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# Recent advances in polysaccharide-based *in situ* forming hydrogels

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**Abstract**

Polysaccharides comprise an important class of natural polymers; they are abundant, diverse, polyfunctional, typically benign, and are biodegradable. Using polysaccharides to design *in situ* forming hydrogels is an attractive and important field of study since many polysaccharide-based hydrogels exhibit desirable characteristics including self-healing, responsiveness to environmental stimuli, and injectability. These characteristics are particularly useful for biomedical applications. This review will discuss recent discoveries in polysaccharide-based *in situ* forming hydrogels, including network architecture designs, curing mechanisms, physical and chemical properties, and potential applications.

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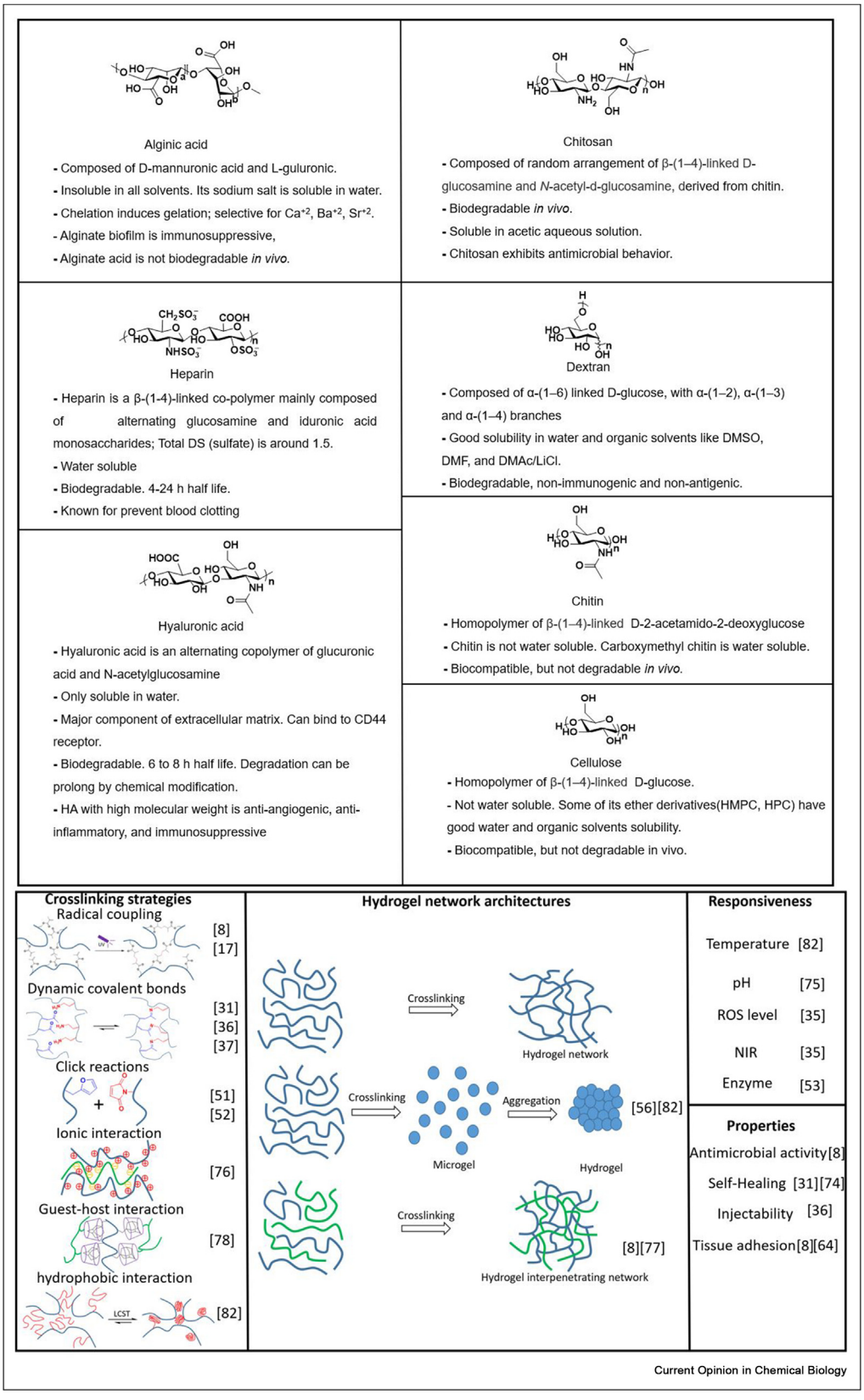
**Keywords**

Polysaccharides, Hydrogels, Self-healing, Biodegradable, In situ forming, Injectable, Benign.

## Introduction

Hydrogels are networks formed by crosslinking hydrophilic polymers and are therefore capable of absorbing great amounts of water [1]. In the 1960s, hydrogels were introduced in contact lenses, where their soft and highly aqueous nature has proved quite effective for protecting sensitive eye tissue. Since then, application of hydrogels has increased tremendously in a broad range of fields, especially for biomedical [2] and pharmaceutical [3] applications. Natural polymers like polysaccharides (Figure 1) are important building blocks for hydrogel preparation due to their hydrophilicity, generally low toxicity, and ability to be modified in versatile fashion. They are biocompatible and biodegradable in many situations and toward many tissues [4]. In recent decades, research has shifted from implantable to injectable hydrogels that enable gel formation at the desired injection site [5]. *In situ*-forming hydrogels are particularly attractive for this purpose as they are usually injectable and can be introduced to the targeted location by minimally invasive procedures. *In-situ* is a Latin expression which means 'on-site' or 'in-position'. *In situ*-forming hydrogels form spontaneously or with external stimuli at the application site, which is of particular value for in-body hydrogel applications. This class of hydrogel can be prepared by several strategies, including ultraviolet (UV)-induced crosslinking, creating non-reversible covalent linkages, and using reversible physical or chemical interactions to create the polymer network (Figure 1). Due to these inherent benefits and their versatile potential for chemical modifications, researchers have explored *in situ*-forming polysaccharide hydrogels and their application in fields including tissue engineering [6], controlled drug release [7], and wound healing [8]. This review will evaluate recent discoveries in the field of polysaccharide-based *in situ*-forming hydrogels, including preparation strategy, functionality, and potential applications. It should be noted that not every polysaccharide is alike, and not every polysaccharide is best-suited for every application. A few guidelines may be useful. Cellulose, for example, is attractive in many ways as a basis for derivatives that can gel; it is inexpensive, vastly abundant, and quite edible (dietary in fact). It is well-suited for hydrogels to be used for personal care, agriculture, indeed most application areas except for inside the body medical applications. A principle for materials in medicine is 'what goes in must come out'; in other words, there must be a way for the material eventually to be cleared from the body. As humans do not have cellulase enzymes inside their bodies, cellulose is generally not a good choice for inside the body hydrogels. On the other hand, polysaccharides like dextran [9], pullulan [10], curdlan [11], chitosan [12], and amylose [13] are readily cleared from the body and generally have low toxicity, so may be good choices for building inside the body hydrogels (as well as those to serve in other applications). Another general consideration for applications of hydrogels in medicine, especially

**Figure 1**




Chemical structures and properties of polysaccharides and their hydrogel crosslinking strategies, network architectures, properties, and responsiveness.

for inside the body, is whether the polysaccharide can be expected to elicit an immune response. Most polysaccharides do not elicit strong immune responses. Even specific sequences from pathogenic exopolysaccharides, often used in vaccine development, do not elicit strong responses and are typically used in conjunction with adjuvants. The polysaccharides listed above, including cellulose, typically do not elicit strong immune responses and are not of concern in that respect. Linear dextran, for example, is routinely, successfully administered to patients intravenously, for uses including the maintenance of osmotic balance, delivery of iron, or emergent treatment of severe plasma loss [14]. Chitosan is a special case, not because it typically elicits immune responses itself, but because it is purified from shells of marine crustaceans that also contain a large amount of shellfish protein [15]. It is imperative to use highly pure, medical grade chitosan for inside the body applications since contaminating shellfish proteins can indeed cause severe immune response.

## Chemically crosslinked *in situ*-forming hydrogels

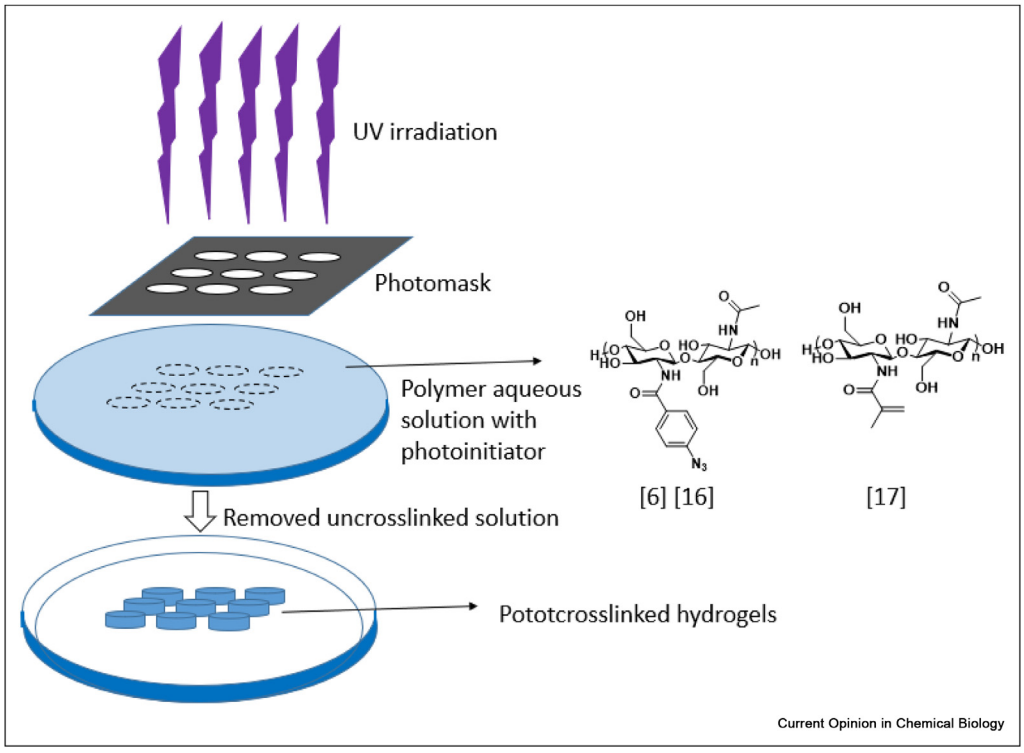
### Photo-induced radical coupling

*In situ* photo-polymerization and photo-crosslinking methods have been investigated to prepare hydrogels for biomedical and other applications. Such hydrogels have been used in various microfabrication methods including photolithography, micro-molding, and bioprinting. For example, attachment of the UV-responsive 4-azidobenzoic acid to chitosan via an amide linkage (Figure 2) affords a cell-seeding hydrogel that has been used in UV lithography, as first reported by Fukuda et al. [6]. The material was used to fabricate a cell-seeded (human hepatoblastoma cell) hydrogel microstructure, creating a promising platform for implantable bioartificial organs. This UV-crosslinkable azido chitosan was also reported as a scaffold for bone tissue engineering [16]. In recent years, efforts have been made to achieve biodegradable hydrogels with specific properties, such as rapid curing or excellent mechanical performance. Zhou [17] reported a water soluble, photo-crosslinkable, injectable chitosan derivative for cell-laden microgels. Chitosan was chemoselectively *N*-acylated using methacrylic anhydride, and the resulting unsaturated amides were readily cured within 60s *in vivo* to form a microgel. Despite the curing reaction, the hydrogel remained injectable. Improving the physical properties of UV-curable hydrogels is challenging as this usually impairs biodegradability. In an attempt to address this issue, Lee reported using photo-curable, methacrylated chitosan, and catechol-modified chitosan to prepare a double network hydrogel, crosslinked by UV-induced C-C bond formation as well as by catechol-$Fe^{3+}$ chelation [8]. This interpenetrating network reinforced hydrogel was reported to display high ductility, biodegradability, and antimicrobial activity, all useful for healing bacteria-infected wounds. Two-photon polymerization is the most precise lithography technique, affording complex 3D microstructures with high resolution. Chichkov reported using this advanced, costly method to react methacrylate-modified hyaluronic acid (HA) with polyethylene glycol (PEG) diacrylate to form 3D hydrogel geometries like girds and rings, reproducibly, with dimensions variable from 10 to 2000 μm. Such scaffolds are particularly useful for tissue engineering, often showing desired biocompatibility in particular tissues and circumstances [18]. Overall, though photo-curable hydrogels have been developed, limitations should be noted. Importantly, photosensitizers/photo-initiators used in photo-curing may be cytotoxic [19].

### Schiff-base and other reactions to form dynamic covalent bonds

Dynamic covalent bonds define an important class of *in situ* forming hydrogels. Among these dynamic reactions, imine formation is most frequently used in hydrogel design. Imine formation is reversible in the aqueous hydrogel environment, occurring by reaction between an amine and an aldehyde or ketone, requiring no catalyst [20]. This dynamic nature favors self-healing [21] and injectability, thus imine-crosslinked hydrogels have shown potential in applications like sequential drug delivery [7] and tissue engineering. Bloodgood [22] reported the preparation of imine-based hydrogels from polysaccharides utilizing sodium periodate to oxidize the vicinal hydroxyl groups on C2 and C3, thereby creating ring-opened dialdehydes (Figure 3**a**). Any aldehyde groups within the hydrogel not involved in crosslinking imine bonds can be used for conjugating active small molecules that possess appropriate chemical moieties. HA [23], dextran [19], chondroitin sulfate [24], cellulose sulfates [25], cellulose nanocrystals (CNCs) [26], pectin [26,27], starch [28], and chitosan [27] have been oxidized to prepare hydrogels via this general and simple approach. Limitations include the fact that periodate oxidation of monosaccharide vicinal diols opens the ring, compromising the rigidity of the native polysaccharide backbone, reducing degree of polymerization and strength properties, and decreasing polysaccharide stability. Functionalization of the polysaccharide with ketones is an alternative route for preparing Schiff base-crosslinked polysaccharide hydrogels. Liu et al. [29] reported the preparation of a hydrogel using cellulose acetoacetate. The acetoacetate ketone groups can react with chitosan amines, thereby forming imine-crosslinked hydrogels. Recently, Nichols et al. [30] and Chen et al. [31] reported a versatile method that can afford ketone-substituted polysaccharides. In this strategy (Figure 3**b**), the terminal 2-hydroxypropyl groups of oligo(hydroxypropyl) polysaccharides are chemoselectively oxidized to ketones using household bleach (NaOCl) as oxidant. The oxidized hydroxypropyl polysaccharides readily react with amine containing polymers like chitosan and Jeffamines [32] to form imine-linked hydrogels. The

**Figure 2**




UV curable chitosan-based hydrogels. UV, ultraviolet.

resulting hydrogels were shown to display attractive properties, including self-healing, injectability, pH-responsiveness, thermal responsiveness, and tunable modulus. Due to the inherent multifunctionality of polysaccharides, post-modification can be applied to polysaccharide blocks, affording antimicrobial [33] and UV-curing reinforced [34] hydrogels. Xu et al. [35] reported a hydrogel that was crosslinked by Schiff base formation, triggered by near-infrared (NIR) light. An HA derivative bearing hydrazide and photosensitizer was prepared and crosslinked by reaction with a reactive oxygen species (ROS)-cleavable dialdehyde. When the hydrogel was further exposed to NIR light, the increasing ROS level caused cleavage of the S-S linkage, resulting in hydrogel degradation and achieving remote-triggered drug release.

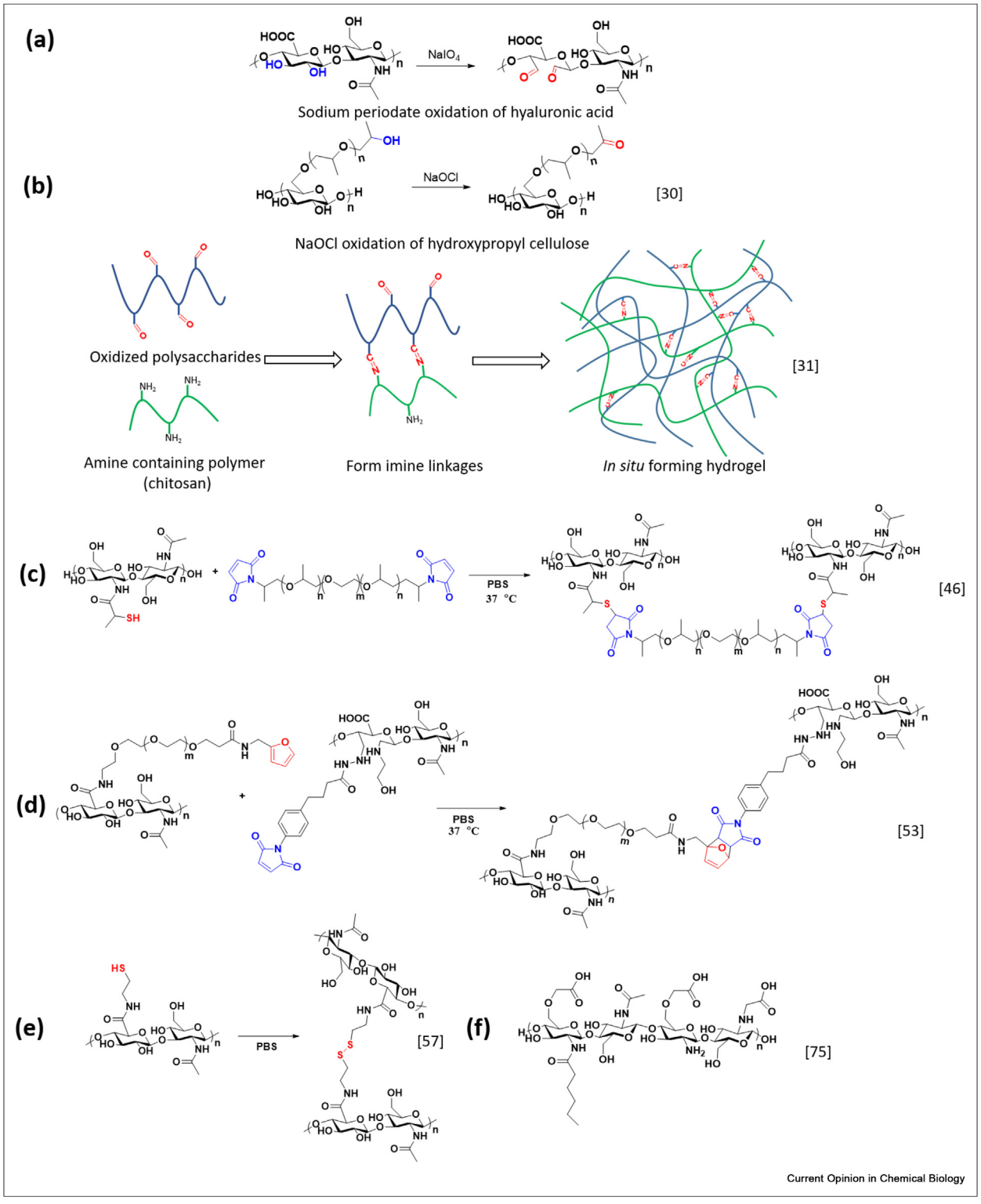
Dynamic covalent bonds other than imines have also been used to prepare *in situ*-forming hydrogels. Ding et al. [36] reported a hydrogel formed *in situ* by reacting two dextrans modified with cyanoacetate and benzaldehyde, respectively. Dynamic covalent C=C links were formed by histidine-catalyzed Knoevenagel condensation of the substituents. The resulting hydrogel was found to protect cells during injection and then degrade to allow space for encapsulated cell growth. Wang et al. [37] reported a starch/polyvinyl alcohol hydrogel cured by boronic ester formation, a reversible dynamic covalent bond that afforded rapid self-healing property both in air and underwater.

Polysaccharide hydrogels crosslinked solely by dynamic covalent bonds usually exhibit weak physical properties. To address this issue, researchers have introduced fillers [40] and second networks [34] to reinforce the hydrogel. However, these methods may impair the benign nature of the hydrogel and may degrade important properties like self-healing and injectability.

### Michael addition

Michael reactions [41] involve 1, 4-addition of nucleophiles to α, β-unsaturated carbonyl groups; typical nucleophiles include thiols and amines [42,43]. These reactions are typically rapid and chemoselective even under physiological conditions, producing no toxic or hazardous by-products (indeed, as a condensation, Michael reaction produces no by-products at all). Moreover, due to their well-controlled sol-gel transitions, hydrogels cured by Michael addition have been intensively investigated in the field of biomedical applications. Polysaccharides like HA [44] and heparin [45] were functionalized with thiols, vinyl sulfones, or aminoethyl methacrylate to prepare substrates for creating hydrogels. Cells and drugs could be loaded to the hydrogel by

**Figure 3**




**a**) and (**b**) Selective oxidation of polysaccharides and resulting imine cured hydrogel, (**c**) Michael addition between thiol and maleimide [46], (**d**) HA hydrogel crosslinked by aqueous Diels-Alder chemistry, (**e**) disulfide crosslinked HA hydrogel [57], (**f**) chemical structure of carboxymethyl hexanoyl chitosan [75]. HA, hyaluronic acid.

simply mixing with the precursor solution. Extracel$^{TM}$ is a typical hydrogel crosslinked by Michael addition between a thiol appended carboxymethyl HA and diacrylated PEG [46] (Figure 3**c**). Gelation could be modulated by pH and concentration of the polysaccharide solutions. Werner reported a glycosaminoglycan hydrogel that was prepared by reacting heparin-maleimide conjugates with cysteine-terminated, multi-armed PEG [47]. This hydrogel exhibited tunable *in vivo* degradation rate and stiffness and did not induce a strong immune response in mice. A similar curing approach was applied to thiolactic acid-modified chitosan [48]. Due to the many benefits of hydrogels crosslinked by Michael addition, this class of materials has been widely applied, including in tissue engineering [49], drug delivery [50], and bioadhesion [51].

### Diels-Alder reaction

Diels-Alder reactions are attractive for preparing *in situ* forming polysaccharide hydrogels, in some cases meeting all the criteria of a click reaction [52]. They are fast, produce no by-products, are highly specific, and often are quantitative. Diels-Alder reaction between furan and maleimide [53] is, for example, a one-step hydrogel-forming approach that is free of catalyst or initiator, and the linkage is reversible via a heating-cooling cycle (Figure 3**d**). Hu reported modification of HAs with furan and maleimide motifs respectively, and these were reacted with each other by Diels-Alder chemistry to afford an injectable nanogel [54]. This Diels-Alder condensation of HA derivatives can occur in aqueous media, thus permitting direct protein and cell encapsulation. These Diels Alder HA hydrogels were used in cell culture and for releasing proteins, with excellent performance [38]. A similar approach has been applied to chitin [55], where furyl chitin was cured by reaction with maleimide-terminated PEG, and the resulting hydrogel was used as a cell culture substrate. Chen reported a dextran-based hydrogel prepared by reacting fulvene-modified dextran with dichloromaleic acid end-capped PEG. The resulting hydrogel self-healed at 37 °C and exhibited low toxicity [56].

### Redox reactions to form disulfide bonds

Disulfide bonds are common in natural proteins and are frequently utilized in hydrogel crosslinking. The S-S linkage is formed by mild oxidation of sulfhydryl motifs and can be cleaved by mild reducing agents; both can occur under physiological conditions. Li reported coating liposomes with thiolated chitosan. These nanoparticles with thiols at the surface can be cured at 37 °C in the presence of β-glycerophosphate, thereby forming liposomal hydrogels *in situ* in a physiological-mimicking environment [57]. Gautrot used cysteamine-coupled thiolated HA to prepare a disulfide cured photoresponsive hydrogel (Figure 3**e**), showing that its modulus could be tuned by controlling the balance between thiol photooxidation and radical-mediated disulfide photodegradation. The hydrogel exhibited self-healing behavior and was able to encapsulate living cells due to the mild conditions of gel formation [39]. One drawback was that thiol oxidation by air or peroxide required hours to achieve gelation. In order to permit usefully rapid gelation, Wang employed pyridyl dithiol modified HA to crosslink with PEG-dithiol via thiol exchange; in this way, gelation was complete within 10 min. However, in such thiol exchange strategies, there will be concern that by-product formation and side-reactions (e.g. of endogenous proteins with disulfide linkages with the hydrogel components or by-products) could lead to undesired biological responses [58].

### Catechol-catechol reaction

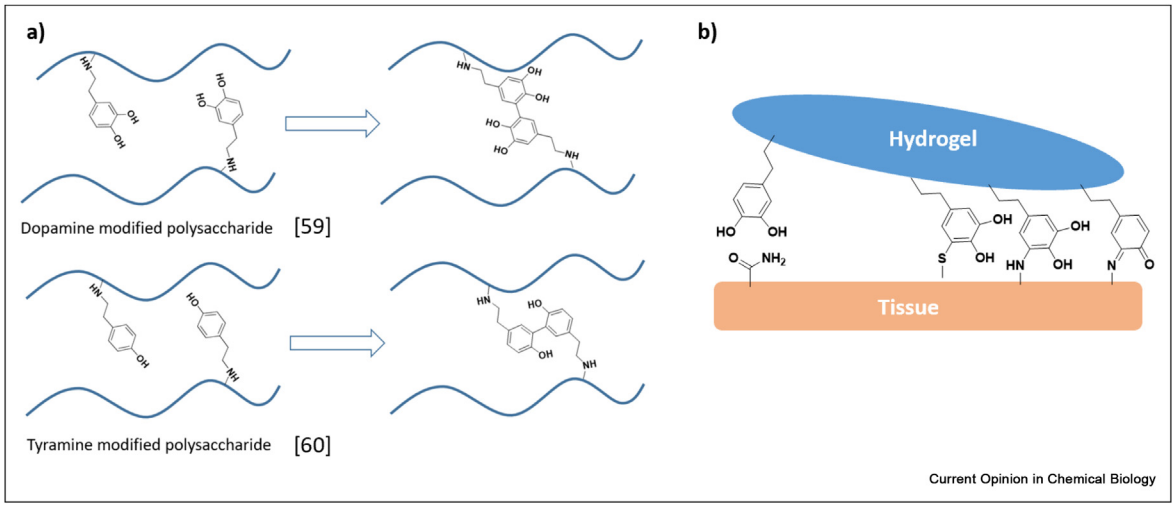
Recently, a mussel-inspired crosslinking strategy has been studied intensively for the preparation of polysaccharide-based hydrogels. In this approach, polysaccharides are modified with catechol motifs and then crosslinked by catechol-catechol reaction (Figure 4) [59]. Zhou et al. [59] reported a catechol-conjugated HA prepared by Schiff base reaction between oxidized HA and dopamine, with oxidative curing triggered by sodium periodate. Bi et al. [60] coupled tyramine with carboxymethyl chitin by amide linkages to afford catechol conjugated polysaccharides; these were oxidatively crosslinked, induced by horseradish peroxidase and $H_2O_2$. Chi [61] reported an HA hydrogel crosslinked by dopamine-modified ε-polylysine via the combination of Schiff base and horseradish peroxidase-mediated catechol-catechol reaction. Hydrogels cured by oxidative condensations of catechols usually exhibit excellent tissue adhesion and biocompatibility under the conditions of use.

## Physically crosslinked *in situ* forming hydrogels

### Electrostatic interaction

Crosslinking polymers by electrostatic interaction is an important route to *in situ* forming hydrogels. Alginate is a natural anionic polysaccharide commercially isolated from kelp, a copolymer of D-mannuronic acid (M) and L-guluronic acid (G) monosaccharides. It has valuable properties for food, pharmaceutical, and industrial applications [62]. Like many ionic polysaccharides, alginate can form hydrogels by interaction with multivalent cations including $Mg^{2+}$, $Ca^{2+}$, and $Ba^{2+}$; G blocks in alginates are particularly well-suited geometrically and chemically for chelating divalent metal ions like $Ca^{+2}$ [63]. This class of hydrogels, some of which are employed in FDA-approved [64] medical products, have been widely used in cell encapsulation, drug delivery, and tissue engineering. The mechanical performance and gelation time of alginate hydrogels can be controlled by the concentration and type of cation used. Muo and Ma [65] reported that the rates of alginate hydrogel formation increased with the increasing concentrations

**Figure 4**




**a**) Polysaccharide hydrogels crosslinked by catechol-catechol reaction, (**b**) potential bioadhesion mechanisms.

of $CaCO_3$ and $CaSO_4$. Moreover, hydrogel mechanical properties are affected by the concentration, molecular weight, and M/G ratio of the alginate employed. Resmi et al. [66] reported an injectable hydrogel via borax complexation of vicinal diols in oxidized alginate, which was further cured by imine linkages with gelatin. The two curing mechanisms worked together to enhance the hydrogel mechanical performance. However, as divalent ionic crosslinks can be destroyed by ion exchange with monovalent ions in body fluids, cation-crosslinked alginate hydrogels may not be sufficiently stable in the human body, depending on the duration required for the particular application [67]. This is of particular importance for exciting applications like alginate-based hydrogels for immunoisolation of islets of Langerhans, as a potential cure for type I diabetes [68].

Chitosan is biocompatible in many situations and toward many tissues, and biodegrades both *in vivo* [69] and in the environment (environmental degradation slows as DS(Ac) decreases) [70]. Chitosan $pK_A$ is roughly 6, [71], thus it is significantly positively charged below pH 6 by protonation of the C-2 amines. Poly(-cationic) chitosan can thereby readily form hydrogels with anionic polymers [72]. Polyol salts that have a single anionic head, such as salt derivatives of sorbitol, glycerol, and fructose, have been used to prepare hydrogels with chitosan [73,74]. These chitosan/polyol hydrogels exhibit useful properties including pH and temperature responsiveness. Mi reported an injectable *in situ*-forming hydrogel based on the blends of carboxymethyl hexanoyl chitosan (Figure 3**f**) with low molecular weight HA, with no curing agents or thermal treatment. Hydrogel formation was by tracked by fluorescence resonance energy transfer, indicating that the polysaccharide blends first form micelles via ionic interaction, then self-assemble into hydrogels [75]. Lv et al. [76] prepared a polyelectrolyte complex hydrogel from carboxymethyl chitosan and alginate, where gelation was assisted by the pH drop resulting from the hydrolysis of D-glucono-δ-lactone. They discovered that the addition of chitosan oligomers and D-glucono-δ-lactone induced a two-stage gelling process, greatly altered the microstructure, and enhanced hydrogel physical properties. This interesting phenomenon was explained by the protonation of chitosan oligosaccharide (pKa 5.8), driven by the hydrolysis of D-glucono-δ-lactone to the ring-opened carboxylic acid. The resulting positively charged oligomers interact ionically with carboxylates of alginate and carboxymethyl chitosan, leading to the second stage gelation. Zhang et al. [77] reported a chitosan/poly(sulfobetaine) double network hydrogel crosslinked by citric ion chelation and hydrophobic interaction. The fully physically crosslinked hydrogel displayed high stretchability (1400%) and the ability to self-heal.

## Host-guest interactions

Cyclodextrins appended to one hydrogel partner can form host-guest interactions, or inclusion complexes, with hydrophobic substituents on the other hydrogel partner, for example with adamantane or cholesterol moieties. Such methods have been used for preparing *in situ*-forming hydrogels for decades. Current related studies mainly focus on introducing new functions to this type of hydrogel. Zhang et al. [78] reported an injectable hydrogel combining chitosan-*g*-adamantane, chitosan-*g*-cyclodextrin, and β-cyclodextrin modified graphene oxide. This injectable, self-healing hydrogel can be heated by exposure to NIR light due to the presence of graphene oxide, affording it photothermal antibacterial activity *in vivo*. Zhang reported a

self-healing, electroconductive hydrogel prepared by host-guest interaction between β-cyclodextrin and adamantane substituents. The hydrogel was prepared using a β-cyclodextrin-epichlorohydrin polymer and adamantane-modified, sulfated alginate/poly(3,4-ethylenedioxythiophene) nanoparticles, where the sulfated alginate was meant to mimic the glycosaminoglycans of the extracellular matrix (ECM), and poly(3,4-ethylenedioxythiophene) incorporated conductivity. The electroconductive environment induced Myoblast C2C12 cells to form myotube-like structures. Moreover, cell release could be triggered by adding monomeric β-cyclodextrin [79]. Ikkala reported a stiff and healable CNC/polyvinyl alcohol composite hydrogel. The CNC surface was modified with polymethacrylate brushes bearing naphthyl motifs (guest 1) via surface initiated ATRP, and polyvinyl alcohol was modified with viologen (guest 2). They were crosslinked by cucurbituril (host) through host-guest interactions. This three-component recognition afforded the hydrogel the ability to undergo rapid sol-gel transition (less than 6s) and self-healing [80].

### Hydrophobic interaction and hydrogen bonding

Polysaccharide-based hydrogels can form *in situ* by hydrogen bonding or hydrophobic non-covalent interactions. Gelation of this class of hydrogel can be controlled by temperature or pH, permitting active encapsulation, injection, and triggered release. In order to manipulate intermolecular interactions, various polymers were grafted onto polysaccharides. For instance, polymerization of acrylic acid and acrylamide in the presence of chitosan was reported to afford a graft copolymer that formed a robust chitosan-based self-healing hydrogel, presumably by a combination of hydrogen bonding and ionic interactions [81]. Polysaccharides grafted with polymers that display lower critical solution temperatures can be crosslinked by strong hydrophobic interaction at temperatures above the lower critical solution temperatures. Li [82] reported an injectable hydrogel prepared by alginate-*g*-poly(*N*-isopropylacrylamide). The thermoresponsive graft copolymer formed micelles at 37 °C and a hydrogel at concentrations of 7.4 wt%. A similar hydrogel was prepared by grafting poly(ε-caprolactone-*co*-lactide)-*b*-poly(ethylene glycol)-*b*-poly(ε-caprolactone-*co*-lactide) and *O*-phosphorylethanolamine onto alginate [83]. The hydrogel could be injected at desired locations as a scaffold for bone engineering. It should be noted that the attachment of moieties onto polysaccharides by classical radical polymerization may not be suitable for biomedical applications for a number of reasons, including the difficulty of properly characterizing such products, repeatability, and the difficulty of being assured of the quantitative removal of unreacted monomers. 'Living' polymerization techniques, particularly using selectively grafted initiators, may be more suitable for such purposes [84].

## Applications

Important applications of polysaccharide-based *in situ* forming hydrogels include as ECM mimics, in wound healing and in cell/drug delivery. Polysaccharide-based materials have several inherent advantages in these fields. First, polysaccharides are natural materials and so are biodegradable. Several polysaccharides, including chitosan [17], dextran [85], pullulan [86], amylose [87], and partly oxidized alginate [66], have been shown to be biodegradable *in vivo*. This is crucial since approval agencies want to see that a polysaccharide-based material placed inside the body has a route by which it can eventually be cleared from the body. Glycosaminoglycans may be particularly useful since they are ubiquitous in humans, should therefore not elicit immune responses, and are fully biodegradable *in vivo*. Second, polysaccharides have many chemical moieties that are suitable for chemical modification, hydroxyl groups for all polysaccharides, and for some polysaccharides also carboxyl or amine functional groups. Such modification can impart functionality and responsiveness. Many natural polysaccharides are economically isolated from natural sources, and derivatives of some of them (prominently cellulose and amylose) are commercially available at low to moderate cost; these sustainable-based materials are suitable for designing hydrogels and modulating their properties. With this combination of properties and characteristics, polysaccharides are extremely useful building blocks for *in situ*-forming hydrogels. They also have advantages as hydrogel components versus competitive polymers like PEG (very limited ability to modify chemically) and polypeptides (complicated synthesis, high cost, typically more immunogenic).

*In situ* forming hydrogels, particularly those based on polysaccharides, can have properties similar to those of physiological microenvironments such as the ECM, thus enhancing utility for tissue engineering. Photocrosslinked, *in situ*-forming hydrogels are frequently fabricated into microarrays and patterned microstructures to mimic the ECM, and are used for cell culture or co-culture [6,17]. Hydrogels formed by Diels-Alder chemistry, Michael addition, disulfide bonds, or imine formation can also be suitable scaffolds for tissue engineering. Using glycoaminoglycans as hydrogel components can limit the resulting immune response, with low immune cell infiltration [47]. Tuning the hydrogel modulus to match the stiffness of target tissue promotes cell proliferation and suppresses immunogenic effects. Moreover, control of hydrogel degradation kinetics can be achieved by modifying polysaccharide backbone structure [59].

Polysaccharide-based *in situ* forming hydrogels have also been used for wound treatment and skin repair. Strong adhesion to tissue can be achieved by attaching

dopamine motifs to the hydrogel, allowing retention of the material at the desired site [59]. To prevent infection of the wound, the hydrogel can be modified with antimicrobial moieties, such as quaternary ammonium salts, or combined with antimicrobial polymers like ε-polylysine [61]. Moreover, hydrogel toughness is important in cases where the wound treatment site may be exposed to high stress and strain. To achieve excellent physical performance, interpenetrating networks, for example those formed by the combination of covalent bonds and chelation, have been used to enhance hydrogel toughness, thereby preventing failure at the service location [8].

*In situ* forming hydrogels can be quite valuable for the delivery of drugs, particularly for targeting and slow release. The drug-loaded hydrogel can be directly injected at the desired location, filling even irregular spaces, and potentially providing prolonged [57], sequential [7], or on demand drug release. Drugs can be physically entrapped in the hydrogel and therefore release is controlled by diffusion, or the drug can be covalently bound to the polysaccharide(s) [30]. Drug release from hydrogels designed to be stimulus-responsive can be triggered by external stimuli, including ROS level, pH [75], temperature [82], and NIR irradiation [35], to serve different purposes.

## Conclusion and perspectives

The benign natures of *in situ*-forming, polysaccharide-based hydrogels make them attractive materials. Those described herein target biomedical applications including tissue engineering, drug delivery, and cell encapsulation. Recent discoveries in material science and polysaccharide chemistry have led to functional, responsive polysaccharide hydrogels. The imparted functionalities allow tailored performance, in some cases triggered by stimuli that can include temperature, UV and NIR light, pH, enzymes, and reactive oxygen species. However, impediments remain to biomedical application of polysaccharide-based, *in situ*-forming hydrogels. One issue is potential toxicity. For hydrogels based on modified polysaccharides, newly introduced motifs and their degradation products may bring unexpected toxicity concerns. Moreover, there are several desirable properties that have not been successfully realized by this type of hydrogel or that still have room to improve. For instance, rapid gelation in a controlled manner and fast self-healing are appealing properties for *in situ*-forming hydrogels. Moreover, hydrogels with physical and biological properties that vary along a spatial gradient would be attractive for tissue engineering and controlled drug release. In addition, hydrogels that could be injected and then would turn into more robust materials would be valuable for applications involving high stress and strain. Finding solutions to these issues will greatly broaden the application range of polysaccharide-based, *in situ* forming hydrogels.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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This work presents a pure chitosan-based hydrogel, crosslinked by blue light irradiation and catechol-$Fe^{3+}$ chelation. The two independent networks give the hydrogel robust mechanical performance. In addition, the hydrogel displays good antimicrobial activity, high tissue adhesion, and inherent hemostatic property, making it suitable for wound treatment.

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