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Novel nanoparticle materials for drug/food delivery-polysaccharides

DOI: 10.1515/psr-2016-0053

1 Introduction

As condensation polymers, polysaccharides are generally termed as glycans, in which more than ten monosaccharide units are mutually joined together by *O*-glycosidic bonds [1]. The general formula of polysaccharides can be represented as $(C_6H_{10}O_5)_n$ where $40 \leq n \leq 3000$ [2, 3]. Polysaccharides are often quite heterogeneous, with structures ranging from linear to highly branched.

Polysaccharides are the most abundant resources in nature that are commonly applied in daily life, including cellulose, pectin, chitosan, starch, etc. Depending on the type of monosaccharide building units, polysaccharides can be divided into homopolysaccharide (also called homoglycan, containing the same type of monosaccharide) and heteropolysaccharide (or heteroglycan, composed of more than one type of monosaccharide) based on the definition given in the International Union of Pure and Applied Chemistry (IUPAC). Both homopolysaccharides and heteropolysaccharides may possess homo-linkages or hetero-linkages with respect to configuration and/ or linkage position. These macromolecules can also be divided into storage polysaccharides (such as starch and glycogen) and structural polysaccharides (such as cellulose and chitin) based on their biological functions [4]. Starch is made up of a mixture of amylose and amylopectin. The amylose content in starch depends on the biological source, accounting about 20–30% in most starch [5]. Amylose is linear chain glucan with α-1,4 linkage, whereas amylopectin consists of several side chains on *O*-6 site of the glucose in α-1,4 backbone with α-1,6-bonds occurring every 24 to 30 glucose units. Starch is used as a storage polysaccharide in plants, while glycogen serves as the form of energy storage in animals and fungi [6]. The structure of glycogen is similar to amylopectin but is more extensively branched and compact than starch [7]. As a hyperbranched biopolymer, glycogen consists of linear glucose chains with side chains branching off every ten glucoses or so. Overall, most of the storage polysaccharides are hyperbranched molecules.

As the most common structural polysaccharide, cellulose is the structural component of the primary cell wall of plants and considered as the most abundant natural resource. This type of polysaccharide consist of a linear chain ranging from one hundred to over ten thousand β-1,4 linked D-glucose units [8]. The other common structural polysaccharide is chitin, which is a long-chain polymer of *N*-acetylglucosamine [9]. Chitin is the main component of the cell walls of fungi, the exoskeletons of arthropods and insects, the radulas ofmollusks, and the beaks and internal shells of cephalopods [10]. Chitin plays the role of cellulose in structure, but serves as keratin in function. As a naturally occurring polymer, chitin has also been proven its usefulness in medical and industrial applications.

Some studies have described several health benefits of polysaccharides including immunomodulatory, antitumor, antimicrobial effects and hypocholesterolemic effects [11–13]. With excellent properties (including highly stable, safe, nontoxic, hydrophilic and biodegradable) and abundant resources, polysaccharides have been studied and applied in biomaterial fields. Particularly, the application of polysaccharides in nanoparticle delivery systems (NPDSs) has attracted increasing attention in recent decades.

Nanoparticles are defined as solid, colloidal particles consisting of macromoleculeswith a size in the range of 10–1000 nm[14]. Nanoparticlematerial is an important application field of nanotechnology, which has been applied in various fields such as food, feed, biomedical sciences, and drug/ gene delivery systems [15–17]. In particular, NPDSs have been attracting increasing attention recently and are became a focus. NPDSs are generally referred to as nanometric carriers with various morphologies, including nanospheres, nanocapsules, nanomicelles, nanoliposomes, and nanodrugs, etc. [18]. Presently, nanoparticles have been widely employed to deliver food ingredients, drugs, polypeptides, proteins, genes, and other biomolecules.

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2 Nanoparticles for delivery systems

Previous studies and applications of nanoparticles weremainly focused on drug delivery, because nanoparticles can entrap drugs or biomolecules into their interior structures and/ or absorb drugs or biomolecules onto their exterior surfaces. Therefore, the most important application of nanoparticles was nanovectors. Additionally, previous studies have summarized various outstanding advantages of nanoparticle drug delivery systems, including [19]: (1) they can pass through the smallest capillary vessels because of their ultra-tiny volume and avoid rapid clearance by phagocytes so that their duration in blood stream is greatly prolonged; (2) they can penetrate cells and tissue gaps to arrive at target organs such as liver, spleen, lung, spinal cord and lymph; (3) they could show controlled release properties due to the biodegradability, pH, ion and/ or temperature sensibility of materials; (4) they can improve the utility of drugs and reduce toxic side effects; etc.

With these superiorities, nanoparticle drug delivery systems have been widely studied in biological, medical and pharmaceutical applications. Meanwhile, nanoparticles have also attracted increasing attention for food delivery systems to provide maximum protection for sensitive food components against oxidation, enzyme degradation and pH before reaching the target [20, 21]. Several bioactive food components have been found effective in treatment of coronary heart diseases, inflammation, and immune disorder etc. in corporation with the diet [21, 22]. They are mainly grouped into isoprenoids, fatty acids, proteins and amino acids, polysaccharides and minerals. However, some of these components have poor properties, such as instability during digestion, poor solubility and bioavailability, ingredient interactions, and unpleasant taste. Thereby, nanoparticles were employed to improve the bioavailability of bioactive food ingredients, provide maximum stability, introduce controlled/ target release of encapsulated compound during mastication and digestion for efficient absorption into the body system.

Based on the method of preparation, nanoparticles can be designed and constructed to possess different properties and release characteristics for the best delivery or encapsulation of the therapeutic agent [23]. The nanoparticles currently used and studied as nanovectors can be grouped into three main classes or "generations" [24, 25]. The first class focuses on a passive delivery system for the target site. For example, the size of particles could enable the driving systems to the tumor site, but not specific recognition of the targets [26]. The second class of nanovectors includes additional functional groups that allow for molecular recognition of the target tissue. These functional groups include ligands, aptamers, and small peptides that bind to specific target-cell surface markers or surface markers expressed in the disease microenvironment [27]. Meanwhile, pH-sensitive polymers are included in this category. Finally, the third class aimed to successfully overcome the natural barriers that the vector needs to efficiently deliver the drug to the target site. This goal will only be reached by a "multistage" approach, and such a system has been recently reported [28]. Currently, particles of the first generation have been approved by FDA for their use in metastatic breast cancer [29]. Numerous clinical trials are also ongoing for the targeted second class nanovectors, particularly in cancer applications [30].

Compared with nondegradable materials, biodegradable systems have some advantages in the application of nanoparticles, including nontoxic, biotolerable, biocompatible, biodegradable, and water-soluble properties. Polysaccharides, as the most popular natural biopolymer, have their unique features in developing nanoparticles [31–33].

3 Polysaccharides and their nanoparticles

A variety of polysaccharides have been modified with various reactants and investigated for the synthesis and application of nanoparticles using various methods [34–36]. From the viewpoint of polyelectrolyte, polysaccharides can be divided into nonpolyelectrolytes (including starch, dextran, cellulose, etc.) and polyelectrolytes as listed in Table 1, the latter can be further divided into positively charged polysaccharides (chitosan) and negatively charged polysaccharides (alginate, pectin, hyaluronic acid, etc.).

Tab. 8.1: Polysaccharides in the preparation of nanoparticle delivery systems.

Polysaccharides	Molecular weight (Da)	Source	Application in nanoparticles
Starch	Amylose: 10^7–10^8 Da Amylopectin: 10^6–10^7 Da	Higher plants	Nanocarrier for parenteral drug delivery [37], for colon-specific delivery [38], for insulin controlled release [39], for siRNA delivery [40], etc.
Cellulose	10^4–10^6 Da	Cell wall of green plants, many forms of algae and the oomycetes	Nanodelivery for hydrophobic active ingredients [41], for oral protein [42], for anticancer drugs [43], etc.
Dextran	10^7–10^8 Da	Product of many bacterial strains and enzymatic product of cell-free culture supernatant	Controlled release of growth factors for wound healing and skin regeneration [44], drug nanocarrier [45], delivery for delicate bioactive molecules [46], etc.
Cyclodextrin	2×10^3–10^7 Da	Enzymatic degradation of starch derived from potatoes, corn, rice and other sources	Nanocarrier for drug release control [47], for gene and drug delivery [48], etc.
Pullulan	From thousands to 2 000 000 Da	Bacterial homopolysaccharide production from starch by the fungus *Aureobasidium pullulans*	Drug and gene delivery [49], protein delivery [50], tissue engineering [51], wound healing [52], etc.
Guar gum	NA	Extraction of the seeds of *Cyamopsis tetragonoloba*	Nanocomposite for drug release [53]
Chitosan	3800–20 000 Da	Shells of crab, shrimp and krill; cell walls of fungi	Nanocarrier in drug delivery system [54], nanocontainer for hydrophobic drugs [55], nanoparticles complex with other molecules as discussed below, etc.
Alginate	200 000–500 000 Da	Extraction from cell walls and intercellular spaces of marine brown algae	Nanocarrier gene delivery [56], for protein delivery [57], for drug delivery [58]; scaffolds in tissue engineering [59], etc.
Pectin	50 000–180 000 Da	Cell wall of all plants	Facilitation of the delivery of specific sequences of amino acids [60], drugs [61]; wound healing scaffolds [62], etc.
Hyaluronic acid	Can reach as high as 10^7 Da	Vertebrate organisms	Nanoparticles for wound healing and skin regeneration [44], for siRNA delivery [63], anticancer drug delivery [64], etc.

3.1 Nonpolyelectrolyte polysaccharides

3.1.1 Starch and its derivation

Starch-based nanoparticles have attracted increasing attention due to their good hydrophilicity, biocompatibility and biodegradability. Starch is made up of two main structural components: amylose and amylopectin [65, 66], the former consists of a linear backbone of α-1,4-linked glucose with/without a low level of branching with a α-1,6-linkage, while amylopectin is a highly branched form of 'amylose' [39, 67] (Figure 1). As the second most abundant biomaterial in nature, starch has been modified with various reactants by way of chemical reaction with hydroxyl groups in the starch molecule for preparation of nanoparticles [68].

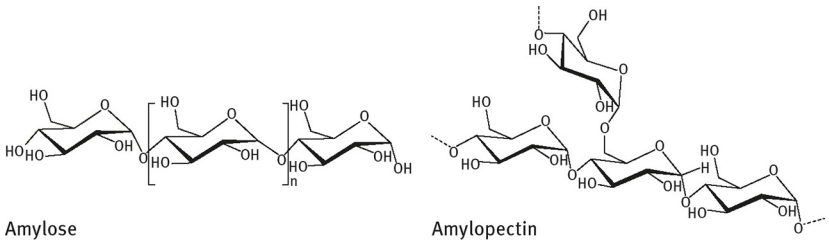

Figure 1: Chemical structure of starch (adapted from [2]).

The hydrophobic derivative of starch by grafting hydrophobic poly (lactic acid) chains (PLA) was prepared to nanoparticles through crosslinked method and used for drug delivery taking Indomethacin as the model drug [69, 70]. Besides, propyl-starch nanoparticles were prepared to entrap docetaxel for cancer therapy and revealed high encapsulation efficiency [71, 72].

3.1.2 Cellulose and its derivation

Cellulose is the most abundant polysaccharide available on Earth with the formula of $(C_6H_{10}O_5)_n$ [73–75]. The cellulose molecule is formed by a linear chain of β-1,4-linked D-glucose units with different lengths (Figure 2). Due to the insolubility of cellulose, nanoparticles are usually prepared from its derivative. Several studies have been conducted on the Poly (ε-caprolactone) (PCL) and poly (L-lactic acid) (PLLA) modification of soluble cellulose and its derivate [76–78]. Besides, chitosan or its oligomer could complex carboxymethyl cellulose (CMC, anionic derivative of cellulose) to form stable cationic nanoparticles for coating with plasmid DNA in genetic immunization [79].

3.1.3 Dextran and its derivation

Dextrans are a class of polysaccharides consisting of a linear backbone with mainly α-1,6-linked glucose, and a variable amount of α (1→2), α (1→3) and α (1→4) branched linkages [80] (Figure 3). Currently, dextran is

widely applied in fields of chemical, pharmaceutical, clinical and food industry playing the function of adjuvant, emulsifier, carrier, drug, stabilizer, and thickener of jam and ice cream [81, 82]. Dextrans are colloidal, hydrophilic and water-soluble substances, and can be decomposed in human feces due to bacterial action [44]. Hence, various drug-dextran prodrugs could be able to keep integrity in stomach and the small intestine but release in colon [83].

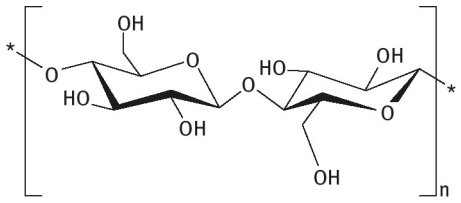

Figure 2: Chemical structure of cellulose (adapted from [2]).

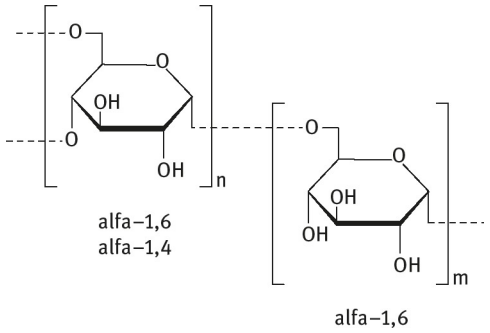

Figure 3: Chemical structure of dextran (adapted from [2]).

Dextrans have been modified to extend their surface-active properties and potential applications in pharmacy, biochemistry and medicine. Either water-soluble or water-insoluble dextran derivatives have been prepared based on the extent of modification. The water-insoluble derivatives could be solubilized in organic solvents like tetrahydrofuran or dichloromethane saturated with water [84–86]. With food availability, biocompatibility and biodegradability, dextran has been widely selected as promising biomaterial in the preparation of nanovectors.

3.1.4 Cyclodextrins and their derivation

Cyclodextrins (CDs) are cyclic oligosaccharides composed of at least five α-1,4-linked glucopyranose units in a rigid $^4C_1$ chair conformation, prepared by enzymatic degradation of starch. The most common CDs contain 6–8 D-glucose units and are known as α-CD, β-CD, and γ-CD, respectively, as shown in Figure 4 [87–89]. CDs are neither hydrolyzed nor absorbed in stomach and small intestine, but are absorbed in the large intestine so that the vast microflora present in the colon could degrade them into small saccharides [90, 91]. This property ensures CDs as a colon targeting carrier.

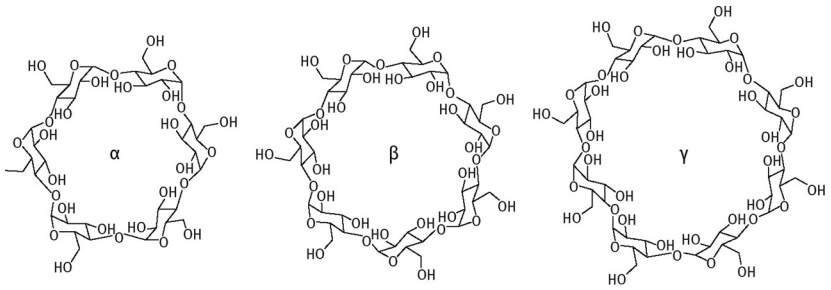

Figure 4: Chemical structure of cyclodextrins.

Cyclodextrins are considered to be the most widely explored materials applied for nanoparticle formation. CD-based nanoparticles were usually prepared by self-assembling method, because the three-dimensional ring structure of CDs allows for encapsulation of hydrophobic molecules within the oligosaccharide cavity [92–94]. Harada et al. have reported that αCD-based nanoparticles could be formed with poly(ethylene glycol) (PEG) for drug delivery [95]. Besides, modified CDs have been used to prepare nanoparticles for delivery of small interfering RNA (siRNA), as well as controlled release of antimalarial artemisinin [96].

3.1.5 Pullulan

Pullulan is a linear glucan produced from starch by the fungus *Aureobasidium pullulans* [97, 98]. The backbone of pullulan is formed by maltotriose units (α-1,4-D-glucopyranose) through α-1,6 glycosidic linkage in a ratio of 2 : 1 (Figure 5). Due to existence of an α-1,6 linkage in the molecule, pullulan tends to perform as a random flexible coil in aqueous solution, which may result in its biodegradability and it has high adhesion, structural flexibility and solubility [99].

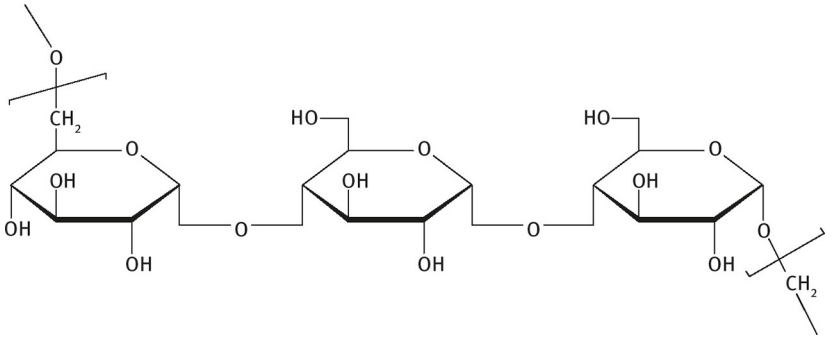

Figure 5: Chemical structure of pullulan (adapted from [2]).

The FDA has approved pullulan for various applications, due to its hemocompatible, nonimmunogenic, and noncarcinogenic properties [100]. The applications of pullulan extend to a variety of fields: in biomedical fields as drug and gene delivery [101], tissue engineering [102], and wound healing [103]; in pharmaceuticals as a coating agent [49, 52]; in foods and beverages as a filler; as an edible, mostly tasteless polymer, as well as edible films [104, 105].

Pullulan needs to be modified with hydrophobic molecules for self-assembling in water solution which will then behave as carriers of agents. Hydrophobic molecules including cholesterol, hexadecanol, vitamin H, etc. have been used to derive pullulan to obtain amphiphilic micelles [19]. The partially hydrophobized pullulan shows unique association and potential application in delivery systems. For example, both cholesterol-pullulan and a copolymer of N-isopropylacrylamide and N-[4(1-pyrenyl)butyl]-N-noctadecylacrylamide, and hexadecyl group-bearing pullulan have been self-assembly prepared for nanoparticle delivery carriers [106–108]. Pullulan acetate (PA) is the other important hydrophobized pullulan, which can form self-aggregation nanoparticles as well as its modified materials [109, 110].

3.1.6 Guar gum

Guar gum, also called guaran, is formed by a linear chain of β-1,4-D-mannopyranosyl residues with branching points at *O*-6 site having α-D-galactopyranosyl units as the side chains (Figure 6) [111]. With water solubility, guar gum is a nonionic natural polysaccharide derived from the seeds of *Cyamopsistetra gonolobusis*.

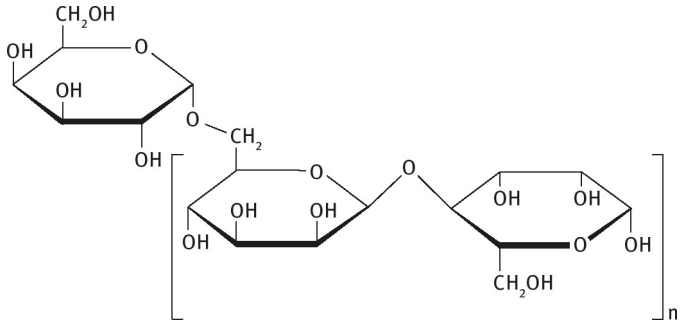

Figure 6: Chemical structure of guar gum.

Guar gum hydrates in cold water to form highly viscous colloidal dispersions or sols [112]. Guar gum solution is stable under pH range 5–7, but extreme pH and high temperature conditions (e.g. pH 3 at 50 °C) can degrade its structure [113]. With properties of being nontoxic, highly viscous and easily available, guar gumis commonly used for various applications: in food industry as thickener for sauces, ice creams, etc.; in pharmaceuticals as binder and disintegrant for solid dosage and as hydrophilic matrix for oral controlled release dosage

[111, 112]. Besides, guar gum has been extensively applied in colon-specific drug delivery due to its drug sustained release property and susceptibility to microbial degradation in the colon [114]. In addition, guar gum has been found to be a better stabilizer of the nanoparticles [113].

3.2 Positively charged polyelectrolyte polysaccharides

3.2.1 Chitin and chitosan

Chitin is the main component of fungal cell walls, the exoskeleton of crustaceans (such as crabs and shrimp) and insects [115–117]. As shown in Figure 7, chitin consists of a linear chain of *N* -acetylglucosamine. The role of chitin is analogous to cellulose in structure and to keratin in function. As the deacetylation product of chitin, chitosan is composed of glucosamine and*N* -acetylglucosamine by β-1,4-glycosidic bonds forming linear backbone. Chitosan is produced industrially by alkali treatment to hydrolyze the amino-acetyl groups of chitin with the degree of deacetylation (%DD) in range 60–100%[117, 118].

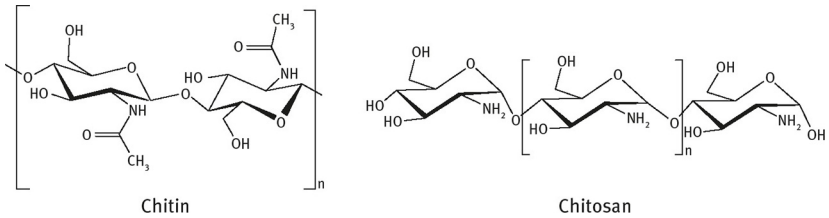

Figure 7: Chemical structure of chitin and chitosan.

Chitosan is considered as a biocompatible, biodegradable and nontoxic biomaterial and widely applied in pharmaceutical and biomedical fields [119]. In the field of nanomedicine, chitosan has received considerable attention as vector in novel bioadhesive drug delivery systems which prolong the residence time of the drugs at the site of absorption and increase the drug bioavailability [54]. However, this biopolymer can only solubilize in diluted acidic aqueous solution (pH < 6.5) for the glucosamine units converting into a soluble form with protonated amine groups [120]. The insolubility in water and organic solvents limited its application, but chitosan could be hydrophobically modified to obtain nanoparticles and applied as nanocarriers for drugs due to their biocompatibility *in vivo* [121]. Experimental *in vitro* and *in vivo* results show chitosan as a promising nanocarrier for controlled release of various drugs with excellent encapsulated efficacy.

3.3 Negatively charged polyelectrolyte polysaccharides

3.3.1 Alginate

Alginate is an anionic linear polysaccharide derived from cell walls and intercellular spaces of marine brown algae. The structure of alginate consists of a backbone of β-1,4-linked D-mannuronic acid (M unit) and α-1,4-linked L-guluronic acid (G unit) arranged of various compositions and sequences depending on the source of the alginate (Figure 8). M block segments provide linear and flexible conformation, while G block segments serve folded and rigid structural conformations [122]. Moreover, the ratio of M unit against G unit has been reported to affect its physicochemical properties, as well as its further applications [100].

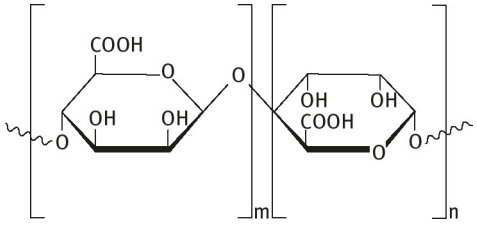

Figure 8: Chemical structure of alginate.

Alginate is a biopolymer with biocompatible, nonimmunogenic, nontoxic and biodegradable properties [123]. A large number of free hydroxyl and carboxyl groups in the backbone of alginate may be modified to achieve solubility, hydrophobicity, physicochemical and biological characteristics, as well as various potential applications [122]. The applications of alginate have extended to various industries: as food additive and

thickener in food industry [124]; as scaffolds in tissue engineering [125]; and as controlled drug release devices in biomedicine [126]. Besides, previous studies have indicated that the muco-adhesive, biocompatible and biodegradable properties of alginate make it an important and hopeful tool in the preparation of controlled drug-delivery systems achieving an enhanced drug bioavailability [124, 126].

3.3.2 Pectin

Pectin exists in the cell wall of plants to function as cell adhesion. Pectins are a family of complex polysaccharides containing 1,4-linked D-galacturonic acid residues and were usually divided into homogalacturonans (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II), xylogalacturonan (XGA) and apiogalacturonan (AGA) based on their structural features [61, 127]. The common pectin type is HG as shown in Figure 9.

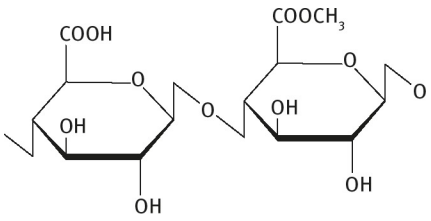

Figure 9: Chemical structure of pectin.

Pectin can resist the degradation in the physiological environment of the stomach and the small intestine, but can be decomposed by pectinases secreted by microflora of the human colon [128]. Thanks to these properties, pectin could function as prebiotics and delivery vector for components from the mouth to the colon [129, 130]. However, pectin cannot protect its encapsulated components during its delivery through the stomach and small intestine due to its high water solubility [129, 130]. Hence, studies mainly focused on pectin derivatives with water resistant and enzymatic degradation. For this purpose, calcium pectinate was deeply studied as a drug carrier for colon-specific delivery because this complex can reduce the solubility of pectin and keep stable in low pH environment [128]. Besides, pectin has been combined with other polymers, including 4-aminothiophenol [131], chitosan [132], hyaluronic acid [133] or poly (lactide-co-glycolide) [134], showing good results as controlled drug release devices.

3.3.3 Hyaluronic acid

Hyaluronic acid (HA) (also called hyaluronan, hyaluronate) is an anionic polysaccharide composed of repeating disaccharide units of D-glucuronic acid and *N* -acetyl D-glucosamine linked via altering β-1,3 andβ-1,4 glycosidic bonds as shown in Figure 10 [135]. HA has been reported to associate with several cellular processes, including angiogenesis and the regulation of inflammation [136].

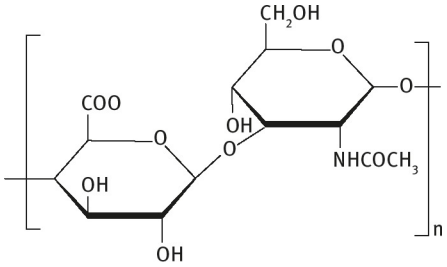

Figure 10: Repeating unit of hyaluronic acid.

As described in previous studies, HA is a biodegradable, bioactive, nonimmunogenic and noncytotoxic polysaccharide [137]. Similar to other glycosaminoglycans, hyaluronan can function as a targeting vector for the delivery of chemotherapeutic agents to cancerous tissues, as many tumors overexpress the hyaluronan CD44 and RHAMM receptors [138]. As a drug delivery carrier, HA has several advantages including the negligible nonspecific interaction with serum components due to its polyanionic characteristics [139] and the highly efficient targeted specific delivery to the liver tissues with HA receptors [140].

3.4 Hyperbranched polysaccharides

The above described polysaccharides are mainly attributed to linear polysaccharides or partially branched polysaccharides. However, hyperbranched polysaccharides (HBPs), including amylopectin, glycogen and some glucans from mushroom cell walls, have attracted increasing attention in the fields of nanotechnology and pharmacology because of their unique structures and properties [141–143]. The spherical architecture of highly branched macromolecules provides numerous terminal units that can be converted into various functional groups leading to novel nanomaterials [32, 144]. HBPs can also form polymeric micelles that are spherical aggregates of amphiphilic blocks of copolymers which enhance water solubility and decrease the toxicity of hydrophobic drugs [145].

Compared with synthetic highly branched polymers, research interest in natural HBPs is emerging in the field of biomaterials due to their nontoxicity, good biocompatibility and biodegradability [19]. Unlike other highly branched polymers, spherical HBPs do not only provide reaction sites for the formation of nanoparticles, but they also protect the nanoparticles in a shell structure with excellent dispersion in water [32, 33, 146]. These unique properties of HBPs can be applied in the fields of drug delivery and controlled release [33, 146]. For example, hyperbranched cationic amylopectin derivatives have been designed for gene delivery with high transfection efficiency and exhibited potential as nonviral gene vectors [147]. HBPs were shown to interact strongly with lectins due to the clustering or multivalent effects of the numerous nonreducing saccharide units on their surfaces [148]. HBPs have also potent bioactivity including immune-modulatory and antitumor effects [31, 149]. Recent studies on HBPs have been focused on obtaining them from natural sources and characterizing their physical properties including solubility, shrinking factors, and rheological properties [150–152].

3.5 Other polysaccharides

Besides, various fungal polysaccharides have attracted increasing attention in the dispersion of nanoparticles. These polysaccharides mainly contain helical chains structures, including lentinan, scleroglucan, schizophyllan, etc. Lentinan is a structural polysaccharide from the fruiting body of *Lentinus edodes*, with a β-(1→3)-D-glucan backbone and two β-(1→6) linked glucoses as side groups every five (1→3)-D-glucoside residues. Lentinan has been identified as triple helical chains in aqueous solutions and single flexible chains in DMSO or high concentration of alkali solutions. These structural properties of lentinan have been applied to disperse silver nanoparticles in water [153]. Triple helical schizophyllan has also been applied as reducing and stabilizing agent to prepare silver nanoparticles [154]. Moreover, $CaCO_3$–lentinan microspheres have been obtained by self-assembly nanoparticles and applied as an anticancer drug carrier [155]. Scleroglucan (SCL) is the other β-(1→3) and β-(1→6) glucan with triple helical chains, produced by fungi of the genus *Sclerotium*. SCL was used to prepare scleroglucan gels for investigation of drug-loading effects on release by using theophylline as the model drug [156]. In addition, SCL-PVA (polyvinylalcohol) hydrogels containing magnetic nanoparticles have been prepared to study drug release behavior [157].

4 Nanoparticle preparation based on polysaccharides

Recent reviews have presented excellent summaries of the preparation and application of polysaccharide-based nanoparticles, mainly focusing on starch, chitosan, cellulose, pectin, etc. Along with the study of nanoparticles furthering the 'third generation', more polysaccharide-based nanovectors emerged in the field of drug, gene and food delivery systems. Based on structural characteristics, polysaccharide nanoparticles are prepared mainly by four mechanisms, including covalent crosslinking, ionic crosslinking, polyelectrolyte complexation, and self-assembly of hydrophobically modified polysaccharides.

4.1 Covalent crosslinking polysaccharide nanoparticles

The method of covalent crosslinking was first performed on the preparation of chitosan-based nanoparticles. In this method, a crosslinker is necessary to obtain the desired nanoparticles. For example, glutaraldehyde was used as the crosslinker to crosslink chitosan-based nanoparticles [158, 159]. However, the toxicity of glutaraldehyde on cell viability limits its utility in delivery systems. Carbodiimide was then employed as a water-soluble condensation agent and biocompatible crosslinker for covalent crosslinking [160]. With the aid of this crosslinker, natural di- and tricarboxylic acids were used for intermolecular crosslinking of chitosan nanopar-

ticles [160, 161]. Furthermore, hyaluronic acid has also been used to prepare nanoparticles by using a carbodiimide method [162].

The nanoparticles prepared with this method were mainly in the form of polycations, polyanions, and polyampholytes and stable in aqueous media at low pH, neutral, and mild alkaline conditions. In the swollen state, the average size of the particles was in the range of 270–370 nm depending on the pH.

4.2 Ionic crosslinking polysaccharide nanoparticles

Similar to the method of covalent crosslinking, ionic crosslinking aims at charged polysaccharides or modified polysaccharides. However, ionic crosslinking has unique advantages against covalent crosslinking, including mild preparation conditions and simple procedures.

For ionic polysaccharides, low MW of polyanions and polycations could act as ionic crosslinkers for polycationic and polyanionic polysaccharides, respectively. To date, the most widely used polyanion crosslinker is tripolyphosphate (TPP), which was first used to crosslink chitosan nanoparticles in 1997 [163, 164]. TPP is nontoxic and has multivalent anions. It can form a gel by ionic interaction between positively charged amino groups of chitosan and negatively charged counterions of TPP [165].

Currently, chitosan is usually replaced by water-soluble chitosan derivatives to prepare nanoparticles by ionic crosslinking method. Compared with chitosan itself, its derivatives can easily dissolve in neutral aqueous media, avoiding the potential toxicity of acids and hence protecting the bioactivity of loaded biomacromolecules. For instance, N-trimethyl chitosan nanoparticles have been synthesized by ionic crosslinking of N-trimethyl chitosan with TPP and showed an encapsulation efficiency up to 95% and a loading capacity up to 50% (w/w) [159, 166]. Their potential as a carrier system was evaluated for the nasal delivery of proteins, ovalbumin [167]. The following studies indicated the nontoxicity of N-trimethylchitosan/TPP nanoparticles to Calu-3 cells. The absorption properties of N-trimethylchitosan/TPP nanoparticles have also been evaluated by use of *in vitro* (Caco-2 cells) and *ex vivo* (excised rat jejunum) models [168].

Besides, some negatively charged polysaccharides (including alginate, pectin and hyaluronic acid) bearing carboxylic groups can be crosslinked by bivalent calcium ion to form nanoparticles. For example, Ca-alginate nanoparticles have been prepared by water-in-oil reverse microemulsion method (~ 80 nm in size) [169] and ion-induced gelification method (235.5nmin size) [170] to deliver gene and drugs, respectively. The relative bioavailabilities of all drugs encapsulated were significantly higher than oral free drugs. In drug delivery system, all drugs (isoniazid, pyrazinamide, rifampicin) were detected in organs (lungs, liver and spleen) above the minimum inhibitory concentration until 15 days post nebulization, whilst free drugs stayed up to day 1. These inhalable nanoparticles could serve as an ideal carrier for the controlled release of antitubercular drugs.

4.3 Polyelectrolyte complexing polysaccharide nanoparticles

Polysaccharide nanoparticles by polyelectrolyte complexation (PEC) are also based on polysaccharides or their derivatives with charge. PEC has received increasing attention compared with other nanoparticle preparation techniques, because PEC-based nanoparticles have several characteristics favorable for cellular uptake and colloidal stability, including suitable diameter and surface charge, spherical morphology and a low polydispersity index (PdI), and so on [171]. Besides, nanoparticles prepared by this method not only avoid many kinds of aggression in harsh conditions (such as organic solvents and sonication during preparation), but also keep the stability and biological activity of the encapsulated agents in completely aqueous condition and in ambient temperature [172, 173].

As shown in Figure 11, polysaccharides can form PEC with oppositely charged polymers by intermolecular electrostatic interaction. Currently, chitosan is the only natural polycationic polysaccharide to form nanoparticles in this method, for its water-soluble and biocompatible properties [175]. Chitosan-based PEC nanoparticles can be synthesized with many negative polymers, including polysaccharides [176], peptides [177], polyacrylic acid family [178], and so on [179, 180].

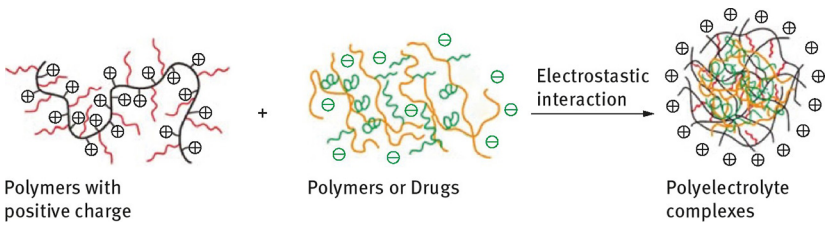

Figure 11: The schematic illustration of the nanoparticles formed by polyelectrolyte complexation [174].

4.4 Self-assembly polysaccharide nanoparticles

Self-assembling polysaccharide nanoparticles have moved to the forefront due to their unique advantages, including biocompatibility and stimulus responsiveness. The self-assemblednanoparticles are composed of a core of hydrophobic moieties surrounding by a hydrophilic outer shell, which could serve as protection for the carried hydrophobic agents [181–183]. By grafting with hydrophobic segments, polysaccharides can form amphiphilically polymeric micelles which enhance water solubility and decrease the toxicity of hydrophobic drugs [145].

In aqueous environment, polyamphiphiles spontaneously form micelles or micelle-like aggregates via undergoing intra- or intermolecular associations between hydrophobic moieties, in order to minimize interfacial free energy. In recent years, numerous studies have been carried out to investigate the synthesis and the application of polysaccharide-based self-aggregate nanoparticles as drug delivery systems. Generally, these hydrophobic molecules can be divided into linear [184, 185], cyclic hydrophobic molecules [186, 187], polyacrylate family [188, 189], etc.

5 Applications of polysaccharide-based nanoparticles

In the design of nanoscale carriers, polysaccharides have received considerable attention for their unique properties, including safety, nontoxicity, bioavailability and biocompatibility. Currently, polysaccharide-based nanoparticles have been investigated and applied in medical and food fields as new promising biomaterials.

5.1 Medical applications

5.1.1 Delivery of peptides and proteins

In recent years, some peptides and proteins have been discovered for therapeutic and antigenic bioactivities and attracted considerable attention [190]. However, most of these biologically derived drugs are limited for *in vivo* application by their disadvantages like low stability, short biological half-life and the need to cross biological barriers. From this viewpoint, polysaccharide-based nanoparticles can overcome some of the problems of systemic administration. Currently, polysaccharide-based nanoparticles have been prepared and applied for delivery of several protein drugs, including insulin [191, 192], basic fibroblast growth factor (bFGF) [193, 194] and epidermal growth factor receptor (EGFR) antisense (AS) [195]. Meanwhile, bovine serum albumin (BSA) is a normal model in the preparation of nanoparticles based on polysaccharides [196].

Insulin is one of the most widely applied therapeutic peptides for the treatment of insulin-dependent diabetes mellitus. The normal problems of oral insulin administration are low bioavailability on acidic gastric pH, the enzymatic barrier of the intestinal tract and the physical barrier made up of the intestinal epithelium. Therefore, polysaccharide-based nanoparticles have been prepared for the inclusion of insulin in various studies by different methods, which show good release control and good results in loading efficacy [192]. Nanoparticles based on alginate-alginate coated with chitosan, alginate-dextran have been prepared by the interaction of carboxylic groups of alginate with amino groups of insulin [197, 198]. Insulin-loaded nanoparticles have also been synthesized by ionic crosslinked method using chitosan as the selected polysaccharide and TPP anions as crosslinker agent [199]. Additionally, PEC between chitosan and different polyanions (alginate, glucomannan, and dextran sulfate) has been prepared for insulin inclusion in some studies and shown ~90% association efficiency values. Self-assembling method was also used to prepare insulin-loaded nanoparticles using cholesterol-bearing pullulan as the selected polysaccharide [106]. These insulin-loaded polysaccharide-based nanoparticles showed excellent behavior in evaluation of physiological activity.

5.1.2 Delivery of anticancer drugs

The main problems of cancer chemotherapy are related with the toxicity caused by anticancer drugs on normal tissues and release control of drugs on the target site [200]. Thus, nanoparticles are being extensively investigated as carriers of anticancer drugs, in order to overcome several problems in cancer chemotherapy, including

reducing harmful side effects, enhancing blood circulation time, controlling the release concentration of the drug at the tumor site for a desired time period, thus, increasing therapeutic efficiency. Among the available potential drug carrier systems in nanoscale, polysaccharide-based nanoparticles play an important role and their use with some anticancer drugs shows promising results [201].

Tamoxifen, a drug for hormone dependent breast cancer, has been entrapped into polysaccharide-based nanoparticles to overcome the undesirable side effects and to increase the concentration at the tumor site due to specific recognition for targeting tissue or organ. Tamoxifen-loaded nanoparticles were prepared by Sarmah and coworkers based on guar gum, which is commonly used for colon specific drug delivery in the pharmaceutical industry [111, 202]. As a breast cancer drug, mitoxantrone is positively charged and then has been encapsulated in chitosan-based nanoparticles by ion gelation method using sodium TPP as gelation agent, and obtaining an encapsulation efficacy of 98% [203]. There were also other anticancer drugs delivered by polysaccharide-based nanoparticles, including methotrexate (MTX, a folate antimetabolite) [204], doxorubicin (DOX, an anthracycline ring antibiotic drug) [205], paclitaxel (an anticancer drug) [206], etc.

5.1.3 Nanovectors of nucleic acids and genetic material

Up to now, gene therapy has been applied in many different diseases such as cancer, AIDS, and cardiovascular diseases [207]. Gene therapy aims to transfer genetic materials into specific cells of a patient to repair defective genes responsible for disease development [208]. To transfer the genes to the specific site, genes must escape the processes that affect the disposition of macromolecules and avoid the degradation by serum nuclease.

Small interfering RNAs (siRNAs) have been employed as a novel tool to block the expression of infectious diseases and cancers. However, siRNA suffers particular problems including poor cellular uptake, rapid degradation as well as limited blood stability. For this reason, chitosan-based nanoparticles have been prepared to transfect small interfering RNAs (siRNAs) by modified ionic gelation method with TPP as crosslinker agent [209]. Besides, PEC between chitosan and different polyanions has been used to prepare nanoparticles in order to include nucleic acids, since chitosan-DNA nanoparticles demonstrated low transfection efficiencies and the incorporation of secondary polymers improved the characteristics of these systems [210]. Recently, a new method has been used to prepare a gene nanocarrier based on triple helical β-glucan [211]. The target DNA sequence was firstly bound to polydeoxyadenylic acid (poly (dA)) by disulfide bonds (poly (dA)–SS–DNA). Lentinan was then used to combine the poly (dA)–SS–DNA chain to form a new triple helical conformation and provide protection of the delivered DNA. The target DNA was then delivered into the cell via endocytosis and released from the delivery system by automatic cleavage disulfide bonds in cytoplasm.

5.2 Food applications

Apart from delivery of drugs and genes, polysaccharide-based nanoparticles have also been studied and applied in delivery systems of food bioactives. Antioxidants, probiotics, polyunsaturated fatty acids, and proteins are common bioactives that can be added to food to improve nutritional value, to prevent diseases and to improve overall health [212]. Nanodelivery of these components may improve their stability [213, 214], solubility [215, 216], functionality [217, 218], cellular uptake [219–221], and bioavailability [222–224] and may also provide controlled release [225–227] for better efficacy of the bioactive.

Nanoparticles formed by polysaccharides can deliver a variety of lipophilic bioactives to the colon, maintain their integrity and are kept impermeable within the upper gastrointestinal tract (GIT). It may be necessary to design a nanoparticle or microgel that protects the bioactive component within a food product, but that can release it within the upper GIT so that it can be absorbed. For instance, casein-pectin microgels have been reported to encapsulate polyunsaturated lipids and protect them from oxidation [228], but they will fully dissociate under simulated GIT conditions [229, 230]. In this case, microgel dissociation takes place due to weakening of the electrostatic forces holding them together, as well as digestion of the casein molecules by proteases.

Polysaccharides have also been used as stabilizing agents to stabilize emulsions for providing controlled release, improving entrapment efficiency, and protection from degradation [231, 232]. In this case, the hydrophobic food bioactive is to be dissolved in the internal organic phase of an oil-in-water emulsion, whereas double emulsions are employed for nanodelivery of hydrophilic molecules [233, 234]. Besides, gum arabic maltodextrin was developed to improve the stability and bioavailability of epigallocatechin gallate [235].

6 Conclusions

As reviewed above, so many polysaccharides and their derivatives are employed as one of the most used biomaterials in preparation of nanoparticulate delivery systems. Due to their complex structure, polysaccharides show variability and versatility, which is difficult to reproduce with synthetic polymers. A variety of polysaccharide-based nanoparticles have been obtained by various preparation methods towards three evolution aspects: need for less toxic agents, simplification of the procedures and optimization to improve yield and entrapment efficiency. Now it is possible to choose the best method of preparation and the best suitable polymer to achieve an efficient encapsulation of the drug/gene/food ingredient, taking into account the agent features in this selection. In addition, a variety of novel polysaccharides with specific properties, such as hyperbranched polysaccharides, are being selected to prepared nanoparticles for delivery systems.

Until now, these nanoparticles have been investigated in terms of their physicochemical properties, drug-loading efficiency, *in vitro* toxicity, and comparatively simple *in vivo* tests. Deeper studies, such as the specific interaction of these nanoparticles with human organs, tissues, cells, or biomolecules, as well as how the administration of these systems can affect the metabolism, need to be carried out and focused on in the future. A combination of *in silico*, *in vitro*, and *in vivo* studies is required for the safe application of nanoparticle delivery systems in drugs, genes and food.

Acknowledgements

This article is also available in: Luque/Xu, Biomaterials. De Gruyter (2016), isbn 978–3–11–034230–7.

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