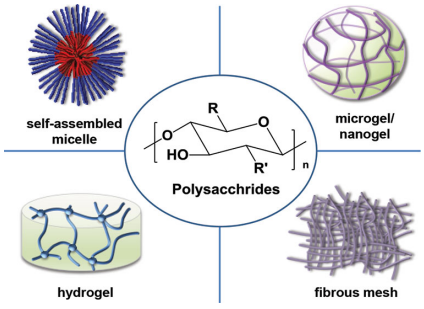
Recent Strategies to Develop Polysaccharide-Based Nanomaterials for Biomedical Applications

Yifen Wen, Jung Kwon Oh*

Polysaccharides are abundant in nature, renewable, nontoxic, and intrinsically biodegradable. They possess a high level of functional groups including hydroxyl, amino, and carboxylic acid groups. These functional groups can be utilized for further modification of polysaccharides with small molecules, polymers, and crosslinkers; the modified polysaccharides have been used as effective building blocks in fabricating novel biomaterials for various biomedical applications such as drug delivery carriers, cell-encapsulating biomaterials, and tissue engineering scaffolds. This review describes recent strategies to modify polysaccharides for the development of polysaccharide-based biomaterials; typically self-assembled micelles, crosslinked microgels/nanogels, three-dimensional hydrogels, and fibrous meshes. In addition, the outlook is briefly discussed on the important aspects for the current and future development of polysaccharide-based biomaterials, particularly tumor-targeting intracellular drug delivery nanocarriers.



1. Introduction

Polysaccharides are naturally occurring polymers or biopolymers. Typical examples of polysaccharides include dextran (DeX), pullulan (PuL), cellulose (CeL), chitosan (CS), hyaluronic acid (HA), alginate (ALG), and many others (Figure 1).[1–6] HA, as a main component of the extracellular matrix, consists of *N*-acetyl-D-glucosamine and D-glucuronic acid. ALG composes β-D-mannuronic acid and α-L-guluronic acid residues. CeL, as a main component of plant cells, has been considered as the most abundant polysaccharide in nature. It consists of a homopolymer of β(1→4) linked D-glucose. With a similar structure, CS is composed of β (1→4) linked 2-amino-deoxy-D-glucan, resulting from the deacetylation of *N*-acetyl-D-glucosamine of chitin (CT). It is almost the only cationic polysaccharide. PuL is a product of starch, formed of α(1→6) linked maltotriosyl units. Dex is composed of α(1→6) glycosidic linkages between D-glucopyranose residues.[7]

Polysaccharides are abundant in nature, renewable, nontoxic, intrinsically biodegradable, and relatively cheap. Furthermore, they possess a high content of functional groups including hydroxyl, amino (i.e., CS), and carboxylic acid groups (i.e., HA and ALG). These functional groups can be utilized for further modification of the polysaccharides. These features have attracted significant attention to develop polysaccharide-based biomaterials for various biomedical applications as drug delivery carriers, cell-encapsulating biomaterials, tissue engineering scaffolds, and regenerative medicine.[8–11] However, polysaccharides also have several drawbacks; they have a broad molecular weight distribution and suffer from batch to batch variability. In addition, most polysaccharides including CeL, ALG, and CS typically show limited solubility in common organic solvents.[12]

Toward those promising applications and to circumvent the drawbacks, various strategies utilizing well-defined organic synthesis and polymerization methods

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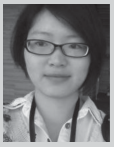
have been extensively explored, and the modified polysaccharides were used as effective building blocks to fabricate self-assembled micelles, crosslinked microgels/nanogels, three-dimensional hydrogels, and fibrous meshes. As examples, polysaccharides have been modified with small hydrophobic molecules or conjugated with hydrophobic polymers to render them amphiphilic for novel core/shell-type micellar aggregates. For the various forms of crosslinked materials (i.e., microgels, nanogels, and hydrogels), polysaccharides have been crosslinked through chemical reactions or physical associations.

In this review, we summarize the recent development of polysaccharide-based biomaterials with a focus on novel approaches for the modification of polysaccharides to synthesize self-assembled micelles, crosslinked microgels, nanogels, hydrogels, and fibrous materials for bio-related applications. This review focuses on polysaccharides with linear structures shown in Figure 1. Cyclodextrin (CD)-based supramolecular assemblies and hydrogels with the focus on recent advances are summarized in a recent review[13] and other reports.[14] In addition, polysaccharide-based hybrid materials containing inorganic metal nanoparticles[15–17] and carbon dots[18] are not covered in this review.



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2. Polysaccharide-Based Self-Assembled Aggregates

For the preparation of self-assembled micellar aggregates, the modification of polysaccharides (hydrophilic or water soluble) with hydrophobic species is required, yielding amphiphilic polysaccharides. The hydrophobic species for the modification are small molecules, oligomers, or high-molecular-weight polymers which bear reactive functional groups. The resulting amphiphilic polysaccharides self-assemble to form micellar aggregates in aqueous solution, consisting of hydrophobic cores surrounded with polysaccharide coronas. A variety of novel strategies have been explored to synthesize amphiphilic polysaccharide-based micellar aggregates.

2.1. Amphiphilic Polysaccharides Modified with Small Hydrophobic Molecules

Various small hydrophobic molecules have been conjugated to polysaccharides to yield amphiphilic polysaccharides. An example includes the conjugation of HA with hydrophobic aminoethyl 5β-cholanomide having a terminal amino group, derived from 5β-cholanic acid, through a carbodiimide coupling reaction. The resulting amphiphilic HA self-assembled to form micellar aggregates surrounded with HA coronas with a diameter = 350–400 nm as potential drug delivery nanocarriers.[19] Other examples include the synthesis of HA conjugated with cholesteryl group,[20] DeX conjugated with terpene (an extract from resin produced by conifer trees),[21] DeX tethered with doxorubicin (Dox; a clinically used anticancer drug),[22] and several others.[23,24] The Dox-conjugated DeX as a biopolymer-based prodrug exhibits improved tumor penetration. These examples of amphiphilic polysaccharides involve the conjugation of polysaccharides with hydrophobic molecules through strong covalent linkages.

In contrast, an introduction of stimuli-responsive cleavable linkages into the design of amphiphilic polysaccharides allows for the synthesis of amphiphilic nanocarriers that can be degraded in response to external triggers (degradable amphiphilic polysaccharides). Such stimuli-responsive cleavage of the labile linkages facilitates the controlled/enhanced release of encapsulated drugs. Examples include an acid-labile cholesteryl-modified PuL[25] and a reduction-responsive Dox-conjugated DeX.[26] Recently, amphiphilic PuL was synthesized by conjugation of pH-sensitive urocanic acid and hydrophobic cholesterol succinate to PuL (Figure 2). It self-assembled to form micellar aggregates with a diameter = 150–300 nm. Due to the presence of pendant urocanic acids, the amphiphilic PuL micelles exhibited pH-responsiveness with a swelling/deswelling transition at around pH 6.5. Such pH responsiveness enabled to enhance the intracellular release of encapsulated anticancer drugs, evidenced by cell viability, confocal laser scanning microscopy, and flow cytometry.[27]

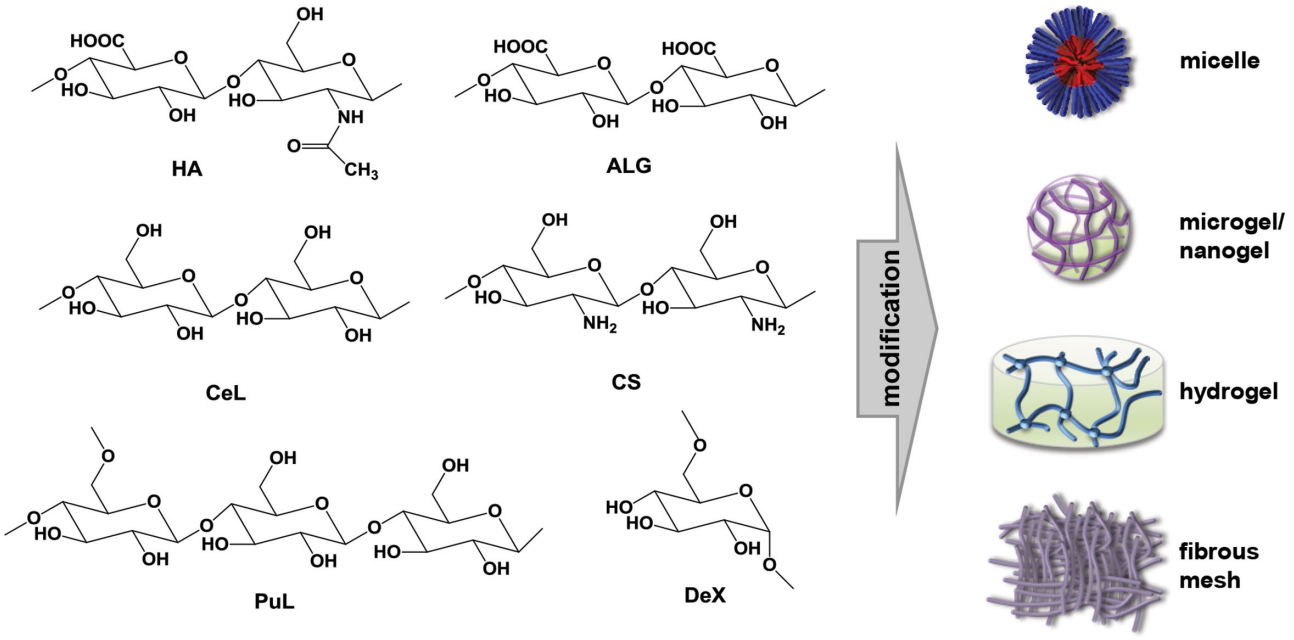

Figure 1. Chemical structures of typical polysaccharides and polysaccharide-based biomaterials, typically self-assembled micelles, crosslinked microgels/nanogels, three-dimensional hydrogels, and fibrous meshes for various biomedical applications such as drug delivery, cell-encapsulation, tissue engineering, and regenerative medicine. Abbreviation of polysaccharides: DeX: dextran, PuL: pullulan, CeL: cellulose, CS: chitosan, ALG: alginate, HA: hyaluronic acid, HPCeL: hydroxypropyl cellulose, ECeL: ethyl cellulose, CMCeL: carboxymethyl cellulose, CT: chitin, and ChS: chondroitin sulfate.

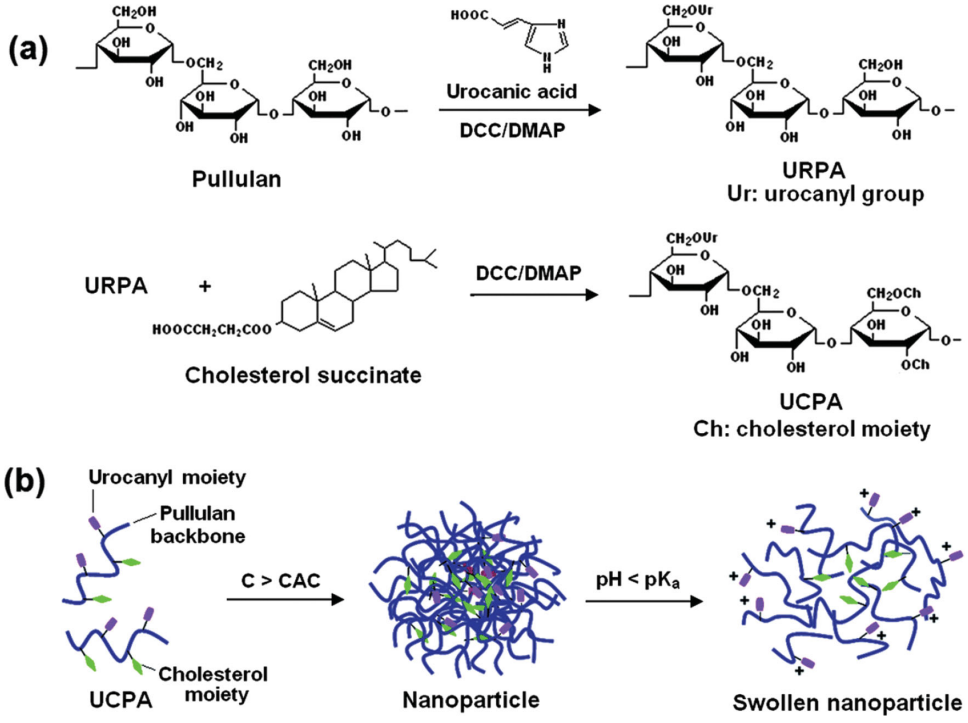

Figure 2. Synthesis, self-assembly, and pH-responsive release of amphiphilic PuL conjugated with urocanic acid and cholesterol succinate via a carbodiimide coupling reaction. Reproduced with permission.[27] Copyright 2014, Royal Society of Chemistry.

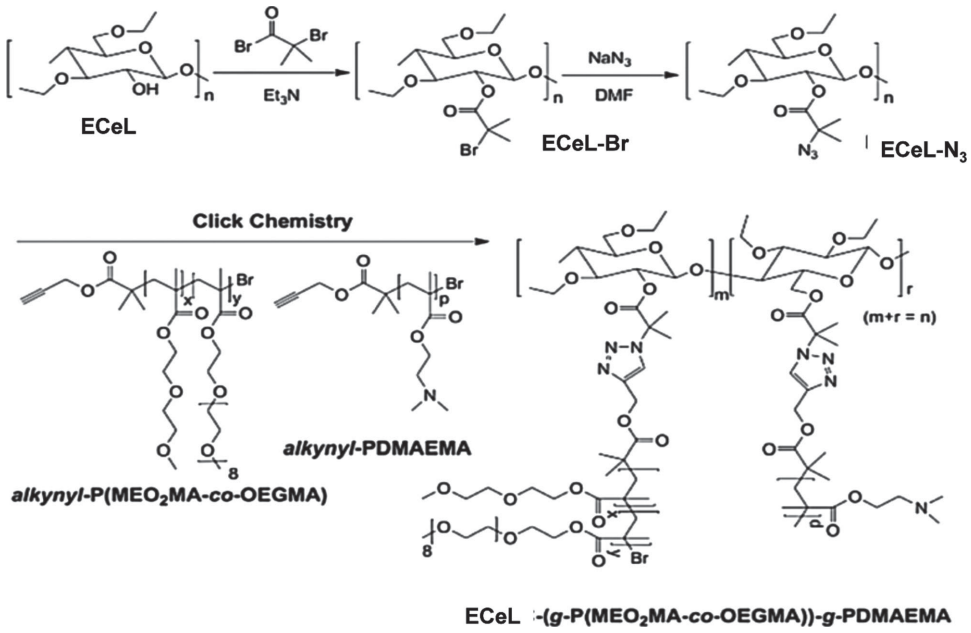

Figure 3. Synthesis of amphiphilic brush-like ECeL grafted with thermoresponsive (co)polymers. Reproduced with permission.[49] Copyright 2012, Elsevier.

2.2. Amphiphilic Polysaccharides Conjugated with Polymers

Numerous approaches have been explored to synthesize polymer-conjugated polysaccharides. One approach is the synthesis of diblock polysaccharides (i.e., polysaccharide-*b*-polymer) where one end of polysaccharides is directly attached to one end of synthetic polymers. This approach requires the modification of polysaccharides to have terminal reactive groups. For example, glycosaminoglycan was oxidized to have terminal aldehyde groups, which were involved in oxime click reaction to form glycosaminoglycan-*b*-poly(ethylene glycol) (PEG) block copolymers. These anionic copolymers enabled to form micelloplexes through ionic interactions with cationic poly(L-lysine) (PLL), a model protein; the resulting micelloplexes can be useful as delivery vehicles for positively charged proteins.[28] However, most approaches involve the synthesis of polymer-grafted polysaccharides though "grafting from" and "grafting to" methods.

The "grafting from" method employs novel polymerization methods such as ring-opening polymerization (ROP),[29,30] controlled radical polymerization (CRP),[31] or oxidative polymerization.[32] Well-defined synthetic polymers with narrow molecular weight distribution ($\bar{M}_w/\bar{M}_n < 1.4$) can be grown from polysaccharide chains, yielding polymer-grafted polysaccharides (polymer-*g*-polysaccharides). ROP is utilized to synthesize biodegradable aliphatic polyesters such as polylactide (PLA), polycaprolactone (PCL), polyglycolide (PGA), and their copolymers grafted from polysaccharides. Pendant hydroxyl (OH) groups in polysaccharides are generally used as initiating species for the ROP of the cyclic monomers. Typical examples include CeL-*g*-PCL,[33] DeX-*g*-PCL,[34] CeL-*g*-(PCL-*b*-PLA),[35] HPCeL-*g*-PCL.[36] CRP is utilized to synthesize poly(meth)acrylates grafted from polysaccharides. Typical CRP methods that have been explored include atom-transfer radical polymerization (ATRP)[37,38] and reversible addition–fragmentation chain transfer (RAFT)[39,40] polymerization. The pendant OH groups in polysaccharides are modified to convert the corresponding initiating moieties such as bromines for ATRP and dithiocarbonyl groups for RAFT polymerization. The detailed synthesis of various polysaccharide-g-polymethacrylates using CRP methods is described in a previous review.[41]

The "grafting to" method utilizes well-known organic reactions such as click-type or condensation reactions of polysaccharides with pre-synthesized polymers. The click-type reactions are highly selective and orthogonal, thus resulting in quantitative conversion under mild conditions.[42–44] A typical click-type reaction is 1,3-cycloaddition of alkynes and azides in the presence of Cu(I) complexes.[45,46] This reaction has been utilized after the modification of polysaccharides with pendant alkyne or azido groups. The modified polysaccharides then react with polymers in the presence of Cu(I) complexes, yielding brush-like, amphiphilic polysaccharides. Most reports describe the modification of polysaccharides including CD,[47] oligosaccharide,[48] ECeL,[49] and CS[50] with azido groups. The resulting azido-containing polysaccharides reacted with alkynyl-labeled PEG, PLA, PCL homopolymers, and their copolymers. As a typical example, Figure 3 illustrates an approach to synthesize amphiphilic ECeL grafted with thermoresponsive polymethacrylates.

ECeL was first modified with 2-bromoisobutyryl bromide to form 2-bromoisobutyryl ECeL (ECeL-Br) via a simple esterification, followed by its reaction with sodium azide (NaN_3), yielding ECeL-N_3. Meanwhile, well-defined alkynyl-poly(*N,N*-dimethylaminoethyl methacrylate (alkynyl-PDMAEMA) and alkynyl-poly(di(ethylene glycol) monomethyl either methacrylate (DEGMA or MEO_2MA)-*co*-oligo(ethylene oxide) monomethyl ether methacrylate (alkenyl-P(DEGMA-*co*-OEOMA)) were synthesized by ATRP in the presence of an alkenyl-labeled bromine ATRP initiator. These thermoresponsive (co)polymers were grafted to ECeL-N_3 via the click reaction. The resultant ECeL-grafted copolymers were double-hydrophilic, thermoresponsive; thus, they self-assembled at temperatures above lower critical solution temperature (LCST) to form aqueous aggregates.

Another click-type reaction is Michael addition reaction. Thiol-ene Michael addition[51–54] has been explored to synthesize a thermoresponsive HA–polymer conjugate, thus HA-*g*-P(DEGMA-*co*-OEOMA) (Figure 4).[55] A pendant maleimide-labeled HA was synthesized by reaction of HA with aminoethylmaleimide. A thiol (HS)-terminated P(DEGMA-*co*-OEOMA) was separately synthesized by RAFT polymerization, followed by aminolysis of terminal RAFT agent in the presence of a primary base. Two polymers reacted via a base-catalyzed Michael addition reaction. The resulting HA-*g*-P(DEGMA-*co*-OEOMA) exhibited tunable thermoresponsive properties with varying

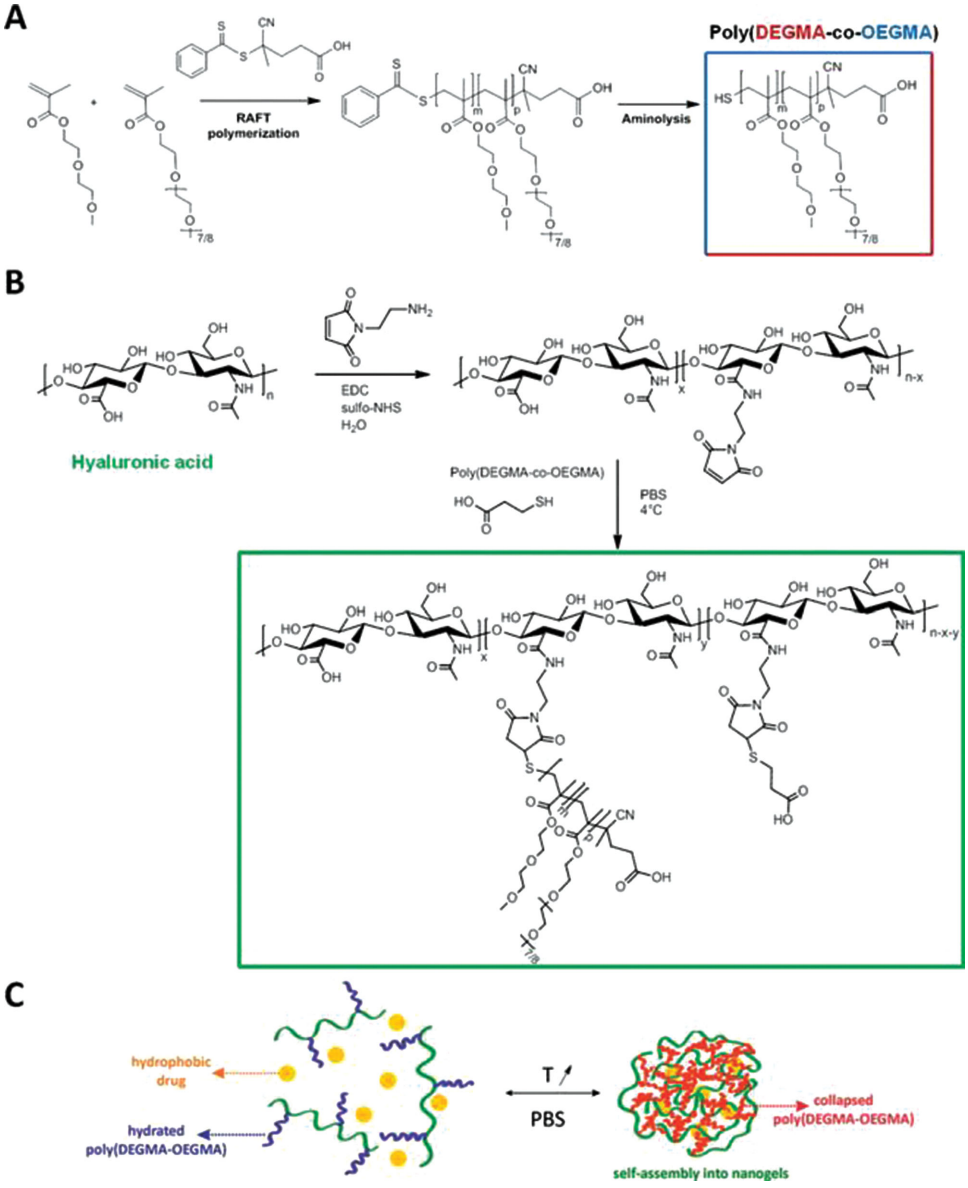

Figure 4. Synthesis of a thermoresponsive HA–polymer conjugate of HA-*g*-P(DEGMA-*co*-OEOMA) via thiol-ene Michael addition reaction and their self-assembly driven by change in temperature. Reproduced with permission.[55] Copyright 2012, Royal Society of Chemistry.

amounts of thermoresponsive P(DEGMA-*co*-OEOMA) block. Further, they self-assembled to form aggregates as a consequence of the hydrophobic transition of P(DEGMA-*co*-OEOMA) block at above LCST.

Facile coupling reactions have also been reported to synthesize amphiphilic polysaccharides. Typical examples include HA-*g*-PLGA in the presence of dicyclohexyl carbodiimide,[56] CD grafted with PEO-*b*-PCL in the presence of *N,N*-carbonyl diimidazole (CDI),[57] β-CD grafted with PCL-*b*-PEO-*b*-PCL triblock copolymer,[58] and HA grafted with a thermoresponsive polymer.[59] Recently, PCL was grafted to HA through a carbodiimide coupling reaction to form PCL–HA, which self-assembled to form anionic micelles in physiological conditions. The anionic HA coronas were ionically interacted with cationic CS to form CS/PCL-HA polyelectrolyte complex aggregates for oral drug delivery.[60]

3. Polysaccharide-Based Crosslinked Microgels/Nanogels

For targeted drug delivery applications in vivo, a challenge of physically aggregated micelles is to retain their colloidal stability upon dilution. After in vivo injection, drug-loaded micelles are significantly diluted by several orders of magnitude in the blood. As a consequence, the micelles are subjected to a local environment far below the critical micellar concentration (CMC). The dilution could result in dissociation of the micelles, leading to the premature of encapsulated cargos. Several strategies have been explored, including the design of block copolymers with a lower CMC[61] and brush-like graft copolymers.[62] A promising strategy is to introduce effective crosslinking chemistry into the synthesis of crosslinked nanogels/microgels based on polysaccharides. The microgels are a class of three-dimensionally crosslinked hydrogels confined in micrometer-sized particles; when the microgels are nanometer-sized, they are known to be nanogels.[7]

3.1. Chemical Crosslinking by Condensation

This method centers on the synthesis of reactive polysaccharides grafted (or conjugated) with small molecules or polymeric chains bearing reactive functional groups. These reactive groups are then involved in the crosslinking reactions to form polysaccharide-based microgels/nanogels in aqueous solutions. HPCeL was modified with poly(acrylic acid) (PAA) by multiple steps; 1) ROP of CL, 2) esterification of the resulting PCL-grafted HPCeL to the corresponding bromide, and 3) chain extension with poly(*t*-butyl methacrylate) (PtBMA) by ATRP, yielding HPCeL grafted with PCL-*b*-PtBMA block copolymer. The following hydrolytic cleavage of *t*-butoxy groups to the COOH groups yielded reactive HPCeL grafted with PCL-*b*-PAA block copolymers. The pendent COOH groups reacted with amino groups of external crosslinkers, allowing for the synthesis of nanogels crosslinked with amide linkages in water.[63] PuL was also modified with vitamin B6 (pyridoxal) by an alkyne–azido click-type reaction to yield pyridoxal phosphate-bearing PuL having aldehydes. The reactive PuL was crosslinked with a protein (lysozyme) containing several amino groups through a Schiff-base reaction with reactive aldehydes in aqueous solution.[64] However, these nanogels could have broad size distribution due to the occurrence of crosslinking in aqueous solution (not in compartmentalized locations). For the preparation of well-defined nanogels with narrow size distribution, the control of concentrations could be important to minimize of undesired inter-chain crosslinking reactions, which leads to the occurrence of large aggregation.

A promising approach to narrow size distribution utilizes self-assembly driven by either amphiphilicity or temperature-change, followed by chemical crosslinking reactions. For the approach, polysaccharides are first modified to be amphiphilic or thermoresponsive; the resulting amphiphilic polysaccharides bearing reactive functional groups self-assemble in aqueous solution to form reactive micellar aggregates. For example, PuL was conjugated with hydrophobic cholesterols and reactive groups. The resulting reactive amphiphilic PuL self-assembled to form nano-assemblies with a diameter = 18 nm in aqueous solution. The reactive acrylate groups were then involved in interparticle crosslinking with a thiol-terminated four-arm star PEG crosslinker through the thiol-ene Michael addition reaction, yielding raspberry-like nanogels with diameter ranging from 40 to 120 nm by varying the initial ratio of $[SH]_0/[acrylate]_0$ groups. The resulting nanogels exhibit the prolonged release profile of encapsulated proteins.[65] HA was also modified with pendant hydrophobic pyrene moieties and reactive hydrazine (—C(O)—NH—NH_2) groups. The reactive HA self-assembled to form aqueous micellar aggregates, and further stabilized by chemical crosslinking (through the formation of hydrazones) of HA chains to form HA-based nanogels.[66]

3.2. In Situ Disulfide-Crosslinking Method

Covalent crosslinking strategy provides enhanced colloidal stability against dilution. However, the use of permanent crosslinkers hampers enhanced/controlled release of encapsulated drugs. An introduction of stimuli-responsive degradation strategy of dynamic covalent bonds (cleavable linkages) in response to external stimuli enables the enhanced drug release.[67–69] In contrast to the addition of external crosslinkers bearing cleavable linkages, in situ disulfide-crosslinking method is more promising in that the method results in the formation of reduction-responsive

disulfide dynamic covalent bonds as crosslinks through two ways: disulfide-thiol exchange reaction and oxidation. The resulting disulfide-crosslinked nanogels exhibit enhanced colloidal stability as well as promoted drug release in response to reduction reactions.

For the disulfide-thiol exchange reaction, polysaccharides were modified with pendant disulfide linkages. For example, lipoic acid (LA) was conjugated to starch[70] and DeX[71] to form LA-starch and LA-DeX, which self-assembled to form micellar aggregates. The partial cleavage of disulfides in response to a catalytic amount of D,L-dithiothreitol (DTT), a reducing agent, resulted in the formation of disulfide-crosslinked nanogels. These nanogels having enhanced colloidal stability as well as reduction-responsive promoted drug release have a potential for tumor-targeted chemotherapy.

For oxidation of pendant thiol groups of copolymers, different strategies have been explored to introduce pendant thiol groups into polysaccharides. A RAFT polymerization and following aminolysis has been examined. The RAFT polymerization allowed for the chain extension of PuL[72] and DeX[73] with poly(*N*-isopropylacrylamide) (PNIPAM), yielding PNIPAM-grafted polysaccharides. The following aminolysis of terminal RAFT agents in the presence of a primary amine resulted in the synthesis of pendant HS-terminated PNIPAM-grafted polysaccharides. At a temperature above the LCST, they self-assembled upon temperature change to form pendant thiol-functionalized micelles, and further to temperature responsive disulfide-crosslinked nanogels upon oxidation. A facile coupling reaction was also examined for the reaction of cysteamine with HPCeL activated with 4-nitrophenyl chloroformate (4-NC) (Figure 5). The resulting thiolated HPCeL (HPCeL-SH) was collapsed to form nanospheres upon the LCST transition. The pendant SH groups in the nanostructures were then oxidized by DMSO, yielding disulfide-crosslinked nanogels with diameter = 72–88 nm.[74] These disulfide-crosslinked nanogels exhibited reduction-responsive degradation in the presence of excess reducing agents.

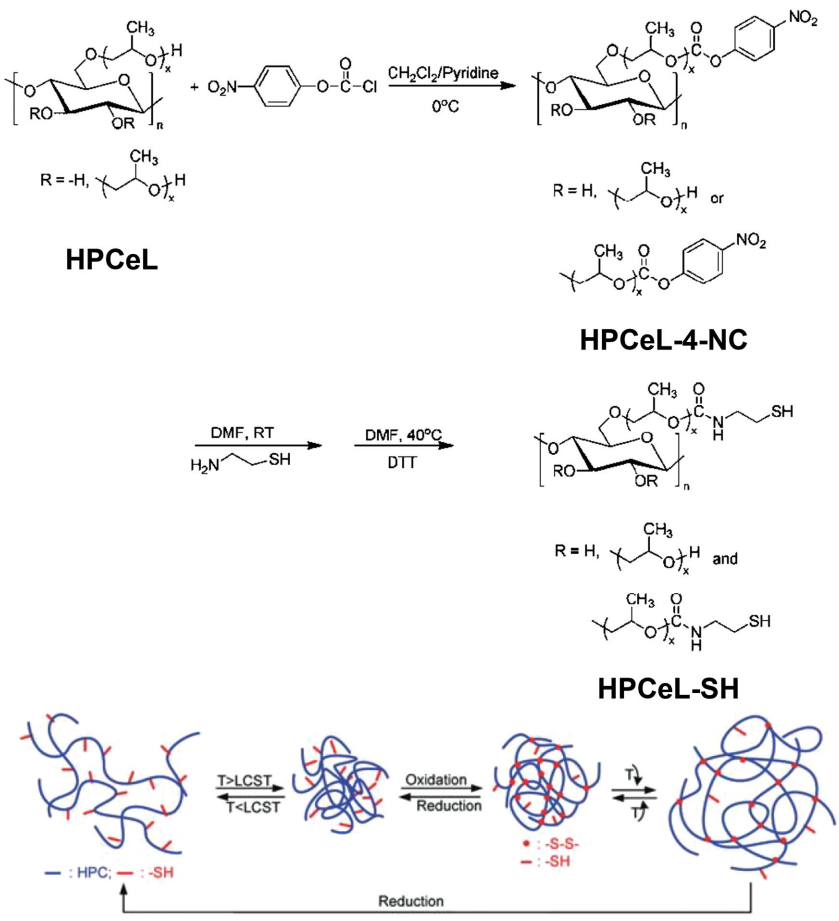

Figure 5. Synthesis and self-assembly above the LCST, and oxidation of thiolated HPCeL to form disulfide-crosslinked nanogels. Adapted with permission.[74] Copyright 2011, Royal Society of Chemistry.

3.3. Aqueous Free Radical Crosslinking Polymerization

Aqueous free radical crosslinking polymerization (FRCP) has been explored to synthesize polysaccharide-based nanogels crosslinked with vinyl polymers (including polymethacrylates). An approach includes the functionalization of polysaccharides with methacrylate moieties. The resulting methacrylated polysaccharides are used as multifunctional crosslinkers for FRCP.[75] Oil-in-water inverse miniemulsion polymerization has been widely explored to synthesize crosslinked nanogels with narrow size distribution due to the occurrence of polymerization in compartmented locations (i.e., inverse miniemulsion).[76] Recently, this approach has been advanced to synthesize enzymatically degradable nanogels by inverse miniemulsion polymerization of acrylamide (AAm) with a methacrylated-modified DeX crosslinker.[77] Further, the interesting technique has been explored for the synthesis of dual enzymatic and light-degradable nanogels (Figure 6).[78]

Another approach utilizes aqueous FRCP of vinyl monomers in the presence of polysaccharides, resulting in the formation of vinyl polymer-grafted polysaccharides. By varying the vinyl monomers, the properties of the grafted

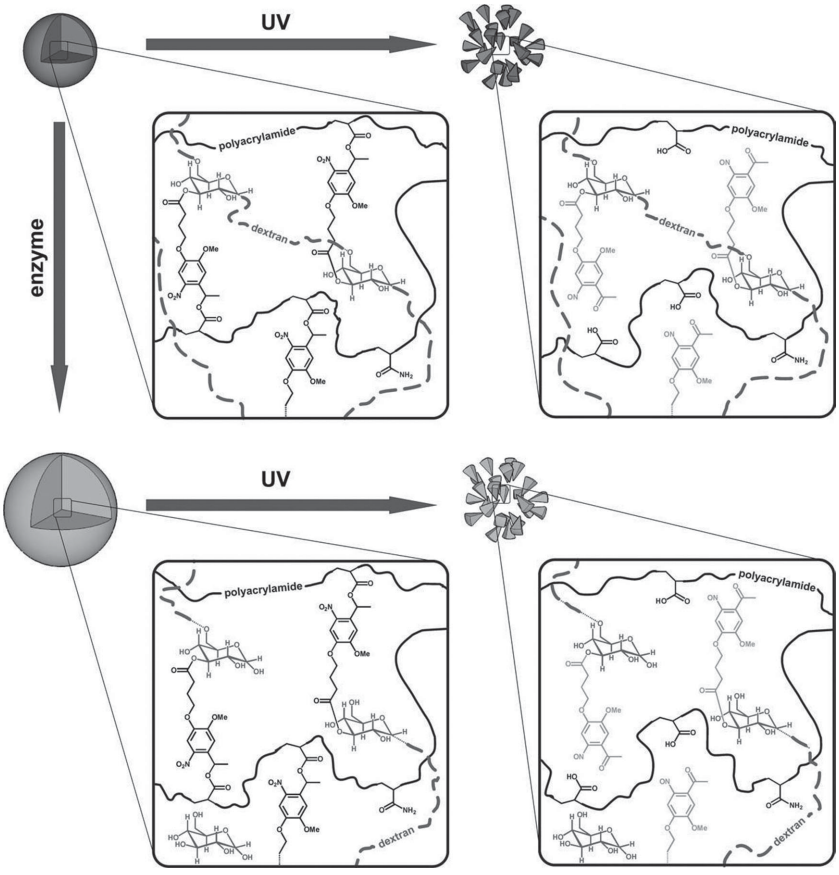

Figure 6. Schematic representation of dual-enzymatic and light-degradable nanogels crosslinked with photo-labile linkage of DeX-*g*-PAAm. Reproduced with permission.[78]

polysaccharide can be tuned. Typical examples of grafted vinyl polymers include PAA for pH response[79] as well as PNIPAM and PAAm for temperature response.[80,81] In the presence of difunctional crosslinkers, this approach allows for the synthesis of crosslinked nanogels such as PNIPAM-grafted CS nanogels.[82–84] Oridon known to be an anticancer agent against liver cancers was loaded into pH-responsive nanogels decorated with galactose ligands. These nanogels show the potential for hepatoma-targeted delivery.[82] Recently, glucose-responsive nanogels based on poly(acrylamidophenylboronic acid) (PAAPBA) were synthesized by a self-assembly-assisted strategy. This method utilizes thermoresponsive properties of PAAPBA; during polymerization at temperatures above LCST, PAAPBA-grafted DeX in the presence of difunctional crosslinker self-assembled to form well-defined nanogels. Since boronic acid moieties incorporated in the nanogels recognize glucose, the nanogels can be useful as potential glucose sensor.[85] More recently, new dual-stimuli reduction and acidic pH-responsive nanogels were developed by a facile aqueous FRCP of OEOMA in the presence of CMCeL and a disulfide-labeled dimethacrylate crosslinker (ssDMA). As show in Figure 7, the nanogels are crosslinked with reductive-responsive disulfide linkages of POEOMA-grafted CMCeL, exhibiting enhanced release of encapsulated anticancer drugs to dual responses: reductive cleavage of disulfide crosslinkers and acidic pH responsive of COOH groups in CMCeL. The intracellular release of anticancer drugs after internalization into HeLa cancer cells, combined with the ability to facile bioconjugation suggest as a promising intracellular nanocarrier platform exhibiting multi-controlled drug release.[86]

3.4. Physical Crosslinking Method

In contrast to chemical crosslinking that allows for the formation of covalently crosslinked nanogels, physical crosslinking yields suprarmolecular nanogels by utilizing non-covalent interactions between polysaccharides and external crosslinkers. Of these interactions including stereocomplexation of PLA-based DeX[87] and host–guest molecular recognition of β-CD with DeX,[88,89] the ionic interaction has been widely explored to synthesize polysaccharide-based nanogels. Without further modification, CS possessing pendant amino groups interacted with anionic crosslinkers such as tripolyphosphate (TPP)/ALG (containing pendant COOH groups) for ionic gelation. The resulting CS/TPP/ALG nanogels were evaluated for insulin release[90] or cell response;[91] however, they had relatively large diameters ($d > 250$ nm) due to the formation of more expanded structures. Such a large size could have a short blood circulation due to uptake by RES. Smaller sized CS-based nanogels were synthesized by ionic complexation of CS with PEO-*b*-poly(sodium 2-(acrylamindo)-2-methylpropanesulfonate), followed by an addition of genipin. Genipin is an irridoid glucoside extracted from Gardenia, and reacts with primary amine groups. The resulting nanogels had a diameter ≈50 nm in the swollen state as determined by dynamic light scattering (DLS) and ≈20 nm in the dry state measured by transmission electron microscopy (TEM).[92]

In addition, further modification of polysaccharides is required for ionic gelation. DeX was modified with PEG and cystamine to yield PEO-DeX-ss-NH_2. The protonated amino groups interacted with negatively charged indocyanine green (ICG), a tricarbocyamine dye, to form ICG/DeX nanogels. Since ICG is an FDA-approved near infrared (NIR) fluorescent dye to be used in clinics, the resulting

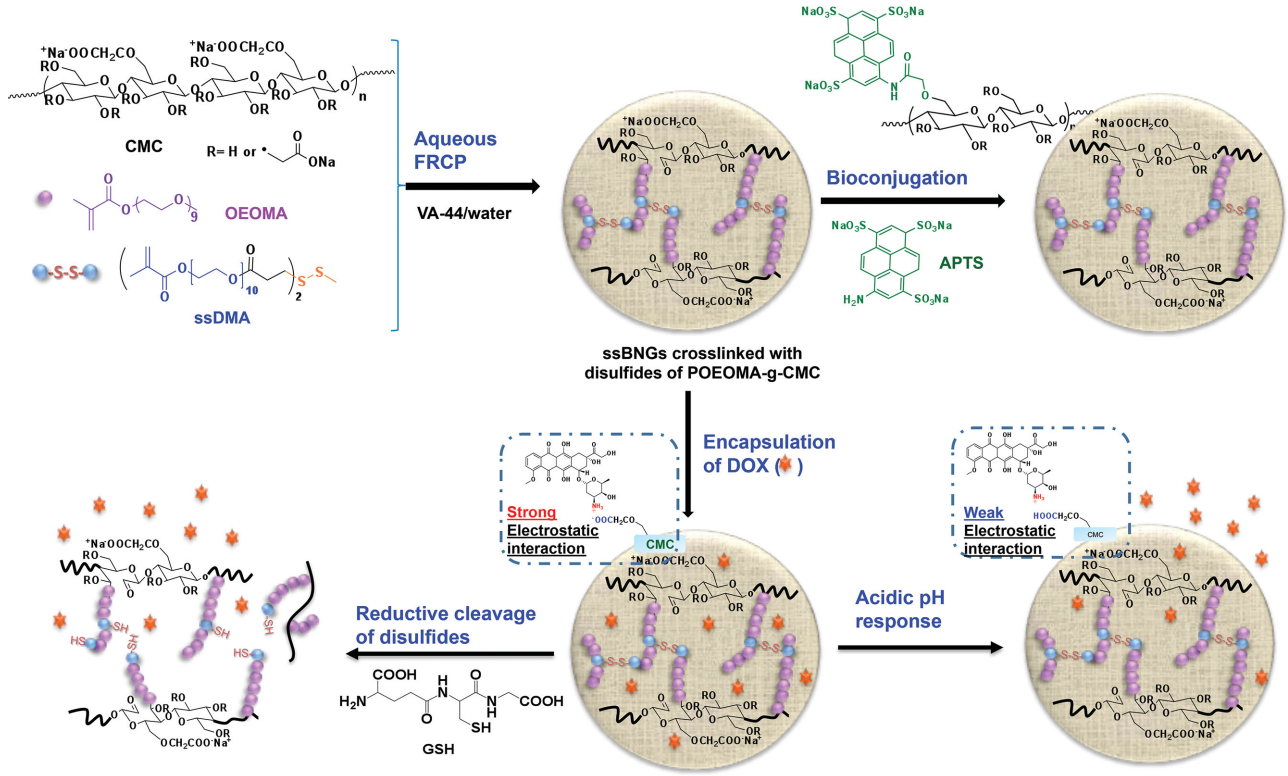

Figure 7. Synthesis and dual reduction and acidic pH-responsive Dox release of nanogels crosslinked with disulfide linkages of POEOMA-grafted CMCeL. Reproduced with permission.[86] Copyright 2014, Royal Society of Chemistry.

nanogels are useful for NIR imaging and photothermal therapy.[93] In another report, wood CeL was modified with carboxymethyl groups, yielding negatively charged CMCeL. Wood CeL was also quarternized with ammonium salts, yielding positively charged CeL (QCeL). Mixing of the CeL-based polysaccharides in aqueous solutions resulted in the formation of ionically crosslinked CeL-based nanogels for protein delivery. Their sizes could be modulated by adjusting either CMCeL and QCeL in the feed, ranging from 150 to 800 nm in diameter.[94]

4. Polysaccharided-Based Hydrogels

4.1. Physical Crosslinking Method

Similar to crosslinked nanogels, several approaches to synthesize physically crosslinked supramolecular hydrogels have been explored. Typical approaches include ionic interactions, host–guest inclusion complexation, and thermoresponsive sol–gel transition.

Ionic interaction includes the interaction of ionic polysaccharides with a broad selection of ionic crosslinkers.[95] Examples include ALG bearing COOH groups with Ca^{2+} and Fe^{3+} ionic species[96–99] and HA bearing COOH groups with gelatin,[100] yielding hydrogels. In contrast to homogeneous hydrogels, a dual-structure hydrogel was also reported. The amino groups of CS were modified, and the formed amphiphilic CS self-assembled to form CS-based nanostructures dispersed in aqueous ALG solution. By adding Ca^{2+} crosslinkers through ionic interactions, the resulting mixtures turned to hydrogels composed of CS-based nanostructures embedded in ALG-hydrogels. The dual-structure gels enabled to encapsulate hydrophobic drug in self-assembled nanostructured cores. Additionally, the hydrogels exhibit self-healing properties in the presence of glycerol.[101] Other examples include HA with cationic PLL[102] and CS with anionic azopolymers,[103] yielding multilayer films.

Host–guest inclusion complexation utilizes CD with a hydrophobic cavity, which is capable of inclusion with guest molecules, typically PEG derivatives. Examples include pyrene-terminated PEG star polymers,[104] cholesterol-derived linear PEG,[105] PEG-PPG-PEG triblock copolymer,[106] and PEG-grafted disulfide-containing poly(amino amine).[107] These CD/polymer inclusion complexes could be useful as injectable smart biomaterials for controlled drug delivery applications.

Temperature-induced sol–gel transition involves the design of thermoresponsive polymers that undergo a volume change due to hydrophobic/hydrophilic transition in respond to temperature change. This property

enables sol–gel transition of the polymers at higher concentrations, resulting in the formation of in situ-formed hydrogels. This approach involves the modification of polysaccharides with thermoresponsive polymers. For example, CS was modified with PEG-based block copolymers of polyalanine, yielding CS-*g*-(PA-*b*-PEG),[108] and poly(L-alanine-*co*-L-phenyl alanine), yielding CS-*g*-(PAF-*b*-PEG).[109] HA was also grafted with PNIPAM by RAFT polymerization, yielding HA-*g*-PNIPAM.[110] Recently, HA was conjugated with dopamine (Figure 8); the resulting HA-dopamine reacted with HS-terminated Pluronic F127 block copolymer to prepare lightly crosslinked HA/Pluronic gel structure based on a click-type catechol-thiol addition. The resulting hydrogels exhibit thermoresponsive sol–gel transition at temperature above 37 °C.[111]

4.2. Chemical Crosslinking Method

Compared to physical crosslinking exhibiting poor ability to tune the mechanical properties, chemical crosslinking possesses several advantages, including facile control of moduli, swelling ratio, and porosity of hydrogels by varying crosslinkers and crosslinking densities.

FRCP has been explored as a robust method to fabricate polysaccharide-based hydrogels. Similar to the preparation of microgels/nanogels of polysaccharides, this approach involves the modification of polysaccharides with vinyl or (meth)acrylate moieties to polymerizable polysaccharides. Typical examples include methacrylated ChS,[112] methacrylated HA,[113] and methacrylated CT,[114] as well as xanthan gum functionalized with maleic anhydride[115] and acetylated galactoglucomannan functionalized with alkenes.[116] Different from the microgels/nanogels, however, these polymerizable polysaccharides are polymerized in aqueous solutions, mostly photopolymerized upon UV irradiation, to form highly crosslinked hydrogels for tissue engineering.

Facile coupling reactions or condensation reactions have also been explored to fabricate polysaccharide-based hydrogels in mild conditions. For the click-type thiol-ene reaction, polysaccharides are modified with either SH or vinyl groups. As illustrated in Figure 9, HA was modified with cysteamine to form SH-modified HA. In addition, hyperbranched thermoresponsive copolymers labeled with acrylate groups were synthesized by ATRP of thermoresponsive OEOMA in the presence of a difunctional methacrylate (DMA). These copolymers reacted with SH-HA through Michael addition reaction, yielding thiol-ene crosslinked hydrogels in aqueous solution.[117,118] Other examples include thiol-ene crosslinking reactions of maleimide modified DeX with thiol-modified β-CD[119] and methacrylate-modified HA with DTT.[120] In addition

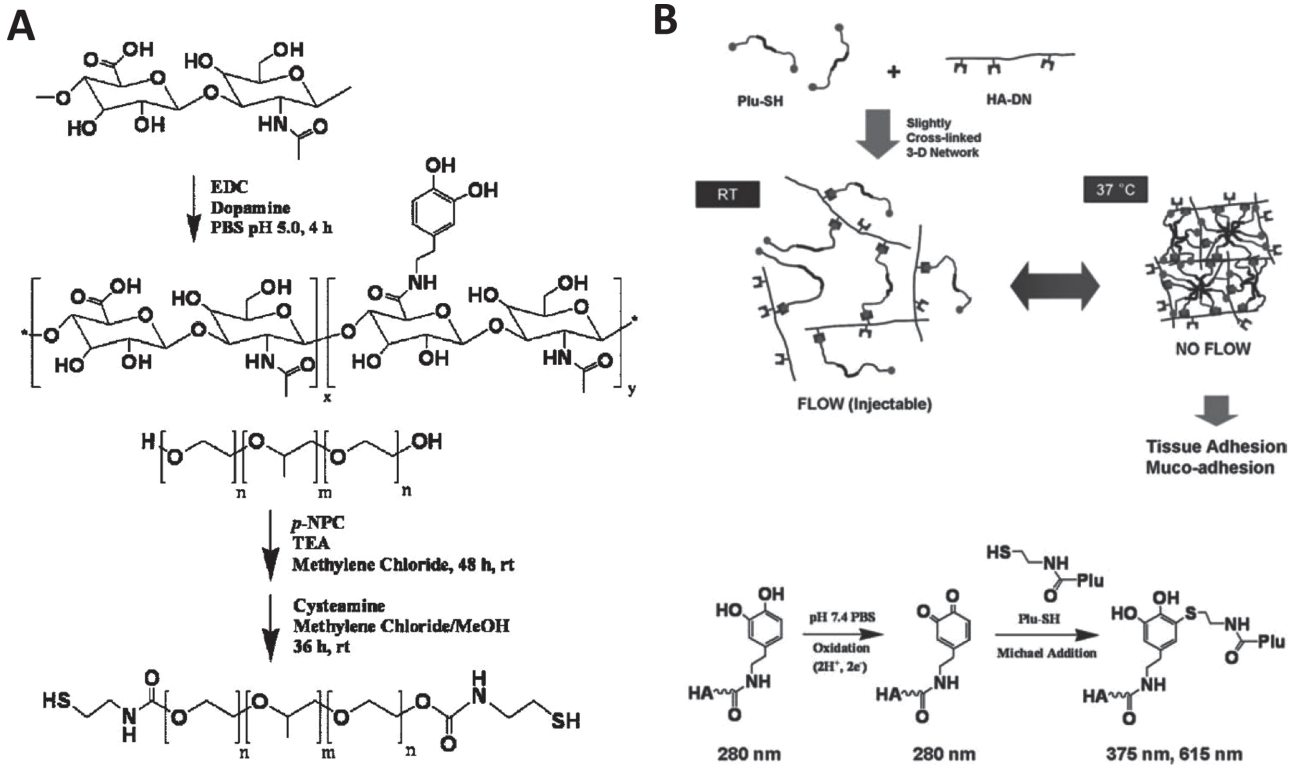

Figure 8. Synthesis (A) and sol–gel transition (B) of HA-dopamine and SH-terminated Pluronic F127. Reproduced with permission.[111] Copyright 2010, Royal Society of Chemistry.

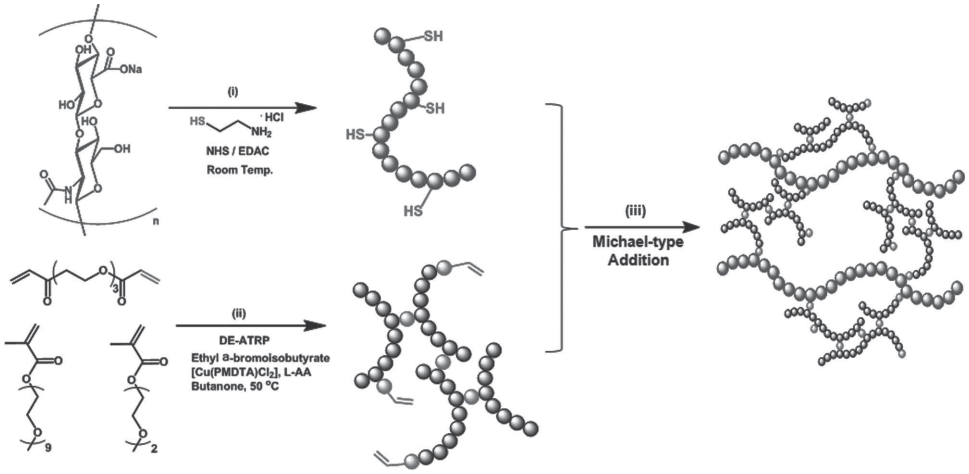

Figure 9. Synthetic scheme for Michael addition reaction of SH-modified HA with hyperbranched thermoresponsive copolymers labeled with acrylate groups to yield thiol-ene crosslinked hydrogels in aqueous solution. Reproduced with permission.[117]

to thiol-ene reaction, copper-free alkyne–azido reaction,[121] Schiff base reaction,[122–124] and Diels–Alder reaction[125] have been explored.

Oxidative crosslinking of dopamine-conjugated polysaccharides provides an alternative route to synthesis of crosslinked hydrogels. An example includes the preparation of calcium-free ALG hydrogels in the presence of $NaIO_4$, an oxidizing agent for catechol-conjugated ALG.[126] Another example is rutin-releasing CS-based hydrogels composed of rutin-conjugated CS-PEG-tyramine crosslinked by enzymatically-catalyzed oxidation of phenol groups.[127]

5. Polysacchride-Based Fibrous Materials

Electrospinning is a powerful method to fabricate polymer substrates into fibrous materials with nanoscale diameters. These fibers possess high specific surface areas, controlled compositions, and high porosities; thus they have found their application in various biomedical fields.[128,129] Due to the unique features, electrospun polysaccharide-based fibers enable the encapsulation of diverse therapeutic cargo. Thus, the resulting drug-loaded nanofibers have been considered as effective drug delivery carriers. Examples include cellulose acetate phthalate fibers for semen-induced anti-HIV (human immunodeficiency virus) vaginal drug delivery[130] as well as a sodium ALG/PEG blend fibers containing ibuprofen for pulsatile drug release[131] and ammonium ALG fibers carrying antibiotics and enzymes.[132] Furthermore, the surfaces of electrospun polysaccharide nanofibers have been immobilized with proteins[133] or functionalized with lysostaphin[134] for wound healing applications. A challenge to electrospinning of most polysaccharides is associated with their poor solubility in organic solvents. The use of room temperature ionic liquids offers a solution to overcome these difficulties.[135,136]

6. Summary and Outlook

The recent advances in the development of polysaccharide-based biomaterials for bio-related applications are summarized. Polysaccharides possess a high content of hydroxyl, amino, and carboxylic acid groups. These functional groups have been used for modification of polysaccharides, and the resulting modified polysaccharides are extensively explored as effective building blocks to fabricate self-assembled micelles, crosslinked microgels, nanogels, hydrogels, and fibrous materials. Self-assembled micelles were prepared by both "grafting to" and "grafting from" methods. To form well-defined micellar aggregates, the hydrophobic/hydrophilic balance of modified polysaccharides is a key criteria. It can be achieved by tuning the substitution degree of hydrophobic moieties. For the "grafting to" method, click-type reactions such as 1,3-cycloadditon of alkynes and azides and thiol-ene addition have been utilized to modify polysaccharides with small hydrophobic molecules and polymers. For the "grafting from" method, well-known polymer synthesis methods such as ROP and CRP have been utilized. Crosslinked nanomaterials (microgels, nanogels, and hydrogels) were fabricated by both chemical and physical crosslinking reactions. Chemical crosslinking methods include further click-type, facile coupling reactions, and in situ disulfide crosslinking methods as well as FRCP, while physical crosslinking methods include ionic interactions, host–guest inclusion complexation, and thermoresponsive sol–gel transition.

Future development of polysaccharide-based biomaterials, particularly for tumor-targeting intracellular drug delivery, requires a high degree of control over properties.

One property is the controlled/enhanced release of encapsulated therapeutics. Although polysaccharides are biodegraded by enzymatic reactions, the enzymatic degradation is intrinsically slow. Such a slow degradation causes a slow release of encapsulated therapeutics from polysaccharide-based nanocarriers and scaffolds in cellular environments. A promising solution to circumvent the challenge is the multiple stimuli-responsive degradation platform. Multi-stimuli responses to each stimulus can independently and precisely regulate encapsulated drug release. Another property is the narrow size distribution. Although the inverse (mini)emulsion technique has been explored to synthesize polysaccharide-based nanogels with relatively narrow size distribution, harsh conditions are required for the complete removal of residual oil-soluble surfactants remaining in the products. Temperature-driven self-assembly/crosslinking is a promising method that could be further explored for nanogels with narrow size distribution. Combined with these properties, the applicability of these biomaterials in response to cellular components toward tumor-targeting delivery applications in vivo is an exploratory research area.

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