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Review

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Self-assembled polysaccharide nanostructures for controlled-release applications

Abstract: Self-assembling polysaccharide nanostructures have moved to the forefront of many fields due to their wide range of functional properties and unique advantages, including biocompatability and stimulus responsiveness. In particular, the field of controlled release, which involves influencing the location, concentration, and efficacy of active pharmaceutical ingredients (APIs), diagnostics, nutrients, or other bioactive compounds, has benefited from polysaccharide biomaterials. Nanostructure formation, stimulus responsiveness, and controlled-release performance can be engineered through facile chemical functionalization and noncovalent intermolecular interactions. This review discusses polysaccharide nanoparticles, designed for targeted and time-controlled delivery of emerging APIs, with improved *in vivo* retention, stability, solubility, and permeability characteristics. Topics covered include nanoparticles of cyclodextrin and cyclodextrin-containing polymers, hydrophobically modified polysaccharides, polysaccharide nanoparticles that respond to pH, temperature, or light stimulus, polysaccharide prodrug complexes, polysaccharide complexes with lipids and proteins, and other polysaccharide polyelectrolyte complexes.

Keywords: controlled release; polysaccharides; self-assembly; stimulus-responsive nanoparticles.

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1 Introduction

Self-assembly is the spontaneous disorder-to-order transition of molecules driven by noncovalent interactions, such as the van der Waals and hydrogen bonding forces, and the hydrophobic effect. Over the past few decades, the concept of self-assembly has been successfully used in the design of drug delivery systems (DDSs) that are capable of releasing bioactive compounds at therapeutically effective rates and concentrations. Several interesting strategies using nanostructured synthetic polymers have been reported [1].

Polysaccharides are attractive as carriers in drug delivery [2] due to their biological vis-à-vis synthetic origin and biocompatibility. The biocompatibility of polysaccharides is attributed to their hydrophilicity. Indeed, glycosaminoglycan (GAG) polysaccharides, such as water-binding heparan sulfate, are the primary constituents of the glycocalyx that is believed to impart biocompatibility to the endothelial lining of blood vessels.

Two important considerations in drug delivery are enhancing the bioavailability (e.g., by increasing the solubility and stability in plasma) and lowering the toxicity (side effects) of the administered drug. Many of the cancer therapeutics are hydrophobic and toxic. The human body has a tendency to eliminate hydrophobic and toxic compounds through hepatic metabolism. Lipid-soluble hydrophobic drugs tend to be converted to more polar compounds through biotransformations, including oxidation, reduction, and hydrolysis reactions, in the liver. The resulting water-soluble compounds are then excreted by the kidney. Thus, hydrophobic drugs are generally rapidly cleared from the body and have low plasma circulation half-lives (characterized by plasma concentration of the drug vs. time after administration).

The conjugation of a hydrophobic drug with a hydrophilic macromolecule not only increases the plasma concentration of the drug but also increases its plasma circulation time. When a drug molecule is conjugated with the synthetic hydrophilic polymer, poly(ethylene glycol) (PEG), its survival through the first-pass metabolism in the liver has been found to improve greatly [3, 4]. Furthermore, the rate of hepatic breakdown of the PEG-conjugated drug decreases with an increase in the molecular weight of PEG. Hydrophilic nanoparticle carriers increase

the plasma circulation half-lives of hydrophilic therapeutics as well. Water-soluble molecules of sufficiently low molecular weight can pass through the glomerular membrane of the kidney and are thereby eliminated by renal excretion. Conjugation with a polymer lowers filterability of the drug through the glomerular capillary because of an increase in molecular size.

The use of hydrophilic polymers as drug carriers also helps in lowering the toxicity of the drug. The hydrophilic DDSs are less readily absorbed by normal tissues. However, they can accumulate in cancerous tissues through the enhanced permeability and retention (EPR) effect [3, 5, 6]. Cancerous tissues exhibit defective, leaky hypervasculature and deficient lymphatic drainage [2, 3], which results in the passive accumulation of nanometer-sized entities in these tissues. This EPR effect serves in localizing the effect of the drugs to cancerous tissues and decreasing their toxicity on normal tissues.

Polysaccharides are promising alternatives to PEG because they contain a variety of functional groups (hydroxyl, amino, and carboxylic acid) that can be used for drug conjugation and for self-assembly. They have another distinct advantage. Many polysaccharides are polyelectrolytes. The surface charge of polysaccharide carriers can be used to engineer biointeractions such as cellular uptake or glomerular filtration. Cationic polysaccharides promote endocytic uptake by cells, whereas anionic polysaccharides could increase bioavailability by reducing excretion through the glomerular capillary wall. Negatively charged species would be "less filterable" because of the negative charge of the glomerular membrane. Some charge-reversible and pH-responsive polysaccharides [7–9] discussed in this review can be used to achieve both these functionalities.

The size of the polymer-drug conjugate plays an important role in determining half-life in the plasma, bioavailability, and efficacy of the drug. Nanoparticles offer many advantages for drug and gene delivery as well as medical diagnostics and imaging [10]. They are large enough to avoid glomerular permeation and small enough to avoid clearance via uptake by macrophages. In order to escape phagocytosis, the particle diameters must be significantly lower than 500 nm [11]. Their small size allows them to pass through tissue gap and capillaries via tissue diffusion and extravasation. Nanoparticles are also more resistant to hepatic filtration than larger particle delivery systems [3]. Because of these unique properties of polymeric nanoparticles, they have been utilized to control the release of macromolecular drugs, proteins, and vaccines [12, 13] and in other sustained-release applications [12, 14, 15].

Polysaccharides and polysaccharide derivatives that self-assemble to form nanoparticles have been studied extensively as carriers for controlled delivery of active pharmaceutical ingredients (APIs) [16]. This review covers advances in the material synthesis, properties, and applications of self-assembling nanoparticles composed of polysaccharides and polysaccharide complexes. Modification of native polysaccharides to yield amphiphilic molecules is among the most prominent methods for producing self-assembled nanostructures. Amphiphilic polysaccharides self-assemble in response to a tailored shift in the hydrophilic/hydrophobic balance.

Self-assembly of polysaccharide nanoparticles is also possible through electrostatic interaction of ionic polysaccharides of opposite charges [17]. Ionic complexes are attractive for their simplicity and because their self-assembly is responsive to pH and ionic strength. Polysaccharide DDSs can be made responsive to other stimuli, including heat, magnetism, and light [18–23]. In many cases, this response to environmental change is often reversible, thus, a "switching" effect can be built into the polymer nanostructure that will respond to stimulation *in vivo*. This review discusses selected examples of stimuli-sensitive polysaccharide nanoparticles.

Finally, examples of polysaccharide prodrugs, an important class of therapeutic agents, are reviewed. Prodrugs are compounds or complexes that are inactive when initially administered to the body, but get converted to their active forms by normal metabolic processes of the body (or external stimuli). The spontaneous formation of polysaccharide prodrugs is an appealing method for protecting, delivering, and in some cases enhancing the efficacy of APIs. This technique greatly reduces side effects caused by the deleterious administration of drug to non-target tissues, as the prodrug is inactive until triggered by an appropriate stimulus.

The focus of this review is on materials chemistry and physiochemical properties of polysaccharide nanoparticle DDSs; these aspects are discussed in the context of their biological interactions, where possible. We have primarily included reports on polysaccharide-derived nanoparticles, but inclusion complexes consisting of cyclodextrin and cyclodextrin-based polymers are also reviewed. Cyclodextrin (CD) nanoparticles are interesting examples of supramolecular self-assembly in soft matter systems and have significant promise as DDSs. Results of clinical trials of many of the nanoparticle systems reviewed herein are not available, but we have discussed any published *in vitro* assays or pharmacokinetic data acquired using animal models. Polysaccharide nanoparticle platforms, not evaluated in pharmacokinetic experiments,

are included if they demonstrate significant prospects as DDSs. Consistent with the review's scope, the topics discussed herein are sorted by the polysaccharide type and the mechanism of particle self-assembly, rather than any classification based on their biomedical application of the nanoparticles or *in vivo* response.

2 Cyclodextrin-based nanoparticles

Cyclodextrins are among the most widely explored materials used for nanoparticle formation. They are cyclic oligosaccharides composed of at least five 1,4-linked α-D-glucopyranose monomers (see Figure 1), prepared by enzymatic breakdown of starch. The conformation of the glucopyranose units forces the cyclic structures into a hollow-frustum or torus-like shape that features a hydrophilic outer surface and a more lipophilic inner cavity. The three-dimensional ring structure of cyclodextrins allows for sequestration of hydrophobic molecules within the oligosaccharide cavity [21, 24, 25]. The self-assembly of CDs with hydrophilic molecules has also been shown to be feasible. A comprehensive review of self-assembling CD-based host-guest complexes is available [26].

2.1 Cyclodextrin-based nanoparticles for hydrophilic drug complexation

Harada et al. [27] studied the sequestration of polymers such as PEG within CDs. They found that α-cyclodextrins formed complexes with PEG of various molecular weights to give stoichiometric complexes in high yields. When aqueous solutions of PEG (with average molecular weights between 400 and 10,000 g/mol) were added to a saturated aqueous solution of α-CD at room temperature, complexes were obtained as precipitates. Precipitates were not formed with the low molecular weight analogs, ethylene glycol, diethylene glycol, and triethylene glycol, evidently because of the high water solubility of these molecules. X-ray diffraction studies of the dried complexes indicated a columnar structure, formed by penetration of the α-CD cavities by PEG chains. Each α-CD accommodated two monomer units of PEG. β-CD did not form complexes with PEG of any molecular weight because the β-CD was too large to compactly fit a PEG chain. Poly(propylene glycol) or PEG carrying bulky substituents such as 3,5-dinitrobenzoyl groups at both ends did not form complexes with α-CD because these chains could not pass through the α-CD cavity. CD inclusion complexes are commonly formed with poorly water soluble molecules; however, these results indicate that even hydrophilic compounds such as PEG may form inclusion compounds with CD. In the drug delivery field, much research is invested in sequestration of hydrophobic compounds, but structures capable of encapsulating or forming complexes with hydrophilic molecules are also of practical interest. This study by Harada et al. demonstrates that hydrophilic APIs may be included in CD-based nanostructures for subsequent controlled delivery.

2.2 Targeted delivery of siRNA from cyclodextrin nanoparticles

PEG has been found to act as a steric stabilizer for CD-containing polymer nanoparticle systems [28]. In a study

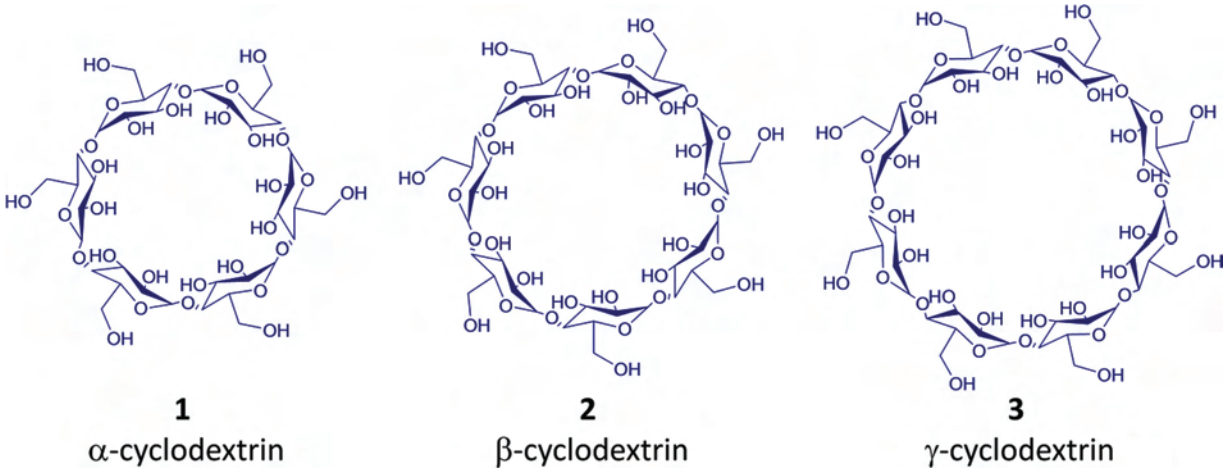
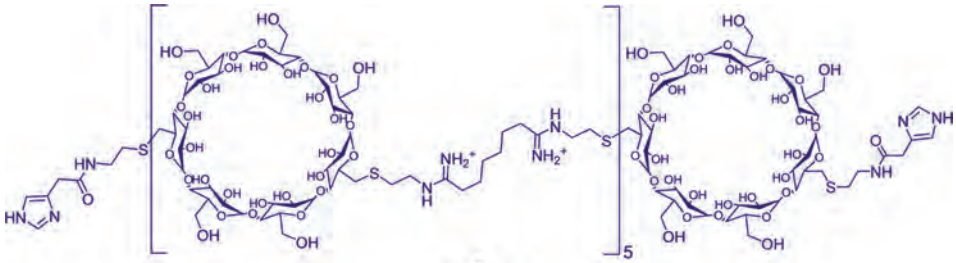


Figure 1 Chemical structures of α, β, and γ cyclodextrins, consisting of six, seven, and eight units of α-D-glucopyranoside, respectively.



4
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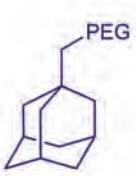
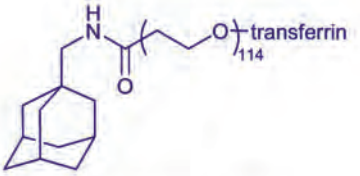


5
Adamantane conjugate of PEG



6
Human transferrin conjugated PEG-adamantane

Figure 2 Components of targeted nanoparticle containing siRNA [28]: a water-soluble, linear cyclodextrin-containing polymer, **4**, an adamantane-PEG conjugate (AD-PEG, PEG MW of about 5000 g/mol), **5**, and the targeting component that is an adamantane conjugate of PEG with human transferrin (Tf) conjugated at the end opposite to adamantane (AD-PEG-Tf), **6**. Nanoparticles are obtained when a solution of these three components is mixed with a solution of siRNA.

by Davis and coworkers [28], a nanoparticle formulation composed of a cyclodextrin polymer (CDP, **4**, Figure 2), PEG, and human transferrin (Tf, used as a targeting ligand), was prepared for the intravenous delivery of synthetic small interfering RNA (siRNA). The CDP was a short polycation that could complex with nucleic acids (polyanions) via electrostatic interactions to form nanoparticles and can protect the polyanion from enzymatic degradation. The β-CD units of the polymer resided on the surface of the nanoparticles and were used for assembling the steric stabilizing agent, AD-PEG (**5**, Figure 2), and the targeting agent, AD-PEG-Tf (**6**, Figure 2). The hydrophobic adamantane (AD) groups of **5** and **6** formed inclusion complexes with CD, tethering PEG and Tf to the nanoparticle surfaces. The several Tf molecules on the surface of the nanoparticles provided multivalent binding to the surface of the cancer cell (with Tf receptors), which would stimulate the entrance of the nanoparticles into the cell. The CDP also contained imidazole end groups that assisted in the endocytic pathway escape and nucleic acid release. The nanoparticle formulations were found to be effective in delivering siRNA against the EWS-FLI1 fusion gene in murine model of Ewing's sarcoma and the ribonucleotide reductase subunit 2 (RRM2) in another murine tumor model [28]. Clinical trials of CD-based nanoparticles encapsulating siRNA and CD-based polymer conjugated to camptothecin have been conducted, but the study results are not available at ClinicalTrials.gov, a database of clinical studies of human participants conducted around the world, maintained by the National Library of Medicine (NLM) at the National Institutes of Health (NIH) [29].

2.3 Multilamellar cyclodextrin structures for controlled release of antimalarial artemisinin

The native structure of cyclodextrins has been used to prepare nanoparticles with interesting morphologies [25, 30]. Choisnard et al. [30] prepared decanoate and hexanoate esters of β-cyclodextrin (β-CDd and β-CDh, respectively) by a thermolysin-catalyzed reaction between β-CD and the corresponding acid chlorides. Thermolysin is a thermostable enzyme that can catalyze the formation of ester linkages. Using cryo-TEM, β-CDh was found to form spherical nanoparticles, while the β-CDd particles presented a multilamellar morphology (see Figure 3) [30].

These unique β-CD nanostructures, along with similarly prepared γ-CD structures, have been investigated as carriers of artemisinin (ART), an antimalarial drug derived from the plant *Artemisia annua* [31]. ART is an effective treatment for parasite strains resistant

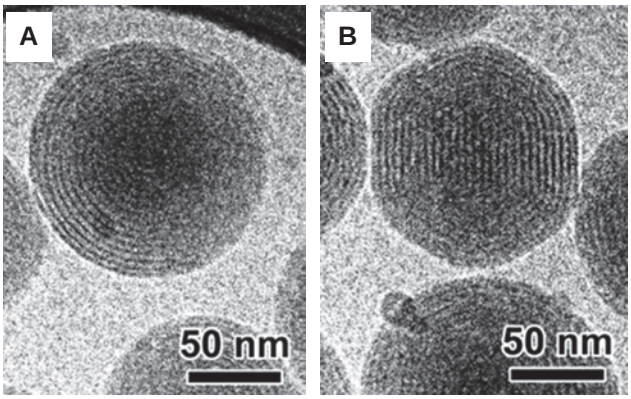


Figure 3 Cryo-transmission electron microscopy (cryo-TEM) images of multilamellar decanoate β-cyclodextrin ester particles in vitreous ice. Concentric β-CDd layers are observable, as is the variation in particle shape (spherical or polygonal). Adapted from ref. [30], Copyright © 2006, with permission from American Chemical Society.

to other antimalarials, but suffers from low solubility in aqueous environments and poor bioavailability [32]. For these reasons, inclusion complexes of ART and CDs have been investigated and found to increase bioavailability [33]. Yaméogo et al. [31] demonstrated successful loading and surface adsorption of ART into PEGylated β- and γ-CD nanospheres and nanoreservoirs by observing an increase in particle size and decrease in ζ-potential. Release of ART, up to 8 days with nanoreservoirs and 10 days with nanospheres, was reported. These ART-containing CD nanoparticles were successful in impeding the growth of *Plasmodium falciparum*, a parasite responsible for malaria in humans. The incorporation and delivery of ART demonstrates the capabilities of this self-assembling CD system as a controlled-release platform for future research.

2.4 Cholic acid-modified cyclodextrin hollow nanospheres

In a study by Liu et al. [25], self-assembling hollow spheres 50–70 nm in diameter were produced using cholic acid modified cyclodextrin and a guest molecule, sodium 1-naphthylamino-4-sulfonate (1,4-SNS). Cholic acid was reacted with mono-(6-aminoethylamino-6-deoxy)-β-CD to obtain compound **7** (Figure 4), in aqueous solution of which, the cholic acid tether was self-included into the lipophilic cavity of β-CD. When **7** was mixed with an equimolar amount of 1,4-SNS, 2D NMR experiments showed that the cholic acid moiety was expelled from the cavity by 1,4-SNS (see **8**, Figure 4). The resulting amphiphilic complex self-assembled in aqueous solutions to form nanospheres with radius of 25–35 nm, and surface pores of 3–10 nm. Replacement of 1,4-SNS with disodium 2,6-naphthalenedisulfonate (2,6-DNS) did not produce the nanospheres. Nanospheres of the 1,4-SNS complexes formed at pH values of 6.2, 7.2, and 10.0, but not at pH 2.2. At the highly acidic pH, complete protonation of the amino group in the tether and the amino group of 1,4-SNS would result in electrostatic repulsion, making it difficult for the guest molecule to be included in the CD cavity (which is necessary for the expulsion of the cholic acid moiety from the cavity, and the formation of nanoparticles). At the intermediate pH values, the degree of protonation would be lower, favoring the inclusion of 1,4-SNS and the exclusion of the cholic acid tether from the CD cavity. At pH 10.0, both the amino groups would be weakly protonated and 1,4-SNS can readily enter the CD cavity, favoring nanostructure formation.

Hollow nanospheres were also prepared by Qin et al. [34], who created β-CD complexes with poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene

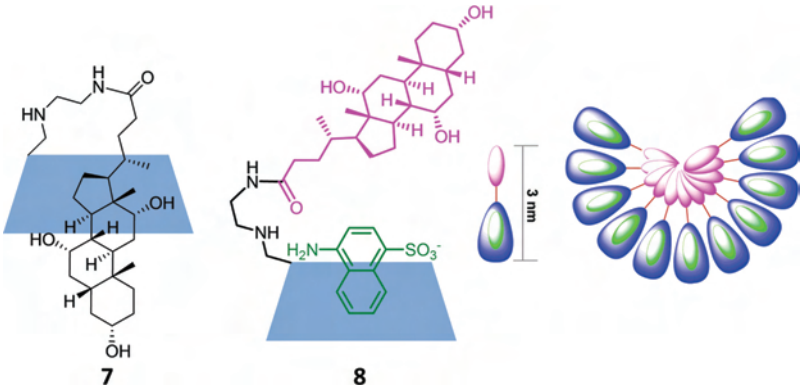


Figure 4 Amphiphilic β-CD derivative with cholic acid tether, **7**, and its 1:1 complex, **8**, with sodium 1-naphthylamino-4-sulfonate (1,4-SNS). The β-CD molecule adopts a hollow frustum conformation, depicted by the blue trapezoid. The "guest," cholic acid moiety, is expelled from the cavity by the addition of 1,4-SNS. The rightmost cartoon is a schematic of the self-assembly of **8** into a sphere, with cholic acid moieties at the core and cyclodextrins as the shell. Adapted from ref. [25], Copyright © 2006, with permission from American Chemical Society.

glycol) (PEG-*b*-PPG-*b*-PEG) block copolymers (Pluronic® surfactants, F127, P65, P104 and P123). The polymer compositions were $EG_{105}PG_{70}EG_{105}$ in F127, $EG_{20}PG_{30}EG_{20}$ in P65, $EG_{20}PG_{54}EG_{20}$ in P104, and $EG_{20}PG_{70}EG_{20}$ in P123, where EG denotes an ethylene glycol mer and PG denotes propylene glycol. In aqueous solutions, the β-CD molecules threaded onto the polymer chains to form inclusion complexes, in which the β-CD moieties were localized primarily on the relatively hydrophobic PPG block. These inclusion complexes further self-assembled to form hollow nanospheres, wherein the hydrophilic PEG blocks lined the inner cavity and the external surface.

The size of the particles was determined by the mass fractions of the hydrophilic EG and hydrophobic PG segments in the Pluronic. When the mass fraction of EG was higher than that of PG, smaller aggregates were formed. The diameters of the β-CD/F127 particles, determined using dynamic light scattering, were 200–400 nm. When P65 was used as the surfactant, larger particles were obtained. The Pluronics, P104 and P123, having lower mass fractions of EG than the Pluronic, P65, resulted in insoluble complexes that precipitated as white powders.

The hydrodynamic diameters of the β-CD/F127 particles were found to be relatively independent of dilution, over a broad range of concentrations, indicating that the aggregates formed by self-assembly of the inclusion complex were fairly stable. In contrast, the micelles of just the F127 surfactant, without β-CD, were found to be unstable upon dilution. The nanospheres were also thermally stable. The β-CD/F127 spheres increased in size (hydrodynamic diameter) in the range of 285–1500 nm when the temperature was increased over a range of about seven to 37°C. However, at 42°C, the solution became transparent, indicating disintegration of the spherical assemblies. The influence of temperature on the self-assembly of the inclusion complexes was reversible.

Because both β-CD and Pluronic are water soluble and biocompatible, the stable hollow spheres (vesicles) developed in this study are promising alternatives to liposomes for drug encapsulation and delivery. A liposome is a synthetic vesicle composed of a continuous lipid bilayer encapsulating an aqueous environment [35]. However, the size of most liposomes is such that they are rapidly cleared from blood due to phagocytosis by cells of the reticuloendothelial system (i.e., the tissue macrophages) [36]. PEGylation of the water-soluble region of the phospholipid is used to increase the circulating half-life of liposomes. Thus, it is expected that the PEGylated hollow nanospheres prepared from Pluronic and β-CD would similarly offer improved circulation half-life, in comparison with non-PEGylated nanoparticles of comparable sizes.

3 Nanoparticles of hydrophobically modified polysaccharides

While cyclodextrins are useful building blocks for preparing hollow structures with a hydrophobic inner cavity, several other modified polysaccharides have also been investigated for this purpose. Naturally available polysaccharides, such as those with structures depicted in Figure 5, can be made amphiphilic through conjugation with hydrophobic molecules. These modified polysaccharides self-assemble in aqueous environments because of the hydrophobic effect of the pendant groups. In many cases, hydrophobic interactions produce micelle-like structures that feature a hydrated outer layer and an isolated hydrophobic core. This type of architecture has proven promising for encapsulation of drugs with low solubility in water [37].

3.1 Doxorubicin encapsulation in carboxymethyl-hexanoyl chitosan nanoparticles

Liu and coworkers [38, 39] prepared amphiphilic chitosan that self-assembled into hollow nanocapsules. The hydrophobic/hydrophilic balance was manipulated through functionalization of chitosan with hydrophilic carboxymethyl and hydrophobic hexanoyl moieties. The resulting polymer, carboxymethyl-hexanoyl chitosan (CHC) (**17**, Figure 6), formed stable colloidal particles with adjustable size that was dependent on concentration of hexanoyl groups. Doxorubicin (DOX), a hydrophobic antineoplastic cancer therapeutic, was found to have an encapsulation efficiency of up to 47% in the CHC nanoparticles. Release of DOX from the CHC particles lasted longer than 7 days at 37°C.

3.2 Carboxymethyl-hexanoyl chitosan nanoparticles for magnolol delivery

Intimal hyperplasia is a problem associated with surgical procedures such as endarterectomy or bypass surgery, or techniques such as balloon angioplasty and stenting, which are used in the treatment of coronary artery disease. These procedures result in a loss of the normal endothelial cell lining of blood vessels, and trigger the overproliferation and migration of the vascular smooth muscle cells (VSMCs) from the media portion of the blood vessel into the intima. (Intima is the blood-contacting innermost surface of the blood vessel consisting of a monolayer of

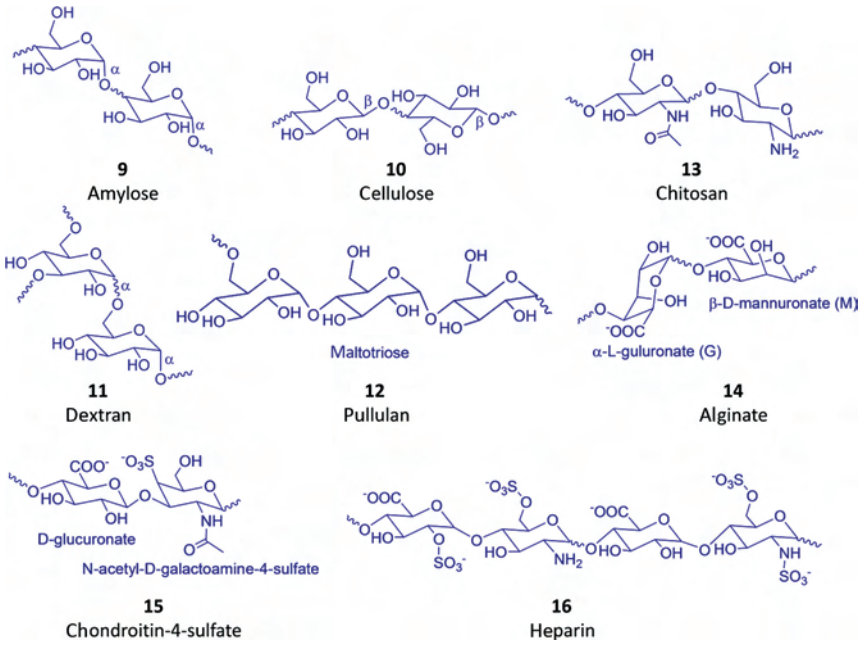


Figure 5 Chemical structures of some natural polysaccharides. Amylose (**9**) and cellulose (**10**) are homopolymers of glucose that differ in their glycosidic bond configuration. Dextran (**11**) is also a glucan, composed of α-1,6 glycosidic linkages; however, it additionally features random branching in the form of α-1,3 bonds. Pullulan (**12**) is a homopolymer of α-1,6 linked maltotriose units. Chitosan (**13**), which is derived from chitin, a homopolymer of *N*-acetylglucosamine, is composed of glucosamine and *N*-acetylglucosamine units and varies in structure depending on its degree of deacetylation. Similarly, alginate (**14**) is composed of covalently linked homopolymeric blocks (of varying lengths, depending on the source of the polysaccharide) of β-D-mannuronate (M) and α-L-guluronate (G) residues, interspersed with sequences of MG dimer residues. Chondroitin sulfate is a sulfated glycosaminoglycan (GAG) consisting of alternating glucuronate and *N*-acetylglucosamine units. Chondroitin-4-sulfate (**15**), which is sulfated at the C4 carbon of the *N*-acetylglucosamine unit, is depicted. Heparin (**16**) is a highly sulfated GAG composed of up to six different disaccharide units, the most common of which are depicted. Chemical structures in the rest of this article are schematic representations without considerations of specific monomer sequence in the copolymers or bond configuration.

endothelial cells. The media consists of concentric layers of smooth muscle cells, surrounding the intima.) This hyperplasia of intima results in a narrowing of the lumen of the injured blood vessel and eventual re-occlusion of the vessel.

Magnolol, 4-allyl-2-(5-allyl-2-hydroxy-phenyl)phenol, a strong antioxidant with approximately 1000 times greater activity than α-tocopherol, has been found to be effective in inhibiting the proliferation of VSMCs [40]. However, this hydroxylated biphenol compound is highly hydrophobic in nature. Its relatively poor solubility in water reduces its bioavailability and clinical efficacy. Conventional liposomal drug carriers were found to have low drug encapsulation efficiency, poor storage stability, rapid clearance from the blood stream, nonspecific uptake by the mononuclear phagocytic system, poor control over release of the drug from the liposome, and rapid drug loss profiles *in vivo* [40]. To overcome these drawbacks, Wang

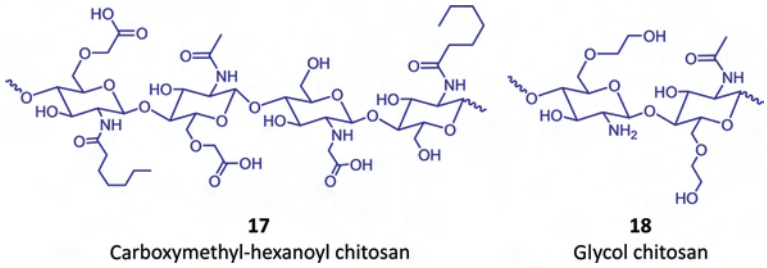


Figure 6 Chemical structures of carboxymethyl-hexanoyl chitosan [39] and glycol chitosan (GC).

et al. [40] prepared CHC (**17**, Figure 6)-based nanoparticles containing magnolol and found an increased antiproliferative effect and effective inhibition of VSMC migration (in comparison with free magnolol), as a result of efficient intracellular delivery of the encapsulated magnolol. They prepared a magnolol-polyvinylpyrrolidone core phase and encapsulated it in an amphiphilic CHC shell, to obtain magnolol-loaded core-shell nanoparticles with average hydrodynamic diameters in the range of 235–420 nm. The nanoparticles exhibited a negative ζ-potential because of the presence of carboxyl groups in the CHC molecules, concentrated in the outer surface of the nanoparticles. The uptake of magnolol-CHC nanoparticles by A10 VSMCs was attributed to macropinocytosis and clathrin-mediated endocytosis (which is initiated by a specific ligand-receptor interaction on the cell surface [11]), upon which the nanoparticles became entrapped in intracellular vesicles (i.e., endosomes) permitting an intracellular release of magnolol.

Other examples of the use of nanoparticles of hydrophobically modified polysaccharides for controlled release of hydrophobic drugs are available [41, 42].

3.3 Nanoparticles of 5β-cholanic acid-modified glycol chitosan and hyaluronic acid for ocular delivery of anti-VEGF

Ocular disorders are of increasing medical concern and affect an estimated 285 million people worldwide, 249 million of whom are afflicted by nonblindness impairments [43]. Among all types of visual impairment, 80% are treatable or can be avoided through healthy diet and lifestyle [43]. While some ocular disorders can be treated with laser surgeries, topical drug administration, transscleral delivery, or intravitreal injection, the application of nanoparticle systems for diagnosis and treatment of these afflictions has the potential to improve efficacy, biocompatibility, and targeting of APIs [44].

The most effective conventional treatment for age-related macular degeneration, ischemic retinal vein occlusions, or diabetic retinopathy is intravitreal injection of anti-vascular-endothelial-growth-factor (anti-VEGF), which combats abnormal angiogenesis [3, 44]. The majority of ocular disorders occur in the retina, which presents a challenge for injected treatments as they are <0.5 mm thick. The human retina constitutes the neural portion of the eye and is composed of multiple layers of neurons and synapses. While intravitreal injections are effective for treating retinal disorders, nanoparticle DDS can provide the ability to target the retina more effectively and improve API retention. The challenge in designing nanoparticles for retinal delivery is penetrating the various membranes and neural layers of the retina all the way to the outermost layer, the retinal pigment epithelium (RPE).

The ability of nanoparticles to distribute throughout the retina, based on their surface properties, was studied by Koo et al. [44]. The various nanoparticles, including those of GC (**18**, Figure 6) and hyaluronic acid (HA), were injected into the vitreous humor of rats, and the distribution was characterized by confocal laser scanning microscopy and TEM. All particle systems investigated were composed of amphiphilic polymers that self-assembled in aqueous media. Polymers such as polyethyleneimine (PEI) and GC were hydrophobically modified with 5β-cholanic acid (see Figure 7). The amphiphilic polymer conjugates self-assembled in aqueous media to form nanoparticles. These nanoparticles were used for intravitreal injection. 5β-Cholanic acid conjugates of hyaluronic acid and human serum albumin (HSA), and heterogeneous particles consisting of blends of the polymers, were also prepared. The polymers were labeled with fluorescent dyes for efficient tracking. The nanoparticles had similar sizes but with different surface properties, which affected their distribution in the vitreous and retinal tissues when administered intravitreally. Nanoparticles consisting of hydrophobically modified GC and its blend with hydrophobically modified PEI were cationic in nature. They were able to penetrate the vitreal barrier but not the inner limiting membrane, which is the first layer of the retina. In contrast, particles composed of the anionic polymers, HA and HSA, were able to distribute throughout the retina, reaching the retinal pigment epithelium [44]. Such nanoparticles could potentially be used as drug or gene carriers for treating retinal and optic nerve disorders such as glaucoma, age-related macular degeneration, and diabetic retinopathy.

3.4 Nanoparticles of cholesterol-modified pullulan for controlled delivery of insulin

Diabetes mellitus is a chronic condition that affects about 347 million people worldwide [45], requiring management of insulin levels in blood. Diabetes manifests as an inability to produce the hormone insulin (type I) or the inability to utilize insulin produced by the pancreas (type II). Controlled delivery of insulin by nanoparticle systems has been investigated as an alternative to conventional treatments of type I diabetes. Orally administered insulin is especially prone to aggregation and proteolysis, thus encapsulation, protection, and release are all important design factors [46].

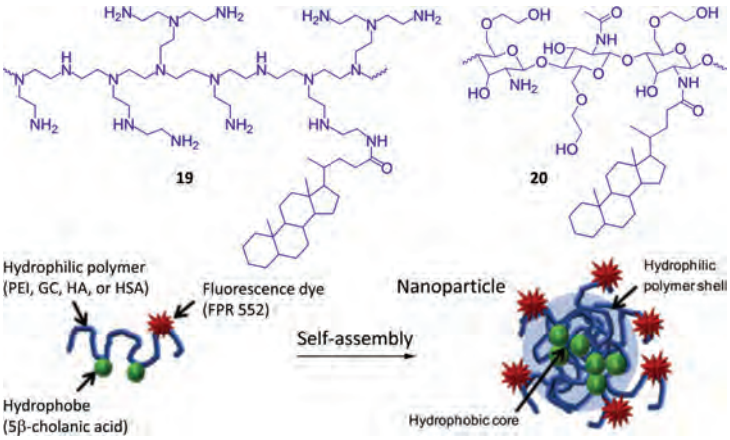


Figure 7 A schematic of self-assembly of fluorescently labeled amphiphilic polymers such as 5β-cholanic acid modified polyethyleneimine, **19**, and 5β-cholanic acid modified glycol chitosan, **20**. Adapted from ref. [44], Copyright © 2012, with permission from Elsevier.

Akiyoshi et al. [46] found that insulin spontaneously complexed with nanoparticles of hydrophobized cholesterol-bearing pullulan (CHP) in water. Pullulan is a polysaccharide consisting of maltotriose units that are connected to each other by α-1,6 glycosidic bonds (cf. **12**, Figure 5). Monodisperse nanoparticles of the polysaccharide were obtained when pullulan (substituted with an average of 2.1 cholesterol units per 100 glucose units) was ultrasonicated in phosphate-buffered saline (PBS) at pH 8.0. When mixed with the nanoparticles in PBS, insulin formed a host-guest complex with CHP resulting in 20–30 nm particles of high colloidal stability. High-performance size-exclusion chromatography (HPSEC) characterization indicated that the uptake of insulin by CHP nanoparticles was rapid and that the particles did not show a significant increase in size, suggesting that the protein was internalized. Immediate release of insulin was only observed when the particles were in the presence of BSA, which caused dissociation of the complexed insulin. Upon intravenous injection in mice, the nanoparticles release insulin and caused a decrease in the plasma glucose concentration, indicating that the released insulin was bioactive and that the nanoparticles protected insulin from enzymatic degradation, oligomerization, or aggregation.

4 Chitosan-based pH-responsive nanoparticles

Chitosan (**13**, Figure 5) is valued in the pharmaceutical and biomedical fields [47] for its mucoadhesive properties, easy chemical modification, antibacterial properties [48] and pH-responsiveness near physiological pH (7.4). The pK_a of the glucosamine monomer in chitosan is ≅6.5 [49]. The cationic nature of chitosan in acidic environments, for example, in the gastrointestinal (GI) tract (highly acidic in the stomach, to about pH 6 in the duodenum [50]) or in the intracellular endosomes (pH 5–6 [51]), is a valuable asset that can be manipulated for pH-responsive controlled release functionality. In addition, chitosan-containing nanostructures may feature a positive surface charge, which is beneficial for cellular uptake or antibacterial activity. Many polysaccharide DDSs incorporate chitosan in their polymer matrix to make use of these properties.

The pH responsiveness of polysaccharides can also be used as a method of nanoparticle assembly. The ability to spontaneously form polysaccharide nanoparticles by manipulating the solution pH is a powerful technique for particle synthesis. Similarly, nanoparticle systems that respond in a predictable way to a change in the environmental pH offer a route to trigger the release of an encapsulated drug. A review of nanoparticles responsive to pH changes, including strategies for delivery in different organs, is available [52].

4.1 Chitosan nanoparticles for lidocaine delivery

Choochottiros et al. [8] designed and synthesized nanoparticles of the pH-sensitive monomethoxy-PEG (mPEG) grafted phthaloylchitosan (**21**, Figure 8) for encapsulation of lidocaine, a hydrophilic amino amide local anesthetic. The nanoparticles formed with a hydrophobic *N*-phthalimido core and hydrophilic mPEG corona. The chitosan

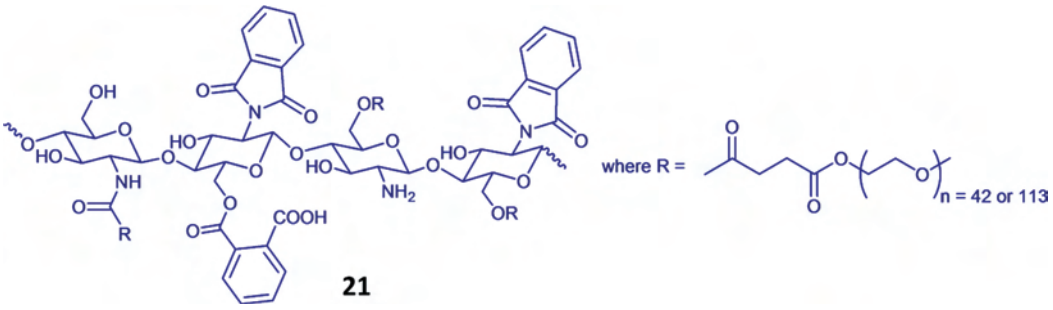


Figure 8 Chitosan grafted with phthalic anhydride and monomethoxy poly(ethylene glycol) (mPEG) [8]. The degree of phthaloyl substitution is about 50%. In aqueous solutions, the polymer self-assembles into spherical particles, with size dependent on the length of the mPEG chains.

degree of deacetylation (DDA) was varied from 80% to 95% and was used to control the extent of phthalimido substitution, which in turn determined the overall hydrophobicity of the modified polysaccharide. In all cases, the phthalimido substitution was about 50%. The substitution of mPEG depended on the mPEG molecular weight and strongly influenced the resulting nanoparticle size. The degree of grafting was significantly higher for the 2000 g/mol mPEG (mPEG2k, 35–60%) than for the 5000 g/mol mPEG (mPEG5k, 7–18%). A bimodal particle size distribution, with particle sizes in the range of 125–350 nm, was observed in aqueous solutions of the mPEG2k-grafted phthaloylchitosan polymers, while smaller-sized monodisperse nanospheres of about 150 nm mean diameter were formed from the mPEG5k-functionalized phthaloylchitosan polymers. Although chitosan is expected to be cationic, the surfaces of the PEGylated phthaloylchitosan particles were found to be negatively charged due to the carboxylic acid groups introduced by phthaloylation (cf. **21**, Figure 8), and their ζ-potentials were in the range of -40 to -50 mV. In some cases, the incorporation of lidocaine led to an increase in particle size to as much as twice the unloaded particle diameter. The uptake of lidocaine indicates that this system offers the potential for delivery of hydrophilic APIs, while the hydrophobic core offers the opportunity for potential incorporation of poorly water-soluble compounds. Lidocaine that is administered orally is rapidly metabolized in the liver. Its encapsulation in the PEGylated phthaloylchitosan nanoparticles may help in increasing its bioavailability.

4.2 Chitosan nanoparticles for methotrexate delivery

Methotrexate (MTX) is a folate inhibitor used for the treatment of certain cancers and autoimmune diseases [53]. Being a structural analog of folic acid, it binds with and inhibits the enzyme dihydrofolate reductase, preventing the formation of tetrahydrofolate. Tetrahydrofolate is essential for purine and pyrimidine synthesis. MTX, therefore, inhibits the production of DNA and RNA and has greater cytotoxic effect on rapidly dividing cells, such as cancerous cells, that replicate their DNA more frequently. Despite high efficacy, MTX suffers from poor solubility and short plasma half-life. For these reasons, nanoparticle DDS have been considered as a means of improving solubility, cellular uptake, and tumor targeting through the EPR effect. Chen et al. [53] prepared chitosan-based nanoparticles to improve delivery of MTX.

The crosslinked chitosan nanoparticles were synthesized using a combination of ionic crosslinking (with sodium triphosphate) and covalent crosslinking (with glutaraldehyde). The imine moieties, formed by the reaction of the amine groups of the polysaccharide with the aldehyde residues of the crosslinker, were reduced to amino groups using sodium borohydride. For longer circulation time in the bloodstream, the crosslinked chitosan nanoparticles were functionalized with mPEG by reacting the hydroxyl and amino groups of chitosan with succinimidyl ester of mPEG (of 2000 g/mol molecular weight). Methotrexate-nanoparticle conjugates were prepared by reacting MTX with the mPEG-chitosan nanoparticles at room temperature, in aqueous phase, in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) hydrochloride. A high encapsulation efficiency (defined as the ratio of the mass of the drug bound to the nanoparticles to that used in the reaction), of about 88%, and a high MTX loading (defined as the mass fraction of MTX in the nanoparticles), of about 44% were obtained. The MTX-mPEG-chitosan nanoparticles had a narrow size distribution with an average diameter of about 210 nm. The particles exhibited a positive surface charge with ζ-potential of about 44 mV. The positive surface charge of

the nanoparticles resulted in higher association and internalization rates by the HeLa (human cervical epithelioid carcinoma) cells with negatively charged cell surfaces. The nanoparticles were found to be far superior to pure MTX in inhibition of growth and proliferation of HeLa cells.

4.3 Chitosan nanoparticles for oral delivery of paclitaxel

Lian et al. [54] have designed a chitosan-based polymer to prepare nanoparticles loaded with paclitaxel (PTX, an antineoplastic mitotic inhibitor cancer treatment drug). In the oral delivery route, the GI mucosal membrane is a primary obstacle that hinders absorption of drugs with poor permeability. The polymer synthesized by Lian et al. consisted of chitosan functionalized with *N*-acetyl cysteine (NAC) and vitamin E succinate (VES) (**22**, Figure 9). PTX-loaded nanoparticles were prepared by a nanoprecipitation technique involving ultrasonication. Particles with hydrodynamic diameters in the range of 220–250 nm, and ζ-potentials (in deionized water) in the range of 50–60 mV, were obtained. Particle formation occurred by hydrophobic interaction between the hydrophobic vitamin E side chains. The oral adsorption of the nanoparticles was investigated in different rat intestinal segments. Compared with PTX solution, the PTX-loaded chitosan-NAC-VES nanoparticles showed higher absorption, by endocytosis, in all parts of the small intestine (duodenum, jejunum, and ileum) and the colon. The nanoparticles offered increased adhesion to, and prolonged residence time in, the intestine because of the electrostatic interaction between the positively charged chitosan and the negatively charged sialic and sulfonic acid of the intestinal mucin, and the formation of covalent S–S bonds by free thiol groups of cysteine groups at the nanoparticle surface and the cysteine domains of the mucosal membrane. The PTX-loaded nanoparticles also showed a significantly improved performance over PTX solution in pharmacokinetic experiments. The plasma concentration of PTX after administration of the PTX-loaded nanoparticles was much higher than that of PTX solution, and the relative bioavailability of the nanoparticle system was 425% compared with that of PTX solution. The enhancement in oral absorption and bioavailability offered by these particles provides encouraging prospects for the development of alternatives to injection-based cancer therapy.

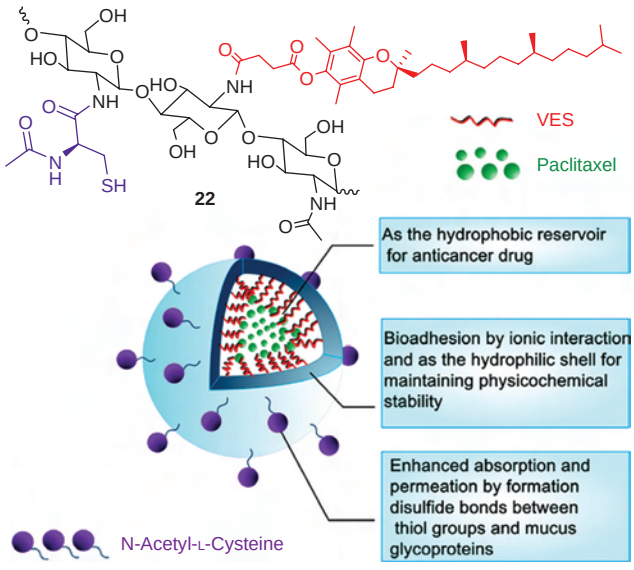


Figure 9 Schematic of chitosan functionalized with vitamin E succinate (VES) and *N*-acetyl-L-cysteine polymer (CVN), and paclitaxel-loaded CVN nanoparticles in aqueous medium. Adapted from ref. [54], Copyright © 2013, with permission from American Chemical Society.

4.4 Chitosan and heparin-based nanoparticles for bFGF delivery

Heparin (**16**, Figure 5) is an important biomedical polysaccharide that is used as anticoagulant and antifouling coating for medical devices. It is a highly sulfated, negatively charged, linear polysaccharide composed of alternating units of glucouronic acid and glucosamine derivatives [55]. Heparin is also known to bind with the basic fibroblast growth factor (bFGF), a growth factor that aids in the wound healing process, angiogenesis, and osteogenesis [56, 57]. An increase in the cellular receptor binding ability of bFGF, and its resistance to denaturation and proteolysis, has been observed when conjugated with heparin [58]. For these reasons, heparin and heparan sulfate have been investigated as constituents for self-assembling nanoparticle delivery systems for growth factors and other drugs.

Chitosan and heparin have both shown promise as particle constituents for wound healing applications [59, 60]. In a recent study, Tang et al. [58] prepared pH-responsive heparinized chitosan-poly(γ-glutamic acid) polyelectrolyte complex nanoparticles that encapsulated bFGF. These conjugate particles were capable of delivering both bFGF and heparin for treatment of ischemia (a restriction in blood supply to a tissue, caused by vascular congestion, resulting in tissue damage due to oxygen and glucose deficiency). Sustained release of bFGF was observed at pH

6.7 (simulating ischemia conditions) followed by disintegration of the particle conjugate at pH 7.4 (healed tissue pH), triggering the release of heparin to prevent potential blood clotting [58].

4.5 Chitosan nanoparticles as immunocontraceptive vaccine carriers

Chua et al. [13] investigated chitosan microparticles and nanoparticles as biocompatible delivery vehicles for peptide and protein-based vaccines. To study the effect of polymer particle size on the interaction of particle-based vaccines with antigen-presenting cells (APCs), thereby influencing and modulating ensuing immune response, they compared the use of chitosan-based nanoparticles and chitosan-based microparticles as vaccine delivery vehicles. The nanoparticles (30–500 nm) and microparticles (1–6 μm), produced by varying the type of surfactant in the synthesis process, were functionalized with chloroacetyl groups for the covalent attachment of thiol-containing peptide and protein antigens. The attached peptide and protein antigens consisted of the luteinizing hormone-releasing hormone (LHRH), a 10-amino acid peptide hormone that is secreted by the hypothalamus and regulates gametogenesis. Antibodies generated against this hormone can lead to the inhibition of reproductive capabilities of vaccinated mammals. In the case of the peptide antigen, the LHRH peptide sequence was combined with an influenza virus-derived 15-amino acid peptide epitope that is recognized by I-E^d restricted CD4+ T cells (helper T cells). The protein antigen, on the other hand, was prepared by conjugating the LHRH peptide to a proprietary carrier protein.

For the generation of cell-mediated immune response, the antigen must first be internalized by APCs, then processed within the APCs by degradative proteolytic mechanisms, and finally presented on the cell membranes of the APCs [in conjugation with class II major histocompatibility complex (MHC) proteins], for recognition of the exposed antigen by helper T cells. Chua et al. found that both nanoparticles and microparticles showed similar abilities *in vitro* to be taken up by murine bone marrow-derived dendritic cells (DCs), which are a type of APCs. When the particle concentration was sufficiently high, up to 75% of the DCs had internalized the particles.

The nanoparticles trafficked from the injection site to the draining lymph nodes, where naive T cells reside, faster than microparticles, but this difference in trafficking rate did not have any significant impact on the ensuing immune response. Inoculation with both types of particles resulted in the desired immune response, namely, the production of high levels of LHRH-specific antibodies. Such polysaccharide-based vaccine delivery vehicles could also prove useful for inducing cell-mediated immune responses against microbial, viral, and tumorigenic protein antigens [13].

4.6 Cytotoxicity of chitosan

The weakly cationic nature of chitosan, uncommon among polysaccharides, offers many benefits for the synthesis and application of chitosan-based nanoparticles as pH-responsive DDS. This is important as most other unmodified polysaccharides are anionic or neutral [61, 62]. However, the positive surface charge may also contribute to the cytotoxicity of these particles, especially in intravenous applications [63]. Cationic polymers such as PEI and poly(L-lysine) (PLL) have been widely studied in gene delivery as carriers for plasmid DNA, for transporting the gene into the nucleus [64]. These polycations possess high transfection efficiency, at the cost of cytotoxicity [65]. The transfection efficiency vs. cytotoxicity of PEI-DNA complexes depends on factors such as PEI molecular weight, degree of branching, ionic strength of solution, ζ-potential, and particle size. In one study, low molecular weight (10,000 g/mol) moderately branched polymer was found to be less toxic in comparison with commercial high molecular PEI [65]. Although the linear polypeptide, PLL, has the advantage that it is biodegradable (whereas PEI is not [66]), PLL-DNA polyplexes were found to rapidly bind to plasma proteins and were cleared from circulation [65].

In a review article, Liu and Yao [67] have discussed the advantages of chitosan and trimethylated chitosan oligomers as gene transfection systems with tunable transfection efficiency and cellular uptake. They reported that chitosan polyelectrolyte complexes formed with DNA (called chitosan-DNA polyplexes) were nontoxic to the Caco-2 human epithelial colorectal carcinoma cell line and the COS-1 monkey kidney fibroblast-like cell line. Conversely, Loretz and Bernkop-Schnürch [68] found that while anionic and neutral nanoparticles caused only minor membrane damage and only slightly altered mitochondrial activity (indicating cell viability), cationic nanoparticles produced severe cytotoxic effects. They reported a strong correlation between ζ-potential and toxicity.

A more recent study suggests that *in vitro* cytotoxicity of chitosan-based nanoparticles is strongly influenced by the pH of the medium in which the nanoparticles

were dispersed [69]. The cytotoxicity of the nanoparticles, toward Caco-2 cells, in Hank's balanced salt solution (HBSS), was greater at pH 6.0 than at pH 7.4. Previous studies had attributed the higher cytotoxicity at pH 6.0 to the higher cationic surface charge of the particles at this pH. However, Loh et al. [69] found that the ζ-potential of the particles were not very different at these pH values (5.3 mV at pH 6.0 and 3.3 mV at pH 7.4). Moreover, cytotoxicity was observed even when Earle's minimum essential medium (EMEM) was used, in which the chitosan nanoparticles exhibited a negative surface charge (ζ-potential of -6.1 mV). They attributed these effects to the large differences in the sizes of the chitosan nanoparticles at the two different pH values. The particles were about an order of magnitude larger in size in the pH 7.4 media than in the pH 6.0 media (e.g., about 333 nm in pH 7.4 HBSS vs. 25 nm in pH 6.0 HBSS). They argued that the smaller nanoparticles were internalized by the Caco-2 cells, by clathrin-mediated endocytosis, and subsequently caused extensive damage to the intracellular organelles. In contrast, the larger particles were poorly taken up into the cytoplasm, despite strong adherence to the cell surface, and therefore, inflicted less damage. They concluded that the cytotoxicity of chitosan nanoparticles was less influenced by positive surface charges than by the particle size.

Another recent study investigated the blood compatibility of chitosan nanoparticles prepared by crosslinking low-molecular weight chitosan [63]. The nanoparticle formation was carried out in an aqueous solution of either acetic acid (pH 3.2) or lactic acid (pH 4.2), in which most of the glucosamine mers of chitosan were protonated. Nanoparticles below 200 nm in size were obtained and were evaluated for their hemolytic activity, platelet aggregation, blood coagulation, and cytokine induction. Nanoparticles prepared in acetic acid showed stronger hemolytic activity, attributed to their higher surface charge density, than the particles prepared in lactic acid and dispersed in saline. The researchers concluded that because of a significant reduction in hemolytic activity, chitosan nanoparticles prepared in lactic acid solution and dispersed in saline, are suitable for parenteral drug delivery.

Regardless of whether size or surface charge density is the primary factor influencing cellular uptake of polymer nanoparticles, there is significant evidence that internalized chitosan nanoparticles pose concerns of cytotoxicity that are similar to those encountered with synthetic polymers such as PEI. While decreasing particle size may improve the efficiency of cell internalization, it may also adversely affect cell viability. The inconsistency in reports on the cytotoxicity of chitosan (cf. ref. [67–69]) indicates that a better understanding of the effects of long-term exposure to the weakly cationic nanoparticles of this polysaccharide is required. Modification of chitosan with chemical groups that might mitigate the polymer's cytotoxicity would also be beneficial in its use as a delivery vehicle.

5 Other stimulus-responsive polysaccharide nanoparticles

5.1 pH-Responsive hydrophobically modified pullulan with acid-labile vinyl ether groups

In a study by Morimoto et al. [70], biodegradable, pH-responsive, cholesteryl-modified, pullulan nanoparticles were prepared for controlled release of proteins. Vinyl ether-cholesterol moieties were grafted onto linear pullulan polymer chains (Figure 10), which subsequently self-assembled at neutral pH forming responsive nanogels. Bovine serum albumin (BSA) was used as a model protein, which rapidly complexed with the nanoparticles at pH 7.4. Upon exposure of the particles to an acidic environment (pH 4.0), hydrolysis of the vinyl ether bonds, linking cholesterol to the pullulan backbone, occurred after a few hours and continued for up to several days. The release of BSA due to particle matrix degradation was confirmed to occur over the same time scale.

5.2 pH-Responsive *N*-acetyl histidine-modified glycol chitosan nanoparticles for uptake by cancer cells

The uptake of nanoparticles by cancer cells is an important step in clinical studies on cancer intravenous therapeutics. Park et al. [71] studied the application of *N*-acetyl histidine-modified glycol chitosan (NAcHis-GC) nanoparticles for intracellular drug delivery to HeLa cells. The NAcHis-GC polymer was obtained by reacting GC (**18**, Figure 6) with *N*-acetyl histidine (NAcHis) in PBS in the presence of EDC hydrochloride and *N*-hydroxysuccinimide (NHS). Because NAcHis is hydrophobic at neutral pH, the NAcHis-GC conjugate formed nanoparticles with mean diameters of 150–250 nm in water. After cellular uptake by endocytosis, the nanoparticles disassembled because of a breakdown of the hydrophobic/hydrophilic balance by the protonation of the imidazole group of NAcHis in the slightly acidic environment of the endosomes. Upon

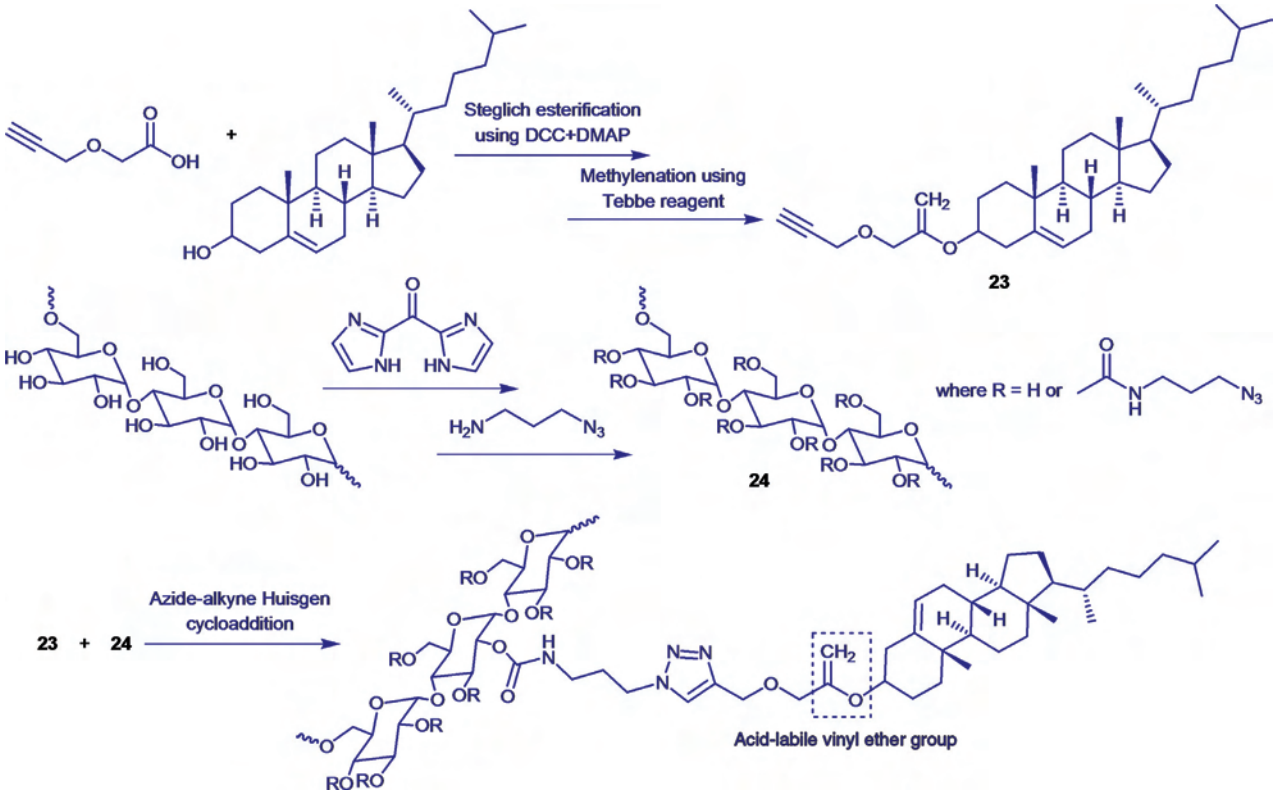


Figure 10 Synthesis of acid-labile cholesterol bearing pullulan. The cholesterol derivative is tethered to the pullulan backbone by vinyl ether groups. The hydrophobically modified polysaccharide self-assembled in aqueous media to form nanoparticles of 10–20 nm diameter that were stable under normal physiological pH conditions but degraded under acidic conditions (pH 4) [70].

internalization, the nanoparticles successfully delivered PTX, reducing cell growth. The endocytic uptake of NAcHis-GC particles loaded with PTX demonstrates a promising technique for delivering poorly soluble drugs into the cytosol of cancerous cells.

5.3 Temperature-responsive chitosan nanoparticles

Chitosan-based nanoparticles designed for encapsulation of hydrophilic drugs were prepared by Chuang et al. [19], by grafting chitosan with poly(*N*-isopropyl acrylamide) (PNIPAM), a well-known temperature-responsive polymer [72, 73] used in DDSs [74–76]. The graft copolymer was obtained by polymerizing *N*-isopropyl acrylamide in the presence of chitosan. Particles were then self-assembled by elevating the solution temperature above the lower critical solution temperature (LCST) causing the PNIPAM grafts to aggregate. The micelles were stabilized using glutaraldehyde crosslinker, and were found to be highly porous, and even hollow under some conditions. The size of the particles formed was tunable through temperature manipulation. Fan et al. [77] used such chitosan-PNIPAM nanoparticles for delivering podophyllotoxin (PPT), an anticancer drug, to tumors. *In vitro* cytotoxicity experiments using human fibroblasts and human cancer cells showed that these cells had higher cytotoxicity than the free PPT at extracellular tumor pH [77].

5.4 Glycol chitosan nanoparticles for photodynamic therapy

Photodynamic therapy (PDT) is a method of treatment used in oncology to target and destroy tumors with minimal damage to proximal healthy tissue [78, 79]. This site-specific treatment involves the targeting of tumor tissues by administration of a photosensitizer (PS) followed by local irradiation of the tumor site by light. The PS absorbs the incident light and transfers energy to surrounding molecular oxygen, generating cytotoxic singlet oxygen species. These species then attack the cancerous cells in the tumor tissue [78]. An extensive

review of PDT diagnosis and treatment using nanoparticles is available [79].

Photosensitizers are often porphyrins, which are hydrophobic, making polysaccharides a logical choice as nanoparticle conjugates for improving solubility and *in vivo* retention of the PS. PDT is not a new technique for the treatment of cancer, but advances have been achieved through conjugation of photosensitizers with polysaccharides [80, 81]. Polysaccharide nanoparticles have been used to improve the therapeutic efficacy of PDT.

Li et al. [82] investigated nanoparticles prepared using heparin-PS conjugates. Pheophorbide A (PhA) was used as the PS, and folate was used as a targeting ligand. Heparin-PhA and folate-heparin-PhA conjugates were found to self-assemble in aqueous solutions, resulting in particles ranging in size from 130 to 170 nm and possessing negatively charged surfaces (ζ-potentials of -20 to -35 mV). The heparin-PhA and folate-heparin-PhA nanoparticles were found to have self-quenching photoactivity in aqueous medium, due to aggregation of the hydrophobic PhA moieties within the nanoparticles. However, the photoactivity was restored upon cellular uptake. The nanoparticles were found to be toxic to HeLa cells when irradiated with 670 nm wavelength light (1.2 J/cm^2 intensity). The cytotoxicity was attributed to enzymatic attack on the heparin backbone and the cleavage of the amide bond between PhA and heparin, resulting in a loss of the quenching effect. Furthermore, the nanoparticles displayed only slight toxicity in the absence of light, for the same concentration and incubation time. The combined effects of self-quenching of the PS, restoration of PS activity when internalized by cells, and targeted delivery using folate ligands lead to improved therapeutic efficiency against tumor cells, while mitigating damage to normal tissues and blood cells. Moreover, the anticoagulant activity of the heparin-PhA and folate-heparin-PhA conjugates was significantly decreased compared to free heparin, thereby potentially reducing the hemorrhagic side effects.

Glycol chitosan has also been shown to form nanoparticles capable of delivering PS to tumor cells [80, 81]. The application of PhA in PDT for cancer treatment was investigated in tumor-bearing mice by Oh et al. [83] using cancer-cell specific nanoparticles that contained PhA-conjugated GC with reducible disulfide bonds (PhA-SS-GC, **25**, Figure 11). The PhA-SS-GC conjugates self-assembled in aqueous solutions to form core-shell-structured nanoparticles with good colloidal stability and switchable photoactivity. In nonreductive environments, the photoactivity of the conjugates was greatly suppressed by the self-quenching effect. However, after uptake by cancer cells, the PS instantaneously dissociated from the polymer backbone

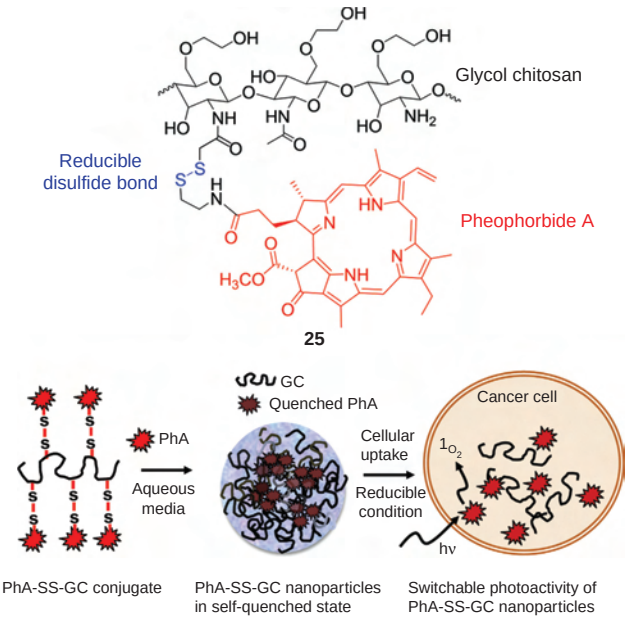


Figure 11 Chemical structure of photosensitizer pheophorbide a (PhA) linked to glycol chitosan (GC) by a reducible disulfide bond. The conjugates (PhA-SS-GC) self-assembled into core-shell nanoparticles in aqueous solution, and PhA was rendered photo-inactive in the particle core. The disulfide bond is cleaved following tumor cell uptake, resulting in the delivery of photoactive PhA. Adapted from ref. [83], Copyright © 2013, with permission from Elsevier.

by reductive cleavage of the disulfide linkages, leading to separation and efficient dequenching of the PS moieties. Thus, the nanoparticles were photoactive and cytotoxic only inside the cancer cells, and not elsewhere. Moreover, the PhA-SS-GC nanoparticles showed longer circulation time *in vivo* compared with free PhA and enhanced tumor-specific targeting behavior through the EPR effect.

6 Polysaccharide prodrug complexes

A prodrug is a therapeutic that has been conjugated or complexed with another molecule or structure, rendering the drug inert when initially administered. The attached substance is usually a particle, ligand, or stabilizer that improves solubility, pilots the drug, protects from the reticuloendothelial system, improves drug efficacy, or provides some other function [84]. The attached drug becomes effective after some objective is met (e.g., arrival at desired site), or the prodrug is exposed to an anticipated stimulus, triggering release. Prodrug complexes are an important class of nanostructured DDS. They have the

unique ability to render a drug inert until desired, which is helpful when toxicity is an issue. These complexes also offer a different mode of release once activated, in comparison with the reservoir or core-shell-type systems, expanding realizable release profile possibilities. Reviews of polymer-drug conjugates [84, 85] and recent clinical studies [86] are available.

6.1 Heparin-doxorubicin conjugates with acid-labile hydrazone linkages

She et al. [9] prepared dendronized heparin conjugated with DOX through acid-labile hydrazone linkages using the reaction scheme shown in Figure 12. Azide-functionalized heparin, **26**, was synthesized in PBS in the presence of EDC hydrochloride and *N*-hydroxysulfosuccinimide (sulfo-NHS). Alkyne-Boc-protected-lysine, **28**, was prepared by reacting 2-propynylamine with Boc-protected lysine, Boc-L-Lys(Boc)-OH, **27**, in the presence of 1-hydroxybenzotriazole (HOBt), *N*,*N*,*N*′,*N*′-tetramethyl-(1H-benzotriazol-1-yl)uranium hexafluorophosphate (HBTU) and *N*,*N*-diisopropylethylamine (DIPEA). Dendron **31** was obtained by reacting the alkyne-lysine, **29**, with Boc-protected lysine, and was further reacted with 4-(*N*′-*tert*-butoxycarbonyl hydrazino)-4-oxo-butanoic acid, **32**, to obtain dendron **33**. Dendron **33** was covalently attached to azido-heparin via Cu(I)-catalyzed azide-alkyne cycloaddition click chemistry.

The amphiphilic conjugate self-assembled to form nanoparticles in aqueous solution, and the release of DOX was found to be sensitive to pH. When only the dendrimers were used as drug or gene delivery vehicles, they were found to be easily eliminated from the body (through extravasation or renal clearance) because of their small sizes. They were also found to exhibit *in vitro* and *in vivo* toxicity. The nanoparticles formed by self-assembly of the dendronized heparin-DOX conjugate, on the other hand, resulted in strong antitumor activity and high antiangiogenesis effects, induced apoptosis of the 4T1 tumor model, and posed no significant toxicity to healthy organs of tumor-bearing and healthy mice. Thus, the desirable properties of dendrimers, including multivalency and versatile surface functionality, were combined with the biocompatibility and longer circulation times of polysaccharide nanoparticles, in a novel way in this study, to produce an interesting platform for conjugate-based drug or gene delivery. Similar acid-labile hydrazole-linked DOX nanoconjugates, based on biodegradable, nontoxic, and non-immunogenic poly(β-L-malic acid), were found to successfully inhibit *in vitro* cancer cell growth of several invasive breast carcinoma cell lines and primary glioma cell lines [87].

6.2 Heparin-paclitaxel conjugates with amino acid spacers

Conventionally, paclitaxel is administered intravenously; however, recent attempts to improve drug efficacy through formation of prodrugs have been made. Wang et al. [88] prepared prodrugs for controlled delivery of PTX by reacting succinylated-heparin with aminoacyl paclitaxel. Three different aminoacyl paclitaxel compounds were investigated: valyl-PTX, leucyl-PTX, and phenylalanyl-PTX. The choice of the amino acid spacer affected the rate of hydrolysis of the ester bond between the amino acid and PTX, thereby providing a mechanism for controlling release kinetics. The leucine spacer was found to exhibit favorable hydrolysis characteristics (characterized using PTX release kinetics) under physiological conditions. The prodrugs self-assembled in water to form spherical nanoparticles 140–185 nm in diameter and with ζ-potentials in the range of -15 to -40 mV. A core-shell architecture with the hydrophobic PTX buried in the core, and the negatively charged carboxylate and sulfate groups exposed on the hydrophilic heparin shell, was proposed. The inhibition of MCF-7 cells *in vitro* was found to be stronger for the prodrug than free PTX. The particle-PTX system also had less anticoagulant activity than free heparin, reducing the risk of hemorrhaging.

7 Polysaccharide nanoparticles prepared by electrostatic self-assembly

7.1 Heparin-based ionic complexes for controlled release of growth factors

An important aspect of regenerative medicine is developing better controlled release devices for the delivery of high efficacy growth factors [89]. Growth factors are molecules that stimulate cellular growth, proliferation, and differentiation. They are usually proteins or steroid hormones. Because native growth factors are bound to, protected, and controlled by the extracellular matrix through heparan sulfate, heparin (which is structurally similar to heparan sulfate) is useful for growth factor delivery.

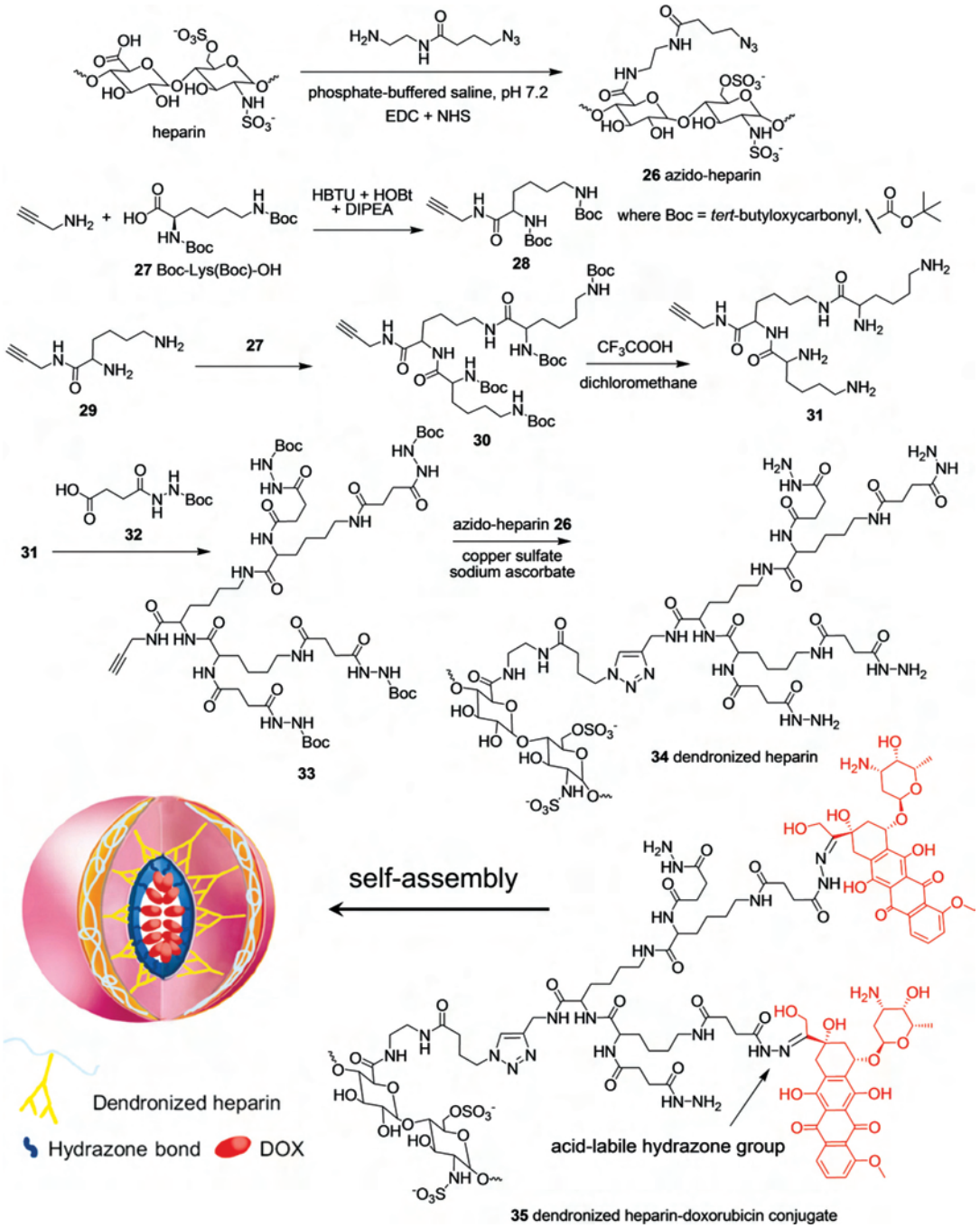


Figure 12 Schematic illustrating the synthesis of dendronized heparin-DOX prodrug conjugates. Adapted from ref. [9], Copyright © 2013, with permission from Elsevier.

However, heparin forms soluble complexes with growth factors, which poses a problem for their controlled release.

In a study by Chu et al. [89], a nanoparticle delivery system was prepared with heparin ionically bound to poly(ethylene argininylaspartate diglyceride) (PEAD, **37**, Figure 13), a biocompatible, biodegradable polycation, obtained by reacting poly(ethylene aspartate diglyceride) (PED, **36**, Figure 13) with t-BOC-protected arginine and subsequent deprotection using trifluoroacetic acid. PED was prepared by polycondensation of ethylene glycol diglycidyl ether and t-BOC-protected aspartic acid, followed by removal of the t-BOC group. Because PEAD had two cationic groups (amine and guanidine) per repeating unit, it interacted strongly with the negatively

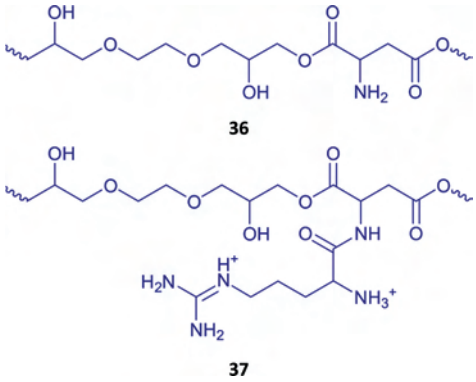


Figure 13 Chemical structure of poly(ethylene aspartate diglyceride) (PED, **36**), and poly(ethylene argininylaspartate diglyceride) (PEAD, **37**).

charged heparin through Coulombic forces. The binding of PEAD to heparin was monitored by ζ-potential titration. Approximately 3.3 PEAD molecules bound to one heparin molecule.

Fibroblast growth factor-2 (FGF-2) and nerve growth factor (NGF) were chosen as model growth factors because of their high and low heparin affinities, respectively. The high charge density of both PEAD and heparin produced nanoparticles with high growth factor encapsulation efficiency (>99%). The PEAD/heparin/FGF-2 nanoparticles had an average hydrodynamic diameter of about 540 nm. These particles showed an initial burst release of approximately 10% after 1 day, followed by a sustained release, at a nearly constant rate, through the end of a 42-day experiment. NGF showed a faster release profile because of its lower affinity for heparin. FGF-2 bioactivity was determined by its stimulatory effects on the proliferation of human aortic endothelial cells. NGF bioactivity was determined by its ability to stimulate neuronal differential of PC-12 cells. The PEAD/heparin complexes were found to maintain the bioactivity of FGF-2 and increased the bioactivity of NGF when compared with bolus delivery.

7.2 Polysaccharide-phospholipid nanoparticles

Ionic complex nanoparticles can also be prepared using ionic polysaccharides and lipids or proteins containing groups opposite in charge to the polysaccharide. Ionic polysaccharide complex particles were prepared from soy lecithin and chitosan in aqueous solution by Sonvico et al. [90]. Lecithin is a mixture of phospholipids, which contain negatively charged phosphate moieties. The mixture of chitosan and lecithin was found to spontaneously form particles through the electrostatic interactions between the positively charged ammonium groups of chitosan and the negatively charged phosphate groups of phospholipids constituting lecithin. The ζ-potential analysis of particles formed at different ratios of chitosan to lecithin revealed a tunable surface charge. An aggregation of particles was observed when the surface charge density was low. The ability of these particles to encapsulate both hydrophobic and hydrophilic material was assessed with progesterone and metoclopramide hydrochloride, respectively. The encapsulation of the hydrophilic metoclopramide hydrochloride was nearly impossible, while that of the lipophilic progesterone was possible with encapsulation efficiency up to about 64% [90]. This particle system provides an interesting particle platform with facile preparation, high encapsulation efficiency of hydrophobic drugs, and pH and ionic strength-dependent aggregation behavior.

7.3 Polysaccharide-protein nanoparticles for delivery of folic acid

Drug delivery nanoparticle systems are being increasingly applied to fields outside of medicine, including delivery of nutrients [14, 91, 92]. Folate is an essential nutrient that is not produced by the human body and, thus, is a dietary requirement. Folate deficiency has been shown to attribute to various conditions including depression, Alzheimer's disease [93], and some carcinomas [94]. Folate has also been shown to be an effective targeting ligand when covalently attached to nanoparticles, for diagnostic and therapeutic applications [95].

The oxidized synthetic version of folate, folic acid, is more stable but insoluble in acidic environments. Many foods and drinks are acidic, thus proper digestion of this vital nutrient can be impaired. Additionally, heat, oxygen, and light can result in degradation of folic acid during food processing and storage. Ding and Yao [14] prepared folic acid-loaded soy protein/soy polysaccharide nanogels that stabilized folic acid under acidic conditions and released it rapidly at neutral pH (similar to that in the intestine). The protein, polysaccharide, and folic acid were mixed in pH4 water and subjected to high-pressure homogenization. Heating the mixture at 90°C resulted in denaturation and gelation of soy protein, resulting in the formation of particles that were 150–200 nm in size. The polysaccharide/protein carrier was not only able to deliver folic acid at neutral pH, but also inhibit the reaction between dissolved oxygen and folic acid during UV irradiation. Folic acid in

solution was degraded completely when irradiated with UV light of 365 nm wavelength, whereas only about 17% of the folic acid loaded in the nanogels degraded after 1 h or irradiation with the UV light. This particle system is ideal for improving solubility, stability, and controlled release characteristics of sensitive nutrients in food and beverage.

7.4 Dextran microtubules

Self-assembling polysaccharide ionic complexes form due to association of oppositely charged polyelectrolytes. When two hydrophilic polymers, dextran-bromoethylamine hydrobromide (Dex-NH_2) and dextran-chloro acetic acid (Dex-COOH), were mixed in pH 4.0 buffer solution, hollow tubules with a diameter between 600 nm and 2 μm and up to 100 μm long were obtained [96]. The dextran derivatives, containing amino groups (Dex-NH_2) and carboxylic acid groups (Dex-COOH), were synthesized by reacting dextran (6000 g/mol) with 2-bromoethylamine and chloroacetic acid, respectively. When aqueous solutions of the dextran-based precursors were mixed, the carboxylate groups (produced by the dissociation of the carboxyl groups of Dex-COOH) formed a polyelectrolyte complex with protonated amino groups of Dex-NH_2 by electrostatic interaction. First, bead-like polyvalent aggregates were formed, which then lined up to form 2D circles due to the alternating negative and positive charges of adjacent beads. These rings directed the growth of the 3D tubule self-assembly in both longitudinal and transverse directions. Microtubular structures were found to form at pH values of 3.0 and 4.0, but not at higher pH values. At pH 7.0, most amino groups in Dex-NH_2 remained unprotonated, and ionic interactions with Dex-COOH were weak. Only sheet-like structures without any distinctive morphology were formed (Figure 14D). This method of forming hollow polysaccharide tubes provides an interesting platform for encapsulation and delivery of bioactive substances in tissue engineering applications. It may be possible to extend this approach to the synthesis of shorter polysaccharide nanotubules to promote cellular uptake in intravenous drug delivery. In one study, nanoparticle shape has been found to have a significant effect on cellular uptake and cell function [97]. Particles with larger aspect ratios were taken up in greater amounts and had faster internalization rates.

7.5 Polysaccharide nanostructures prepared by layer-by-layer (LbL) self-assembly

LbL self-assembly was first introduced by Decher with bipolar amphiphiles [98] and polyelectrolytes [99]. The assembly of polyelectrolytes of opposite charge on a solid

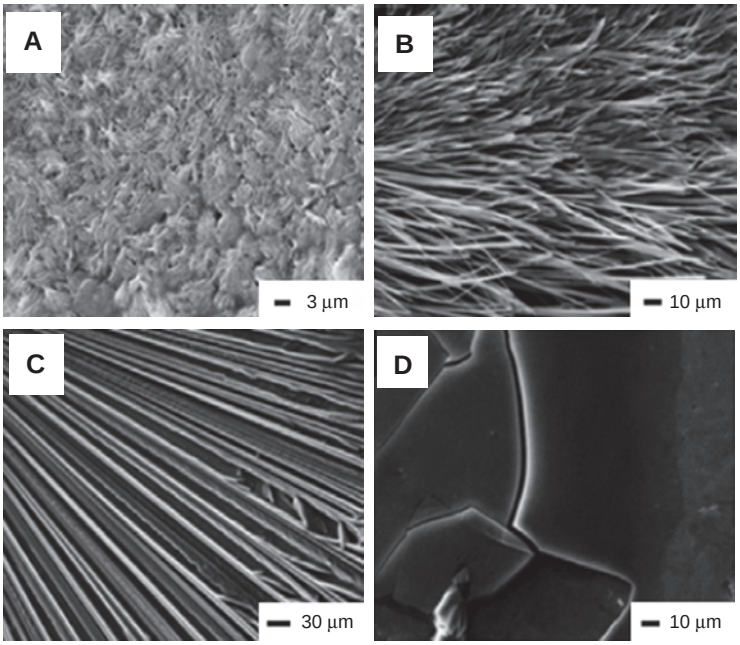


Figure 14 SEM images of dextran modified with 2-bromoethylamine (Dex-BH) and chloroacetic acid (Dex-CA). The formation of self-assembled tubules is apparent at pH 4.0 (B) and 5.0 (C) but not pH 3.0 (A) and 7.0 (D). Reprinted from ref. [96], Copyright © 2009, with permission from American Chemical Society.

particle substrate occurs due to the reversal of surface charge with each adsorbed layer. A recent review of LbL self-assembly of nanocoatings is available [100]. Nanoparticles with multilayer polysaccharide shells can be assembled through alternating application of cationic and anionic polysaccharide coatings to a template. However, hydrogen bonding, hydrophobic interactions, and van der Waals forces have also been used to produce LbL structures [101].

7.5.1 pH-Responsive carrageenan-chitosan hollow nanoparticles

Electrostatic LbL self-assembly of carrageenan, an anionic sulfated polysaccharide, with chitosan was reported by Liu et al. [17]. The LbL self-assembly onto silicon dioxide nanospheres, and the subsequent removal of the template silica particles, produced hollow nanocapsules of alternating polymer layers. The surface of SiO_2 particles was first functionalized with amine groups, and then coated with alternating layers of carrageenan and chitosan in aqueous solution (see Figure 15). The silica core was dissolved using hydrofluoric acid following polysaccharide layer formation. The pH responsiveness of chitosan was maintained in the final nanocapsules, producing hollow particles responsive to both pH and ionic strength.

7.5.2 Polyelectrolyte multilayers for FGF-2 delivery

Tissue engineering applications of LbL thin film coatings have been previously reviewed [101]. FGF-2 is one of several paracrine factors that regulate proliferation, differentiation, angiogenesis, and ossification [102]. However, the rather short plasma half-life of FGF-2 presents a hurdle for *in vivo* delivery of this protein over extended periods. Hence, there is interest in developing biomaterials for sustained release of FGF-2 and protecting it from degradation. Using the fact that noncovalent binding of FGF-2 to sulfated glycosaminoglycans, such as heparin and heparan sulfate, protects it from proteolytic and chemical inactivation (cf. Section 4.4), increasing its half-life almost six-fold, Almodovar et al. [103] investigated the use of polyelectrolyte multilayers (PEM) consisting of heparin and chitosan for FGF-2 delivery. They found that, on PEM-coated tissue-culture polystyrene (TCPS) substrate, FGF-2 adsorbed to heparin-terminated PEMs, and induced greater cell density and a higher proliferation rate of ovine bone marrow-derived mesenchymal stem cells (MSCs), than when FGF-2 was delivered at an optimally mitogenic dose in solution.

7.5.3 LbL assembly of charge reversible polymers for pH-responsive siRNA delivery

Like growth factors, the delivery of siRNA (cf. Section 2.2) is also affected by the short plasma half-life of these double-stranded RNA complexes. Since the discovery of the ability of siRNA to induce RNA interference (post-transcriptional gene silencing, inhibiting gene expression) in mammalian cells, significant research into siRNA as a biodrug therapeutic has been conducted [104]. Naked siRNA is rapidly degraded by serum nucleases. A strategy to protect and deliver siRNA was reported by Han et al. [105]. They prepared gold nanoparticles coated with chitosan as a positively charged template for LbL assembly of pH-responsive, charge-reversible, polyallylamine-citraconic

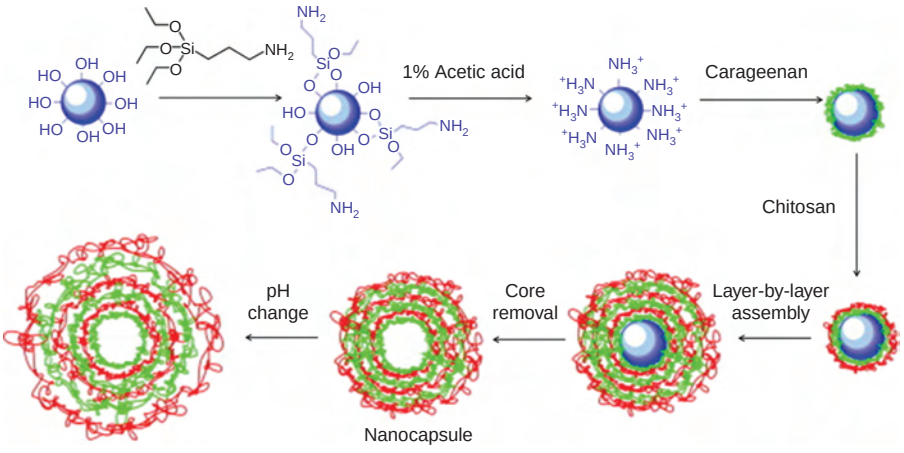


Figure 15 Schematic of the self-assembly of alternating layers of anionic carrageenan and cationic chitosan onto amine-functionalized silica templates. Adapted with permission from ref. [17], Copyright © 2012, with permission from Elsevier.

anhydride (PAH-Cit), and PEI. PAH-Cit is an anionic carboxylate-functionalized polymer (**38**, Figure 16), which can be readily converted to cation polyallylamine (**39**, Figure 16) by amide hydrolysis upon exposure to acidic environments, such as those found within late endosomes and lysosomes [105, 106]. The negatively charged siRNA adsorbed onto the positively charged PEI surface forming siRNA/PEI/PAH-Cit/AuNP-CS nanoparticle complexes. The complexes protected the adsorbed siRNA from enzymatic degradation and were found to have negligible toxicity against HeLa and MCF-7R cells. The delivery of siRNA was mediated by the charge-reversal of PAH-Cit under acidic conditions, which disrupted the LbL structure of the particle complexes, triggering release.

7.5.4 Core-shell nanoparticles with gelatin core and polysaccharide containing PEM shell for epigallocatechin gallate delivery

Approaches involving core-shell nanoparticles are interesting for targeting encapsulated materials to specific sites, and controlling their release and cellular uptake. Plant phytochemicals such as polyphenols are being widely researched as anticancer agents (c.f. Section 3.2). The major challenge with the encapsulation and delivery of these compounds is their short half-life, fast oxidation in basic environments, and low solubility. Shutava et al. [107] loaded gelatin-based nanoparticles with the flavonoid, epigallocatechin gallate (EGCG) using the approach shown in Figure 17. Gelatin-based nanoparticles are known to be relatively safe and effective nonviral gene delivery vehicles with prolonged *in vivo* circulation time and high accumulation at the tumor site [108, 109]. Shutava et al. prepared the gelatin nanoparticles using a two-step process involving dissolution of gelatin in an acidified aqueous solution, precipitation of the polymer by addition of acetone, and crosslinking of the nanoparticles by the reaction of glutaraldehyde with the free amine groups of gelatin. Particle size, distribution, and stability were all influenced by the pH of the preparation medium, and acidic conditions (pH 3) produced dispersions with the smallest particles sizes (200–300 nm) and better colloidal stability. The LbL assembly was carried out in pH 6.0 aqueous solution, in which the ζ-potential of uncoated gelatin core was +20 mV. The nanoparticles were then coated with 5- to 20-nm-thick PEM shells consisting of polycations and polyanions of synthetic or biological

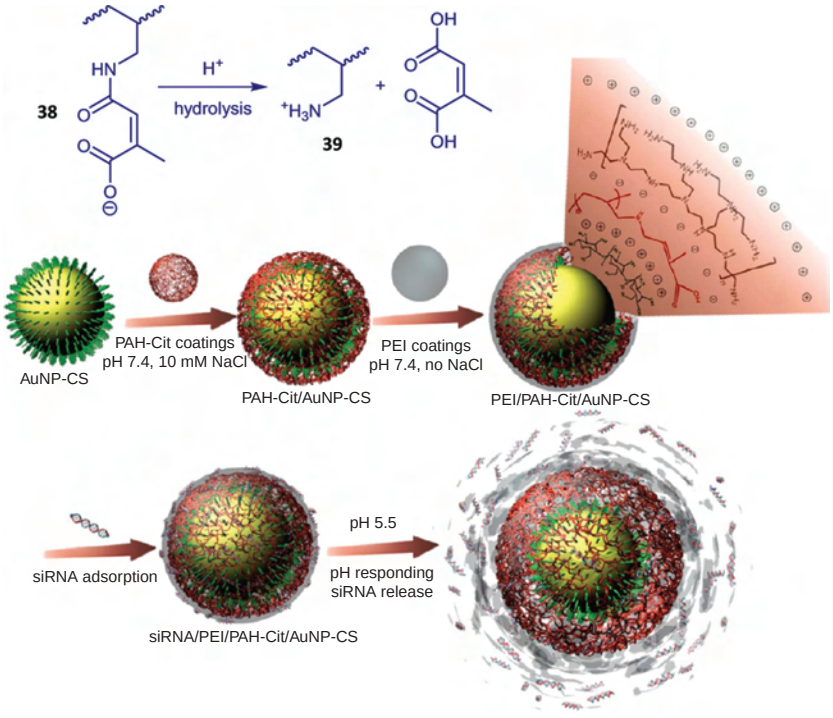


Figure 16 A representation of assembly of alternating layers of polyethylenimine (PEI) and polyallylamine-citraconic anhydride (PAH-Cit) onto gold nanoparticles, surface-modified with chitosan (AuNP-CS). Small interfering RNA (siRNA) was adsorbed onto the PEI surface and released under acidic pH due to breakdown of the LbL structure. Adapted with permission from ref. [105], Copyright © 2012, with permission from American Chemical Society.

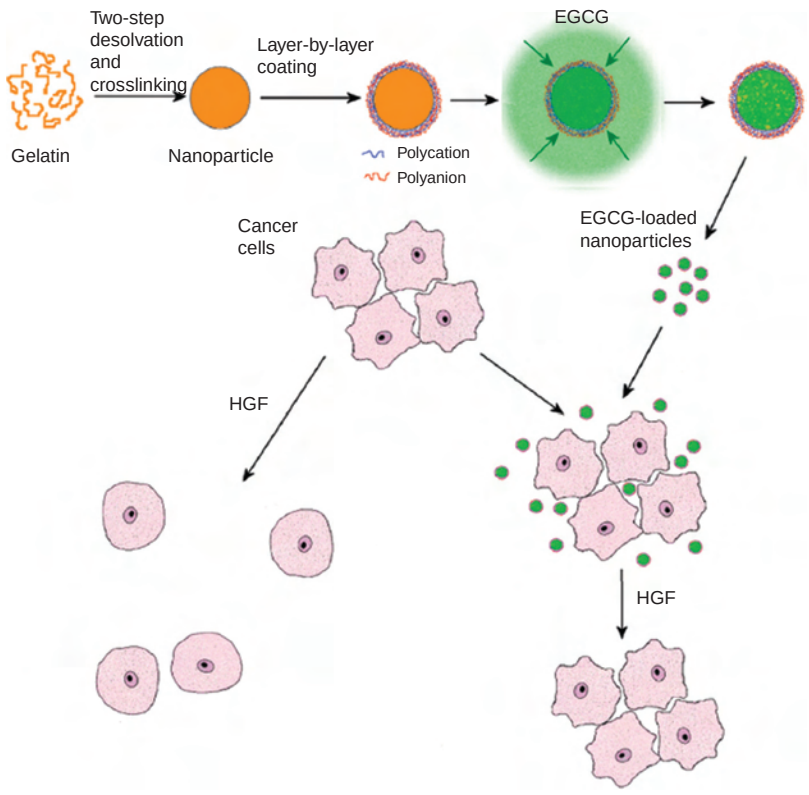


Figure 17 Schematic of the preparation of LbL-coated gelatin nanoparticles containing the natural polyphenolic cancer chemopreventive agent, epigallocatechin gallate (EGCG), and their effect on cancer cells. The nanoparticles were effective in suppressing hepatocyte growth factor (HGF)-induced scattering of cancer cells. Reprinted from ref. [107], Copyright © 2009, with permission from American Chemical Society.

origin. The PEM layers incorporating polysaccharides included dextran sulfate/protamine sulfate, a combination of a strong polyanion and a strong positively charged polypeptide, and carboxymethyl cellulose/gelatin-A, a combination of weak polyanion and a weak positively charged protein. When a weak polyelectrolyte was the outermost layer of the LbL assembly, the nanoparticles were found to aggregate, resulting in an increase in the effective particle diameter and polydispersity. The EGCG was adsorbed onto the coated nanoparticles at pH 6.8 for 48 h. They attributed the reversible binding of EGCG to gelatin by hydrogen bonding interactions.

The nanoparticle-encapsulated EGCG was found to retain its biological activity, as inferred from its ability to block the c-Met/HGF intracellular signaling pathway in the breast cancer cell line MBA-MD-231. In this pathway, the hepatocyte growth factor, HGF, secreted by the cancer cells, activates the proto-oncogenic c-Met cell-membrane receptor, leading to an increase in intracellular signaling and culminating in HGF-induced cell scattering, motility, matrix invasion, and tumor metastasis. The EGCG-containing nanoparticles were found to be capable of blocking HGF-induced signaling at a longer preincubation time than free EGCG, indicating slow release of EGCG from the nanoparticles.

8 Conclusion

Polysaccharides are highly promising platforms for the synthesis of biocompatible nanocarriers of small-molecule and macromolecular drugs for biomedical applications. The ability of oligosaccharides such as cyclodextrins to form inclusion complexes can be used to prepare nanoparticles and nanocapsules for encapsulation of a variety of hydrophobic and hydrophilic drug molecules. Modification of polysaccharides with hydrophobic groups results in amphiphilic polymers that self-assemble in aqueous phase to form nanoparticles. Polysaccharides that feature basic and acidic groups, such as chitosan and heparin, respectively, have been used to form nanoparticles by the formation of ionic complexes. Such nanoparticles are often pH responsive and

can respond to physiological pH differences. These polysaccharides have been further functionalized with acid-labile groups, such as vinyl ether and hydrazone, to make use of the acidic pH of endosomes. The hydroxyl, amino, and carboxylic acid groups in polysaccharides have been used for chemical functionalization, and for binding targeting ligands, such as folate and transferrin, for targeted delivery. These groups have also been used to attach temperature and light responsive moieties. Polysaccharides have been used to deliver a variety of hydrophobic molecules, peptides, and oligonucleotides, in the form of pro-drug complexes, in which the polysaccharide served to increase bioavailability (by increasing water solubility) and to increase retention (evidenced by an increase in the plasma circulation half-life).

The examples discussed in this review demonstrate the many opportunities that self-assembling polysaccharide nanoparticles have to offer to the drug delivery field. Medical diagnostics is a related field that can benefit equally from the advantages of polysaccharide nanoparticles. In particular, stimulus responsive properties are ideal for diagnosing medical conditions. Nanoparticles can be targeted to the tissue site of interest actively or passively then "activated" via external stimulus to release diagnostic agents. As with some cancer therapeutics, diagnostic agents that may be hazardous can be limited to the target tissue preventing adverse side effects. This specificity has been achieved using targeting ligands, such as folate, attached to the surface of micelles [110] and particles composed of modified polysaccharides [111]. The term theranostics [112, 113] has been given to the combined development of therapeutic and diagnostic technologies. Fortunately, there is significant overlap between these two fields, allowing for mutual gain from the future the development of polysaccharide nanostructures.

While polysaccharides are very customizable through grafting and modification, their natural variability in composition and molecular weight can be a disadvantage when compared with well-defined synthetic polymers prepared with techniques such as controlled radical polymerization [114–116], or other synthetic nanostructures such as dendrimers [117] and peptide-based nanoparticles [118–121]. It is necessary to develop techniques for large-scale production of refined polysaccharides, having well-defined molecular weights and chemical compositions, which are suitable for safe application of these materials as DDSs. It may be possible to adapt advanced chemical separation techniques for the purification of natural polysaccharides [122] and to obtain pharmaceutical-grade forms of these polymers. Synthesis of polysaccharides using bioengineering approaches is also a way forward [123–125].

Another concern is the inherent immunogenicity of several polysaccharides (similar to proteins and peptides). The meningococcal polysaccharide vaccine, approved by the US Food and Drug Administration (FDA), makes use of this immunogenic property of the polysaccharide isolated from the cell wall of meningococcal bacteria, for vaccination against infection caused by these bacteria [126]. However, the immunogenicity of a polysaccharide must be suppressed for it to be useful in a DDS designed for nonvaccination purposes. It may be possible to impart the biocompatibility required for parenteral drug delivery using approaches such as PEGylation of the nanoparticle surface.

Although there are several preclinical studies demonstrating the efficacy of chitosan-based parenteral DDS, we are unaware of any clinical trials using chitosan for this purpose. Some laboratory studies discussed in this review have demonstrated the pH and size-dependent toxicity of chitosan nanoparticles. As such, chitosan is not included in the GRAS (generally regarded as safe) list of the FDA. PEGylation of the chitosan nanoparticles might also be effective in eliminating the cytotoxicity of these particles. Polysaccharide DDSs that have proven safe in preclinical trials should be further evaluated in clinical trials.

In spite of some of the aforesaid challenges, there are several success stories in the use of polysaccharides in the pharmaceutical field. A classic example is the evolution of the commercial heparin introduced by William H. Howell and his colleagues in 1924, that was associated with side effects such as headaches, fever, and nausea, to a safer version, without these side effects, in 1937, resulting from the development of a process for extraction and purification of heparin from bovine liver (by Charles Best and his colleagues in 1933) [127].

Successful application of polysaccharide-based nanoparticles in oral delivery systems is perhaps easier than their use in parenteral delivery systems. Indeed, hydrophobically modified celluloses are GRAS materials that are widely used as excipients in the pharmaceutical industry [128]. Starch, which is a mixture of branched (amylopectin) and linear (amylose) polysaccharides composed of repeat glucose units, has been similarly modified to produce materials capable of nanoparticle formation for biomedical applications [129]. As starch is a component of the human diet, starch-based nanoparticles are expected to be quite safe in oral DDS. Alginates are polysaccharides found in brown algae that have many food and pharmaceutical applications [130, 131] because of pH-responsive capabilities. Carrageenan, a natural polysaccharide sourced from red seaweed, is another FDA approved material that has been applied extensively to the

pharmaceutical and food industries [132]. Carrageenan and alginate are both appealing for their anionic character, which provides a simple method for constructing ionic complex or crosslinked nanostructures. It is expected that with the increasing interest in the use of polysaccharide carriers for drug delivery, products that are provenly safe will be widely available in the near future.

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