure. The core compartment of polymeric micelles demonstrates high drug loading capacity and controlled drug release profile. The core of polymeric micelles is usually the hydrophobic microdomains, which can load low molecular weight hydrophobic anticancer drugs, such as paclitaxel [14], doxorubcin [17, 20]. Additionally, hydrophobicized polysaccharide polymeric micelles could also complex with hydrophilic biomacromolecules *via* hydrogen bond and electrostatic force, such as proteins [95], peptides and genes [11, 96]. The controlled release of the loaded drug can be achieved through diffusion from the micelles or by biodegradation of amphiphilic polysaccharide derivatives. The shell of polymeric micelles composed of hydrophilic backbone of polysaccharides is responsible for providing an effective steric protection layer and determines surface hydrophilicity, charge and particle size of the micellar nanocarrier. The presence of reactive groups (-NH_2, -COOH, -OH) in the shell is suitable for derivatization, such as an attachment of PEG or targeting moieties [22, 75, 97-99].

Second, nanoscale dimensions and narrow size distribution of hydrophobicized polysaccharide polymeric micelles permit the efficient accumulation in tumor tissue *via* EPR effect by avoiding rapid clearance by the RES and renal excretion [24, 100]. Generally, the size of particles trapped in the smallest capillaries of the lungs is larger than 5-7 μm, and the liver and spleen, related to the RES uptake, are relevant for particles as large as 4-5 μm [101]. The EPR effect has been exploited as a passive drug targeting strategy for altering the biodistribution of encapsulated drugs to tumor tissues, based on the pathophysiological characteristics of

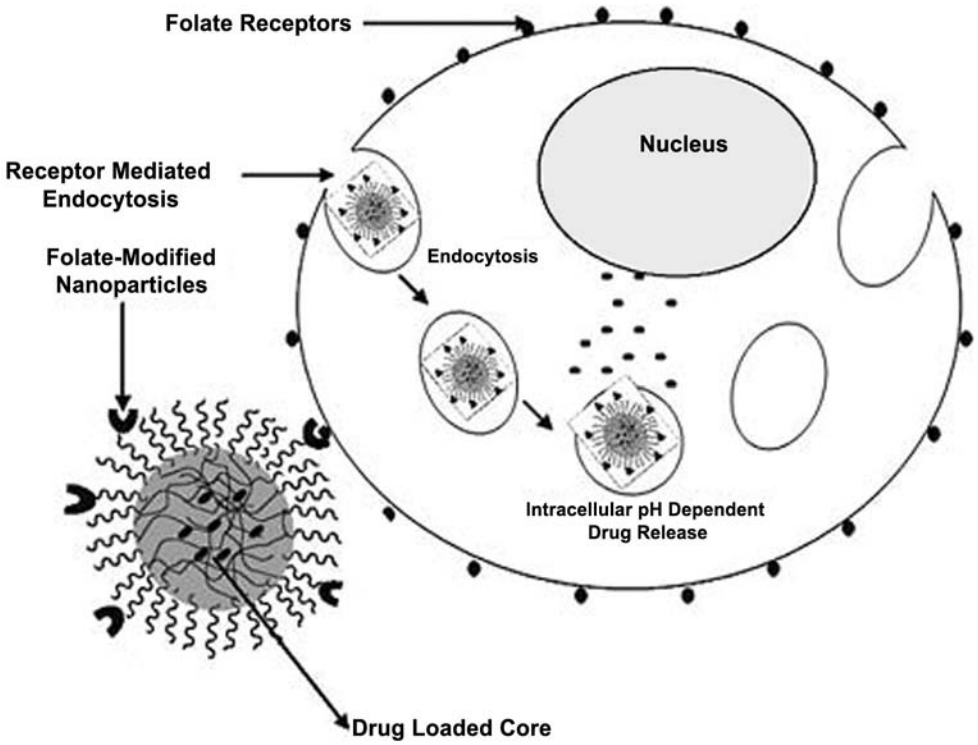

Fig. (3). Schematic illustration of active drug targeting with surface-modified micelles [82].

solid tumor tissues. The characteristics of tumor tissues such as the increased permeability of the vascular system, poor drainage of lymphatic system lead to the passively accumulating preferentially and retaining more of small particles (<500 nm) in tumor tissues than in normal tissues. And side effects of drugs can be reduced due to the preferable uptake of tumor tissues [3, 73]. Hydrophobicized polysaccharide polymeric micelles can passively deliver drugs to tumor tissues *via* EPR effect, due to its small particle size and surface hydrophilicity. Son Y.J. *et al*. investigated the tissue biodistribution of fluorescein isothiocyanate labeled glycol chitosan nanoaggregates (FTC-GC) targeting by EPR effect [24]. FTC-GC nanoaggregates with a diameter of about 250 nm were distributed mainly in kidney, tumor and the liver and were scarcely observed in other tissues. The amount of nanoaggregates distributed into the tumor site was remarkably increased gradually up to 8 days. All the above targeting strategies were stemmed from the EPR effect, passive accumulation in the tumor tissue.

Third, the hydrophobicized polysaccharide polymeric micelles are water-gel matrix composed of highly hydrated outer shells, with good structural stability in the liquid phase. Compared with the traditional low-molecular-weight surfactants and amphiphilic block copolymeric micelles, it has a lower CMC value, therefore possessing a better structural integrity in the physiological condition. When in contact with the bulky body fluid, it does not tend to dissociate. The good structural stability of hydrophobicized polysaccharide polymeric micelles can protect drugs from degradation by the body enzymes. Meanwhile, it can also prevent drug leakage and further improve the drug stability to ensure resulting adequate drug levels in the lesion sites [27, 102].

The CMC and aggregation number are two important parameters affecting the behavior of polymeric micellar solution. CMC indicates the self-aggregation ability of the amphiphilic copolymers and is correlated to the structural stability of micelles *in vitro* and *in vivo*. Self-aggregates formation in aqueous solution can be studied by fluorescence spectroscopy using pyrene as a hydrophobic fluorescence probe. Pyrene is a highly hydrophobic aromatic hydrocarbon and sensitive to the polarity of the surrounding environment. When self-aggregates or other hydrophobic domains are formed in aqueous solution, pyrene partitions preferentially toward the hydrophobic domain afforded by the micellar core experiences a non-polar environment. Consequently, an increase in the fluorescence intensity is observed. CMC can be determined by the variation of the intensity ratio between the first and the third highest energy emission peaks [3]. Generally, the CMC of the hydrophobicized polysaccharide nano-aggregates decreases as the substitution degree of the hydrophobic modified-groups increased. Xu X.Y. *et al*. prepared *N*-succinyl-*N'*-octyl chitosan polymeric micelles and estimated the potential of micelle formation of the derivatives in an aqueous environment [23]. The CMC values decreased from 3.1×10^{-5} to 5.9×10^{-6} g/ml with the increase in substitution degree of octyl chains from 28.6% to 52.5% (28.6-52.5 octyl chains per one hundred glucose residues).

As mentioned previously, self-assembled hydrophobicized polysaccharides in aqueous solution can form multi-core micellar aggregates. So it is essential to investigate the numbers of hydrophobic microdomains per hydrophobicized polysaccharide molecule in the micelles and distribution pattern of microdomains in the micelles. Steady-state fluorescence-quenching method using 1-dodecylpyridinium chloride (DPC) as a quencher, can be applied to determine the aggregation number of micelles consisting of low-molecular-weight surfactants or polymeric amphiphiles [30]. The numbers of hydrophobic microdomain crosslinked to form the hydrophobic core and the aggregation numbers of hydrophobic groups per hydrophobic microdomain depend on the substitution degree of the hydrophobic groups. Park K. *et al*. evaluated the microscopic structure and the aggregation number of heparin-deoxycholic acid conjugate self-assembly [103]. As the degree of substitution of deoxycholic acid groups increased from 6.2% to 9.6%, the aggregation number per hydrophobic microdomain was in the range from 32.5 to 88.1, and 5-9 heparin-deoxycholic acid conjugate per hydrophobic microdomain constituted a multi-core structure within micellar self-aggregates.

5. GENERAL METHODS FOR PREPARATION OF POLYMERIC MICELLES

Self-assembled micelles of hydrophobically modified polysaccharides can be prepared by a variety of techniques, including ultrasound, dialysis and emulsification, depending on the swelling and solubility of modified polysaccharides in water.

5.1. Ultrasound

The polymeric micelles consisting of hydrophobicized polysaccharides with good swelling property can be prepared by ultrasound method. The polymer samples swell in aqueous solution and the polysaccharide long-chain backbone can be dispersed by the ultrasonic energy. The micelles were formed spontaneously by intermolecular and intramolecular hydrophobic interactions of the conjugated hydrophobic groups. The dispersion and particle size of polymeric micelles can be controlled by the ultrasonic power and time. The method is simple and doesn't require adding stabilizer, emulsifier and other agents, however, the stability of the resulting micelles is not satisfied [20, 103].

5.2. Dialysis

Dialysis method is commonly employed for hydrophobicized polysaccharides with poor swelling property. The polymers dissolved completely in a water-miscible solvent (dimethylsulfoxide, dimethylformamide, ethanol, tetrahydrofuran, etc.) or mixed solvents, and then dialyzed against water. With the gradual replacement of soluble solvent by insoluble water, the hydrophobic portion of the polymer associates to form the hydrophobic micellar core, which is capable of incorporating the insoluble drug during preparation. The particle size, distribution and yield of the polymeric micelles are related to the water-miscible organic solvents. The method also doesn't require adding stabilizer, emulsifier and other reagents, especially suitable for polymers with poor swelling property or high hydrophobicity. The particle size of micelles prepared by dialysis method is usually small and evenly distributed [23, 31].

5.3. Emulsification

Hydrophobicized polysaccharide polymeric micelles are mainly prepared by the oil in water (O/W) emulsion method. The hydrophobicized polysaccharide polymers were dissolved in aqueous solution. Oil phase composed of organic solvents (methylene chloride, dichloromethane) was added to the polymer solution while stirring. The O/W emulsion was then homogenized to prepare more evenly distributed droplets. The core-shell polymeric micelles were then formed upon the organic solution evaporation. Some factors of this method, such as type of organic solvent, the volume ratio of oil to water phase, and the homogenized pressure can significantly influence the physicochemical properties of the resulting polymeric micelles. This method can increase the drug loading to a larger extent, but it has not been widely applied recently. Chen X.G. *et al*. prepared linoleic acid-modified chitosan micelles by O/W emulsification technology with methylene chloride as oil phase, which can enhance the formation and stability of the polymeric micelles in the solution with the ratio of methylene chloride/linoleic acid-chitosan solution (v/v) over 4% [104].

6. PHARMACEUTICAL APPLICATION

Nanosized colloidal drug carriers with lower side effects and better therapeutic effect have been successfully used as the delivery system of small-molecule drugs [21, 105, 106], protein drugs and genes [96]. As one class of colloidal drug carriers, self-assembled hydrophobicized polysaccharide polymeric micelles have been widely applied in biotechnology and pharmaceutical studies owing to its unique core-shell structure and good structural stability. Relevant researches mainly focused on the following aspects: First, hydrophobicized polysaccharide polymeric micelles self-assembled in aqueous solution are employed as carriers of hydrophobic small-molecule anticancer agents (paclitaxel, doxorubicin, camptothecin, etc.), and show significant solubilization, sustain drug release and improve stability. Second, hydrophobicized polysaccharide polymeric micelles can complex with peptide and protein drugs by non-covalent interactions (electrostatic force, hydrogen bond and hydrophobic force). Third, hydrophobicized polysaccharide-DNA complex can be used as non-vital vector for gene delivery. It has been found that the presence of hydrophobic groups in the modified polysaccharides enhanced the cellular endocytosis, while reduced the interaction with serum proteins and significantly increased gene transfection efficiency.

6.1. Encapsulation of Hydrophobic Drug

The hydrophobic core in hydrophobicized polysaccharide polymeric micelles can be used as promising carriers of water-insoluble drugs. The hydrophobic drugs can be directly incorporated into the core of micelles by hydrophobic interaction.

Zhao M.D. *et al*. developed paclitaxel (PTX) and doxorubicin (DOX) co-loaded polymeric micelles formed by SA-CSO [18]. The IC_{50} value of PTX and DOX in micelles against drug sensitive cells (A549, MCF-7 and SKOV3) was about 20-fold and 4-7-fold lower compared to that of PTX and DOX solution, respectively. The IC_{50} value of PTX and DOX co-loaded micelles against drug-resistant cells (MCF-7/ADR, SKOV3-TR30) were also lowered, and no clear difference was found between drug-sensitive and drug-resistant cells. The combination chemotherapy of SA-CSO/PTX and SA-CSO/DOX showed synergistic effects in both drug-sensitive and drug-resistant cells.

6.2. Complexation with Proteins

As the development of biotechnology, peptide drugs have exhibited preferential therapeutic effect over the traditional drugs. But there are several disadvantages limiting its application. Firstly, proteins orally administrated tend to be degraded by enzymes in gastrointestinal tract, so it must be administrated parenterally. Secondly, protein drugs must be administered in multiple doses owing to its rapid elimination. Thirdly, most of biomacromolecules can not cross biological barrier easily. So the efficient delivery system of biologically active proteins or enzymes has received considerable attention in modern chemotherapy. The protection of proteins against self-aggregation as well as enzymatic degradation is an important issue for protein delivery system.

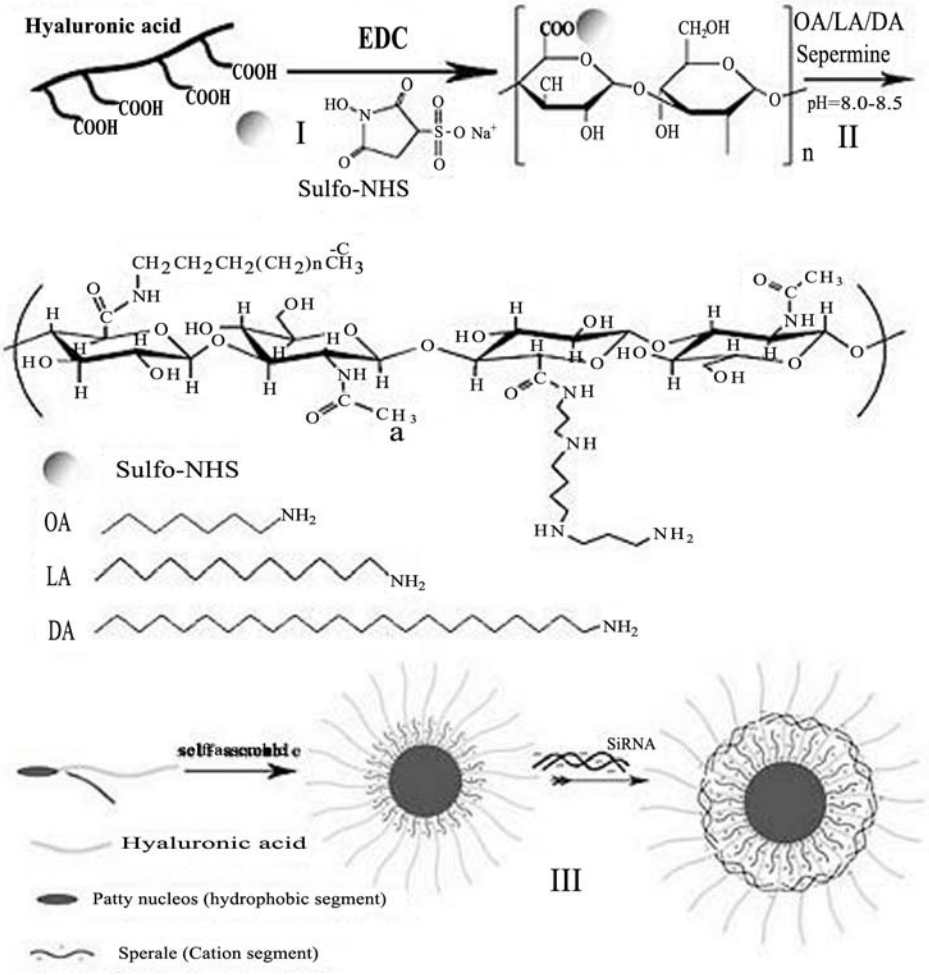

Fig. (4). Schematic representation for (I) the activation of the carboxyl terminal ligands of HA with EDC and sulfo-NHS; (II) the conjugation of alkyl chain to the active eaters of HA and (III) the conjugates complexed with siRNA in aqueous solution [109].

The interior microdomains of hydrophobicized polysaccharides can supply cross-linking sites for complexation with proteins through non-covalent interaction. Hydrophobicized polysaccharide polymeric micelles complexed with proteins can enhance the ability of proteins to resist degeneration and improve their thermal stability. They also can prevent proteins from irreversible denaturation and aggregation [107, 108].

Insulin complexed with cholesterol-bearing pullulan (CHP) in water can form a stable polymeric micelle spontaneously [21]. The thermal denaturation and subsequent aggregation of insulin were effectively decreased upon complexation. The complexed insulin was significantly protected from enzymatic degradation. In addition, the biological activity tests indicated that released insulin was fully bioactive and the bioactivity of complexed insulin was well preserved.

6.3. Complexation with DNA

As a class of non-viral vector, self-assembly polymeric micelles, especially the cationic micelles, can serve as a gene carrier for the treatment of many genetic diseases or diseases caused by infection of exogenous viral genes. Hydrophobicized polysaccharides with cationic segments, such as hydrophobicized chitosan derivatives, due to the controlled complex formation with DNA, can spontaneously complex with DNA through electrostatic interaction and induce condensation of DNA to form a compact structure. Fig. (**4**) illustrated the hyaluronic acid grafted fatty amine and spermine conjugates which could self-assemble in water to form polymeric micelles. Spermine, as the cationic side chain, could complex with the negative charge of siRNA by electrostatic interaction and siRNA was entrapped into the polymeric micelles [109].

The cationic hydrophobicized polysaccharide-DNA complex can enhance the gene transfection efficiency by increasing cell membrane-carrier interaction and membrane destabilization [110, 111]. Furthermore, it also can protect DNA against DNase degradation in body fluids, and release DNA as the active form to improve the transfection efficiency of DNA [11, 112, 113].

Hu F.Q. *et al.* had synthesized SA-CSO, and prepared SA-CSO/DNA complex micelles by condensing the plasmid DNA [18]. The micelles can efficiently protect the condensed DNA from enzymatic degradation by DNase I. The *in vitro* transfection efficiency of SA-CSO micelles using plasmid DNA was higher than that of CSO, and comparable to Lipofectamine^{TM} 2000.

As an efficient gene carrier, deoxycholic acid (DOCA)-modified chitosan nanoparticles showed superior gene condensation and protection from endonuclease attack than the unmodified chitosan. Furthermore, DOCA-modified chitosan showed great potential as gene carrier to reach the high level of gene transfection efficiency, even in the presence of serum. Given the negligible cytotoxic effects, DOCA-modified chitosan oligosaccharides can be considered as potential carriers for efficiently and specifically delivering the target gene [37].

7. CONCLUSIONS AND PROSPECTIVES

As drug delivery nanocarriers, hydrophobicized polysaccharide polymeric micelles provide a series of attractive properties, such as high drug loading, long-circulation in blood, and controlled release profile of the incorporated drugs. The polymeric micelles not only can incorporate hydrophobic drugs, but also complex hydrophilic macromolecules such as proteins, peptides and DNA. Apart from drug loading, polymeric micelles can modify the *in vivo* distribution of drugs through either passive or active targeting strategies. All the above properties contributed to the successful clinical application of hydrophobicized polysaccharide polymeric micelles in the foreseeable future.

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