Thermosensitive Injectable Polysaccharide-based Hydrogels: Gelation Mechanisms, Synthetic Strategies, Biomedical Applications, and Challenges

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In recent years, thermosensitive polysaccharide-based injectable hydrogels have gained increasing attention in biomedical applications, including wound healing, drug delivery, and cartilage repair. These hydrogels have favorable biocompatibility, biodegradability, and tunable physical and chemical properties. Thermosensitive polysaccharide-based injectable hydrogels are a class of intelligent soft matter material. They can undergo a reversible liquid-solid transition when exposed to temperature stimuli. Therefore, their precursor solutions can be accurately inserted into target sites with irregular geometries in a minimally invasive way and then transformed into gels in situ by the organism's temperature stimulation to deliver biologically active molecules. This review summarizes the recent developments of thermosensitive injectable polysaccharide-based hydrogels. The focus is on the mechanism of sol-gel phase transition, as well as the design and preparation of thermosensitive polysaccharides and their applications in biomedical fields. In addition, the outlook of the challenges in biomedical applications is provided at the end of the paper.

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INTRODUCTION

Hydrogels are hydrophilic polymers that form a 3D network structure. They are synthesized from hydrophilic polymers through physical or chemical crosslinking by functionally modifying polymers; hydrogels display remarkable biocompatibility, biodegradability, tissue adhesion, and tunable mechanical properties; in particular, reversible hydrogel networks present many exciting possibilities (Aldakheel *et al.* 2023; Alsareii *et al*. 2023). Therefore, hydrogel materials are extensively employed in various biomedical applications, including wound healing dressings, drug delivery, and cartilage repair (Chen *et al*. 2024). In recent decades, research has shifted from implantable to injectable hydrogels that enable gel formation at the desired injection site. Injectable hydrogels have the benefits of simple handling, minimally invasive administration, and affordable injection expenses (Li *et al*. 2019). Furthermore, injectable hydrogels can fill minor and irregular wounds caused by injuries and can also be implanted into treatment sites that are difficult to access. Equally significant, the injectable hydrogel benefits from gradually undergoing the "sol-gel" phase transition in physiological conditions, thereby

accomplishing precise treatment (Liao *et al*. 2020; Omidian and Chowdhury 2023).

Stimulus-responsive injectable hydrogels are hydrophilic polymer-based materials that undergo sol-gel phase transition when exposed to environmental factors including pH, temperature, electric field, pressure, ionic strength, *etc*. Temperature is a readily available and controllable ecological stimulus that frequently coincides with damage to organisms, among various stimuli (Salehi *et al*. 2023). Developing thermosensitive injectable hydrogels with excellent performance is a hot research topic in biomedicine. The initial state of the substance is liquid (sol state). Upon injection into the organism, a reversible sol-gel state transition can be achieved through slight changes in ambient temperature (Bellotti *et al*. 2021). Polysaccharide-based polymers are the most prominent and extensively studied biomacromolecules in thermosensitive hydrogel formation. This is due to their advantages in biocompatibility, biodegradability, and other physicochemical properties (Tanga *et al*. 2023). Moreover, polysaccharides possess multiple functional groups, including hydroxy, carboxy, and amine groups, which make them suitable for chemical modification with other polymers and maintain the desired properties for a wide range of uses in biomedical (Graham *et al*. 2019). Therefore, natural polysaccharide-based polymers are ideal for creating hydrogel materials for in vivo implantation, and their study in biomedicine has garnered increasing interest (Shen *et al*. 2020). Although there have been numerous commendable reviews on biomedical hydrogels and their applications, only a few presentations have focused on polysaccharide-based thermosensitive injectable hydrogels (Cascone and Lamberti 2020; Daly *et al*. 2020; Sánchez-Cid *et al*. 2022; Tavakoli and Tang 2017).

The current review is distinguished from previous reviews in that it focuses on the contributions from 2015 to 2023 on the progress of the thermosensitive injectable hydrogels based on polysaccharides (Table 1). Specifically, the phase transition mechanism, design, synthesis methods, and biomedical applications are considered in detail.

Table 1. A Summary of Thermoresponsive Polysaccharides, their Properties and Applications

Polymers	Modifications	Sol-gel transition	Applications	Status of development	References
Chitosan	β-glycerol phosphate cross-linked CS	37 °C	Wound dressing	*In vivo*	(Zhu *et al.* 2022)
		37 °C	Tissue engineering	*In vitro*	(Chenite *et al.* 2000)
		37 °C	Diabetic fracture healing	Mouse	(Moradi *et al.* 2023)
		37 °C	Cartilage repair	*In vitro*	(Zheng *et al.* 2022a)
		37 °C	Tissue engineering	*In vitro*	(Bhuiyan et al. 2023)
	Cross-linking of coordination bonds	37 °C	Wound dressing	*In vivo*	(Cao *et al.* 2021)
	Grafting hydroxybutyl groups and catechol	37 °C	Hemostatic agents	Mouse	(Shou *et al.* 2020)

	groups to the CS backbones				
	PNIPAM grafted to CS	37 °C	Drug delivery	*In vitro*	Ziminska *et al.* 2020)
	PEG grafted to CS backbone	37 °C	Wound healing	*In vivo*	(Aldakheel *et al.* 2023
Alginate	PNIPAM grafted to alginate	37 °C	Drug delivery	*In vitro*	(Liu *et al.* 2017)
		27-42 °C	Wound healing	*In vivo*	(Zakerikhoob *et al.* 2021)
		28.9 °C	Wound healing	*In vitro*	(Safakas *et al.* 2023)
Cellulose	Host-guest interactions between the hydrophobic core	37 °C	Drug delivery	Mouse	(Okubo *et al.* 2020)
	Substitution of OH with alkoxypropy groups	27.3-51.2 °C	Drug delivery	*In vitro*	(Dai *et al.* 2019)
	Substitution of OH with butoxypropyl groups	17–43 °C	Drug delivery	*In vitro*	(Tian *et al.* 2016)
	Substitution of OH with isopropoxypropyl groups	21.1-56.1 °C	Drug delivery	*In vitro*	(Tian *et al.* 2016)
Starch	PNIPAM grafted to starch	37 °C	Cancer therapy	Mouse	(Fan *et al.* 2022)
	Substitution of OH with butoxypropyl groups	4.5-32.5 °C	Drug delivery	*In vitro*	(Ju *et al.* 2012)
	Host-guest interactions between the hydrophobic core	31.3-36.5 °C	Drug delivery	*In vitro*	(Pourbadiei *et al.* 2023)

SOL-GEL PHASE TRANSITION MECHANISM OF THERMOSENSITIVE POLYSACCHARIDE-BASED POLYMERS

The sol-gel phase transition mechanism of thermosensitive injectable hydrogels is primarily driven by the alteration in the hydrophilic and hydrophobic equilibrium of the polymer chains (Kotova *et al*. 2023). Amphiphilic thermosensitive polymers are composed of hydrophobic and hydrophilic chain segments, and such polymers self-assemble in water to form micelles due to the hydrophobic association of the hydrophobic chain (Hubbe *et al*. 2017). As temperature rises, the hydrogen bonding between polymer micelle surfaces and water molecules weakens. This reduces the thickness of the hydration layer around the micelles (Zarrintaj *et al*. 2019). Consequently, the micelles come together to form a physical hydrogel. This polymer sol-gel phase transition process is known as the lowest critical solution temperature (LCST) phase transition (Chen *et al*. 2022a). A minor fraction of polysaccharides with the utmost critical solution temperature (UCST) exhibits an inverse phase transition state; the gelation of polysaccharides is favored at lower temperatures (Phunpee *et al*. 2022).

The schematic diagrams of LCST-type and UCST-type phase transitions are shown in Fig. 1. Polysaccharides and their derivatives, including carrageenan, agarose, and cementum, typically experience UCST-type phase transitions. These polysaccharide chemicals are commonly used as thickeners and emulsifiers in food and cosmetics (Kim and Matsunaga 2017). The UCST-type polysaccharide hydrogels need elevated temperatures to stay in a solution state. However, excessively high temperatures can harm the viability of cells and tissues in organisms (Chen *et al*. 2021). In contrast, LCST-type polysaccharide hydrogels are better for injectable hydrogel systems, as they are free-flowing solutions at ambient temperatures and transform into gels at body temperature (Thambi *et al*. 2016). Therefore, LCST-type hydrogels are of greater interest to scholars in biomedical applications. This review will discuss the sol-gel phase transition mechanism of LCST-type hydrogels.

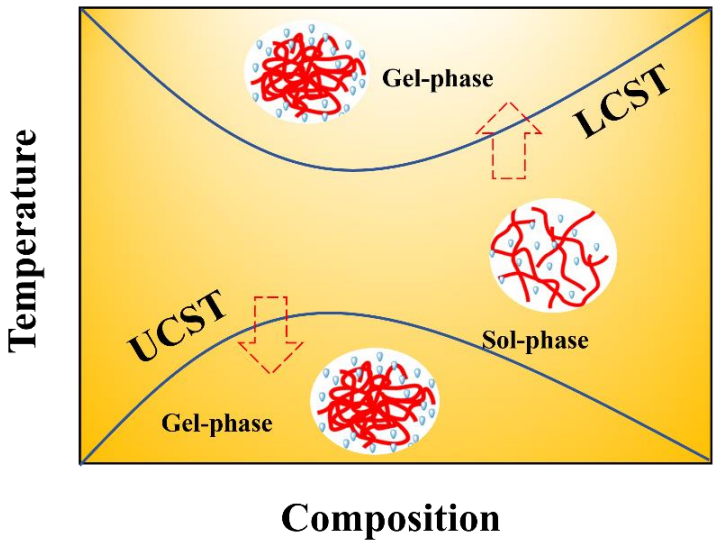

Fig. 1. Schematic diagram of volume phase transition of LCST-type and UCST-type

The sol-gel phase transition mechanism of LCST-type injectable hydrogels can be discussed at the molecular level and thermodynamically: (a) When the ambient temperature is lower than the LCST of the polymer, the hydrophilic effect is the main force. The hydrophilic groups on the polymer molecular chains connect with water molecules through hydrogen bonding, resulting in a sol state (Ryl and Owczarz 2021). However, when injected into warm-blooded animals and the temperature rises above the LCST, hydrophobic interactions become dominant, which contributes to the formation of bulky aggregates by the association of hydrophobic polymer chains, resulting in a gelation phase transition (Li *et al*. 2021). In general, the morphology of hydrogels can be reversibly transformed between sol and gel states because of the dynamic interactions between polymer chains and water molecules (Okubo *et al*. 2020). The analyses mentioned above indicate that the interaction of hydrophobic chain segments of thermosensitive polymers is the primary driving force for the sol-gel phase transition at the molecular level. (b) From the thermodynamic standpoint, according to the Gibbs free energy formula, $\triangle G = \triangle H - T \triangle S$, the association free energy of the polymer chain is directly correlated with enthalpy, entropy, and temperature (Wang *et al*. 2018). In an amphiphilic thermosensitive polymer-water system, when $T < LCST$, the polymer chain stretches and forms hydrogen bonds with

water molecules, resulting in the $\triangle H$ term being negative. Simultaneously, because the water molecules are arranged in a more orderly manner around the polymer chain, $\triangle S$ is negative; therefore, $\triangle G < 0$, resulting in the solubility of the polymer in the sol state (Pan *et al*. 2020). When $T > LCST$, the entropy of the system increases and becomes dominant, *i.e.* ($\triangle H > T\triangle S$). This results in $\triangle G > 0$, representing the gel state and favorable for the association of polymer chains (Zhang *et al*. 2018a). In summary, from the thermodynamic perspective, the sol-gel phase transition of thermosensitive polymers is driven by increased entropy.

DESIGN AND PREPARATION OF THERMOSENSITIVE INJECTABLE HYDROGELS

Small-molecule Functional Group-modified Polysaccharides

In nature, only a few polysaccharides, including agarose, carrageenan, and gellan gum possess thermosensitive properties. Most polysaccharides require chemical or physical modifications to acquire thermosensitive properties (Atanase *et al*. 2017; Kotova *et al*. 2023). Chemical modification strategies can be broadly categorized into two main categories: (a) Modifying polysaccharides through the small-molecule functional group, which is also called polysaccharide derivatization, aims to impart thermosensitive properties to polysaccharides. This is achieved by incorporating small-molecule functional groups, including carboxy and alkyl groups, into their molecular structure (Otto *et al*. 2021); (b) Synthesizing thermosensitive polymer graft polysaccharides. Physical modification strategies primarily involve additive-modified polysaccharides, where additives are utilized to trigger thermosensitive sol-gel transitions in polysaccharide solution systems comprising multiple components without the formation of additional covalent bonds (Loukotová *et al*. 2018; Nishimura et *al*. 2020).

Carboxylated Polysaccharides

The carboxyl group is both a donor and acceptor of hydrogen bonds. Therefore, after the carboxyl groups are introduced to the polysaccharide, the hydrogen bonds between intramolecular and intermolecular chains of polysaccharide are altered, leading to a change in the spatial conformation of the polysaccharide (Fu *et al*. 2024; Li *et al*. 2023). This change is crucial for regulating the thermosensitive properties of carboxylated polysaccharides. Agarose is a natural thermosensitive polysaccharide that reverses a sol-gel phase transition with temperature. It is of the UCST type (Rochas and Lahaye 1989). Furthermore, the temperature hysteresis between the agarose sol temperature ($T_{gel\text{-}sol}$) and gelation temperature ($T_{sol\text{-}gel}$) is significant, with $T_{sol\text{-}gel}$ (15 to 30 °C) being considerably lower than $T_{gel\text{-}sol}$ (60 to 80 °C). The $T_{sol\text{-}gel}$ and $T_{gel\text{-}sol}$ of unmodified agarose and the phase transition rate rely on sources, processing conditions, concentration, and cooling rate (Kim *et al*. 2019; Ooi *et al*. 2016). Introducing charged carboxy groups boosts the electrostatic repulsion among the carboxylated agarose chains. This leads to increased spacing between the chains and a weakening of hydrogen bonding (Chu *et al*. 2020). Consequently, the conformation of agarose shifts from α-helix to β-sheet structure, ultimately impacting its phase transition temperature (Su *et al*. 2013).

As the degree of carboxylation is increased, $T_{sol\text{-}gel}$ has been found to decrease significantly (Forget *et al*. 2013). When the degree of carboxylation was increased from

28% to 93%, $T_{sol\text{-}gel}$ fell from 28 °C to 7 °C, and $T_{gel\text{-}sol}$ decreased from 58 °C to 46 °C. The sol-gel phase transition temperature of agarose can be achieved by adjusting the carbonation level within a broad range of regulations (Arnott *et al*. 1974).

Alkylated Polysaccharides

The design of thermosensitive injectable hydrogels for alkylated polysaccharides relies on balancing the alkyl side chains' hydrophobicity and the polysaccharide backbone's hydrophilicity (Tian *et al*. 2015). Modifying hydrophilic and hydrophobic components in alkylated polysaccharide structures is crucial for designing these hydrogels. Some regulate the hydrophilic component by adjusting the polysaccharide's molecular weight, construction, and hydrophilic modification (Iqbal *et al*. 2023). Similarly, methods to control the hydrophobic part involve managing the carbon chain length, structure, and degree of substitution (DS) of the alkylating reagent on the polysaccharide main chain (Bostanudin *et al*. 2023; Liang *et al*. 2015). Polysaccharides are hydrophilic because they have hydroxy and amino groups that can form hydrogen bonds with water molecules. Modifying polysaccharides through etherification or esterification reactions involves grafting short-chain alkanes onto their backbone. This hydrophobic modification results in polysaccharide derivatives with both hydrophilic and hydrophobic properties (Palacio *et al*. 2018; Rodrigues-Souza *et al*. 2022). When the balance between hydrophilicity and hydrophobicity is achieved, these derivatives exhibit thermosensitive behavior. Many studies showed that alkylated polysaccharides' sol-gel phase transition temperature is primarily influenced by the length and DS of the alkyl carbon chain (Tian *et al*. 2019). The phase transition temperature decreases as the carbon chain length increases and the DS rises. Methylcellulose (MC) is a cellulose product modified using methylation reagents. The LCST of MC typically ranges from 60 to 80 °C and is influenced by the substitution degree of the methyl group (Wang *et al*. 2016; Zhong *et al.* 2020). Isopropoxy or butoxy-modified hydroxyethyl cellulose derivatives can be modulated by adjusting the DS (Dai *et al*. 2019). This results in an LCST range of 21.1 to 56.1 °C for isopropyl-modified derivatives and 17.0 to 43.1 °C for butoxy-modified derivatives (Tian *et al*. 2016). Noteworthily, when the length of the hydrophobic carbon chain is C5 or longer, alkyl hydroxyethyl cellulose becomes insoluble in water, even with low DS. As a result, they do not exhibit thermosensitive properties.

Besides cellulose, other polysaccharides including starch, chitosan, and guar gum can obtain thermosensitive properties through alkylation (Miller *et al*. 1994). Regarding polysaccharide structure, the LCST of branched-chain polysaccharides is lower than that of straight-chain polysaccharides with the same carbon chain length, similar DS, and equal molecular weight (Zheng *et al*. 2022b). For the butoxy-modified polysaccharide, the LCST values were 57.1 °C for butoxy cellulose of straight-chain structure with a DS of 1.43 and 17.5 °C for butoxy starch of branched-chain structure with a DS of 1.31 (Ju *et al*. 2012). Due to hydrophobic chains, Polysaccharides with branched-chain structures aggregate more easily, resulting in lower phase transition temperatures. Interestingly, the effects of the alkyl chain structure are different from that of the polysaccharide chain structure on LCST. In the authors' recent work, it was discovered that the LCST of polysaccharide products with different structures, but the same length of hydrophobic carbon chains is notably higher than that of alkylation-modified polysaccharides with straight-chain form, even with the same DS (Ju *et al*. 2014).

Polymer Grafting Polysaccharide

Synthetic polymer components are generally responsible for the thermosensitive injectable polysaccharide-based graft copolymers. The polysaccharide components, on the other hand, contribute biocompatibility, biodegradability, non-fouling, cell adhesion, and enhanced gel mechanical strength (Darge *et al*. 2021; Jiang *et al*. 2021). Grafting polymers onto the main chain of polysaccharides is the key to designing adjustable LCST thermosensitive polymers. Specifically, this is equivalent to grafting hydrophilic groups on the polysaccharide skeleton, thereby increasing the hydrophilicity of the polysaccharide, increasing the LCST of the polysaccharide-based graft copolymer (Hubbe *et al*. 2021). The thermosensitive copolymer will be compromised if the grafting ratio is inadequate (Mellati *et al*. 2017).

The findings of this research warrant the attention of researchers involved in developing such graft copolymers. The above injectable hydrogel is identical to the conventional graft copolymerization method, which is further subdivided into two processes: 'grafting-from', in which the polymer chain is grown directly onto the polysaccharide backbone, and 'grafting to', in which a specific molecular weight polymer chain segment is synthesized before grafting onto the polysaccharide backbone (Azzam *et al*. 2016). Particularly important among the thermosensitive polymer family is poly(N-alkyl acrylamide); it is also known as poly (N-isopropyl acrylamide) (PNIPAM). Preparing injectable hydrogels from polysaccharides based on PNIPAM has long been a research hotspot (Conzatti *et al*. 2019; Fillaudeau *et al*. 2024).

Currently, the design methods for graft copolymers of polysaccharides with PNIPAM can be categorized into three groups: (a) Polysaccharides are solely graft copolymerized with PNIPAM, namely PS-g-PNIPAM. Hydrophilic polysaccharides increase the $T_{\text{sol-gel}}$ of PS-g-PNIPAM compared to PNIPAM homopolymers (Kanidi *et al*. 2020). (b) Graft copolymerization of polysaccharides with various synthetic-type polymers is a design method divided into two approaches. One approach is to graft copolymers of PNIPAM and other polymers onto the polysaccharide backbone, named [PS-g-(PNIPAM-co-other Polymers)] (Hubbe *et al*. 2023).

The second class is to graft PNIPAM and other polymers individually, specifically (PS-g-PNIPAM-g-other Polymers). The main purpose of introducing other hydrophobic or hydrophilic polymers is to adjust the $T_{\text{sol-gel}}$ of the hydrogels. Introducing hydrophobic polymers decreases the $T_{\text{sol-gel}}$ while adding hydrophilic polymers increases the $T_{\text{sol-gel}}$ (Kurisawa and Yui 1998).

Another way to construct hydrogel systems is by incorporating organic/inorganic molecules, metal ions, enzymes, oligosaccharides, or other polysaccharides through covalent or non-covalent bonds in the design methods of (a) or (b) (Hubbe *et al.* 2008). These functional components provide special functions to the hydrogel. Fan *et al*. (2022) introduced iodine into starch's helical interior using non-covalent bonds (Fig. 2). This formed an iodine-starch complex. Next, they grafted PNIPAM onto the complex to create an injectable hydrogel loaded with iodine starch-g-poly (N-isopropyl acrylamide) (PNSI). This hydrogel combines photo-thermal and anti-infective effects. It is easy to prepare and safe for biological use. It can be applied in clinical settings for oncology therapy and anti-infection purposes.

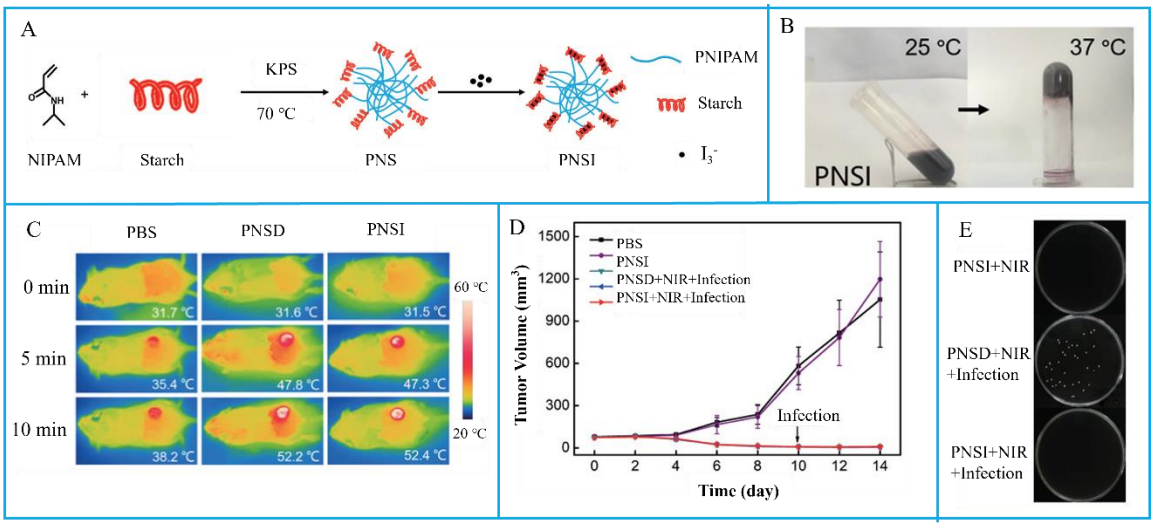

Fig. 2. *In vivo* photothermal antitumor and antibacterial effect of the PNSI hydrogel: (A) Schematic diagram of the preparation of the PNS and PNSI nanogels. (B) Photographs of the PNS and PNSI dispersion at 25 and 37 °C, respectively. (C) Infrared thermal images of the tumor-bearing mice under 808 nm laser (1.0 W cm^{-2}) for 5 and 10 min after injection of PBS, the PNSD, and PNSI dispersion (50 mg mL^{-1}), respectively. (D) Tumor-growth curves of mice after different treatments (n = 5). (E) Pictures of bacterial colony in the skin wound of different groups on the 5th day after S. aureus infection. Figure republished from Fan *et al*. (2022) with permission from Wiley, *Macromolecular Rapid Communications*.

Polyether-type thermosensitive polymers are commonly used to prepare injectable hydrogels of polysaccharide graft copolymers. Polyether-based polymers that are thermosensitive exhibit similar sol-gel principles and copolymer design strategies as PNIPAM-polysaccharide graft copolymers (Schneider *et al*. 2021). In the family of polyether-based polymers, polyethylene glycol (PEG) stands out due to its superior water solubility, resistance to antigens, and low toxicity (Ahmadkhani *et al*. 2017). Polyethylene glycol is frequently utilized to create graft copolymers based on polysaccharides for biomedical uses. The design methods and thermosensitive mechanism of PEG-polysaccharide graft copolymers differ from those of the above copolymers (Que *et al*. 2015). On the one hand, the aldehyde group modifies the end of the molecular chain of PEG molecules. It reacts with the reactive groups on the polysaccharide skeleton to obtain the graft copolymers (Ghanavi *et al*. 2023). On the other hand, unlike PNIPAM and thermosensitive polyether polymers, PEG lacks thermosensitive properties (Filippov *et al*. 2016). The thermosensitive sol-gel phase transition of PEG graft copolymers with polysaccharides is caused by the alteration of hydrogen bonding force between the copolymer and water molecules. For instance, in the case of chitosan and PEG graft copolymer (CS-g-PEG), the poly (ethylene glycol) molecular chain's end was altered with the aldehyde group to create an imine bond with the amino group on the chitosan molecular chain (Rivas-Barbosa *et al*. 2022). This process resulted in the formation of the graft copolymer. Water molecules surrounded the CS-g-PEG chains through hydrogen bonding at low temperatures. The temperature rise disrupted the hydrogen bonds between water molecules and copolymers. This caused water molecules to detach from the surface of CS-g-PEG and increased hydrophobic and hydrogen-bonding interactions between CS-g-PEG chains. As a result, gels were formed (Hao *et al*. 2021).

Additive-modified Polysaccharide

Besides the method mentioned earlier about creating thermosensitive injectable hydrogels through modification of small-molecule functional group and graft copolymerization, another approach involves using small-molecule organic compounds as additives to induce the sol-gel phase transition of polysaccharides at various temperatures, resulting in thermosensitive properties for polysaccharide-additive systems (Bhuiyan *et al*. 2023). The main driving force behind the phase transition of injectable hydrogels is the alteration in coulombic, hydrogen, and hydrophobic bonding forces among water molecules, polysaccharides, and additives (Dhivya *et al*. 2015). The mechanism of action involves small-molecule additives (polyol phosphates, triglyceride derivatives) that weaken hydrogen bonding between polysaccharide and water molecules and enhance hydrophobic bonding interactions between polysaccharide molecules. At this stage, hydrophobic bonding dominates in a certain temperature range, leading to the final phase transition from sol to gel. It should be noted that for additives to induce the sol-gel phase transition, a crucial requirement is a substantial alteration in the solubility of polysaccharides in water due to variations in external factors such as temperature, pH, and ionic strength (Geng *et al*. 2023). This change is the primary driving force behind the phase transition in polysaccharide solution systems. The main function of the additive is to maintain the polysaccharide in a pre-gelatinized state by surrounding it with enough water molecules. This prevents the polysaccharide from directly precipitating out of the solution system (Liu *et al*. 2016). Chitosan is soluble in acidic environments. At a pH above 6.2, chitosan starts to deprotonate and gradually forms flocculent deposits. Chitosan solubility varies over the physiological pH range, making it an ideal raw material for preparing thermosensitive hydrogels with additives in the biomedical field. Chenite's team suggested using a chitosan/β-glycerophosphate (β-GP) aqueous solution to create a thermosensitive injectable hydrogel (Chenite *et al*. 2000). This hydrogel can be formed *in vivo* through the combined effect of various forces that promote the formation of gel states, such as hydrogen bonding, and electrostatic and hydrophobic interactions. The β-glycerophosphate additive plays three roles in the hydrogel system: (a) β-Glycerophosphate is alkaline. Thus, it can raise the pH level of the system to the physiological range. (b) It can ensure that the chitosan solution remains in a sol state at room temperature and within the physiological pH range. (c) The sol-gel phase transition is facilitated with increased temperature.

Functional compounds, whether metal or non-metal (ions or elements), are commonly added to polysaccharide-additive systems (Zhang *et al*. 2018b; Zhou *et al*. 2015). This is done to regulate the gelation rate and phase transition temperature, improve mechanical properties, antibacterial properties, and biocompatibility, or introduce new biomedical functions. Moradi's team incorporated hydroxyethyl cellulose (HEC) and graphene (GO) into the CS/β-GP hydrogel system, resulting in a hydrogel with accelerated sol-gel phase transition rate and a more robust three-dimensional network structure (Moradi *et al*. 2023). Hydroxyethyl cellulose and GO have three critical contributions to this hydrogel system: (a) Enhance structural stability. The electrostatic interaction among HEC, GO, and CS molecular chains enhances the strength of the hydrogel structure. (b) Increase the phase transition rate. GO enhances the hydrophobic effect of the hydrogel system, boosts the mutual aggregation between polymer chains, and speeds up the sol-gel phase transition rate. (c) Improve treatment effectiveness. The hydrogel is utilized for diabetic fracture healing, and the HEC component aids in cell adhesion, growth, and calcium deposition.

BIOMEDICAL APPLICATIONS OF POLYSACCHARIDE-BASED THERMOSENSITIVE INJECTABLE HYDROGELS

Wound Healing

Skin injuries are a common type of body injury. They often result in complex clinical problems due to difficulties in wound healing, mainly when bacterial infections occur repeatedly (Abbas *et al*. 2019; Barbu *et al*. 2021). Therefore, it is crucial to design and synthesize novel wound dressings with antibacterial, hemostatic, and wound-healing capabilities (Dhand *et al*. 2016). At present, enhancing the speed of adhesion of a medical formulation to wounds for better healing efficiency is a significant scientific concern in the field of new materials for wound healing (Hu *et al*. 2020; Jafari *et al*. 2022). Thermosensitive polysaccharide-based injectable hydrogel materials have caught the interest of researchers among various topical wound dressings (Luo *et al*. 2022). Their precursor solutions' fluidic nature allows them to fill wounds with irregular geometries and adhere to surrounding tissues during gelation (Ramasami *et al*. 2019; Raina *et al*. 2022). This helps retain wound moisture, provide gas replenishment, and absorb wound secretions for efficient wound healing (Safakas *et al*. 2023).

The integration and adhesion of biomaterials or implants, such as hydrogels, to the surrounding native tissue is crucial for wound healing (Lee *et al*. 2023). Injectable hydrogels face challenges in adhering to the bleeding site for hemostasis and wound healing due to the moist environment of the human body. Shou *et al*. (2020) designed and synthesized catechol-hydroxy butyl chitosan (HBCS-C) with tissue adhesion properties inspired by the adhesion properties of marine mussels in water (Fig. 3).

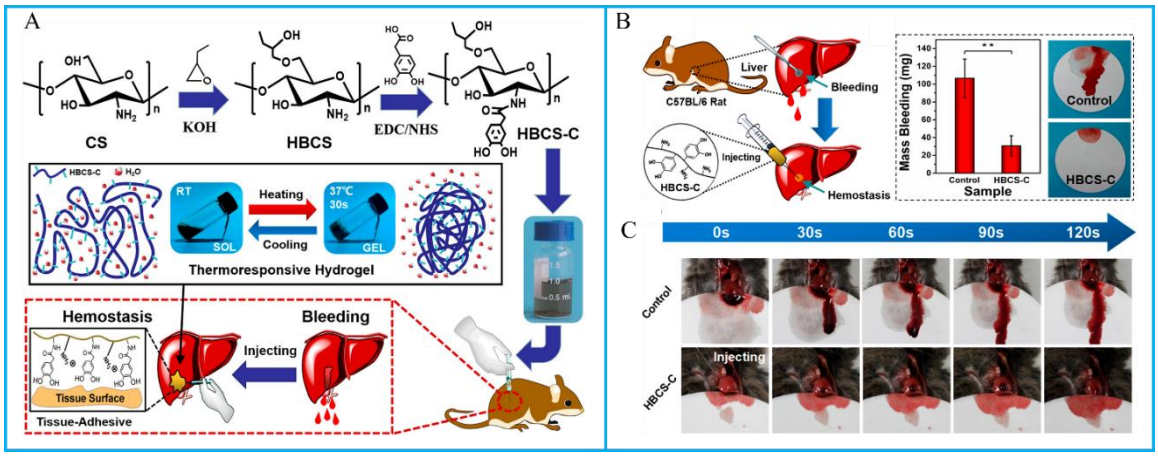

Fig. 3. The wound hemostatic behaviors of HBCS-C hydrogels: (A) Schematic illustration of HBCS-C thermoresponsive adhesive hydrogels. (B) Schematic illustration of hemorrhagic liver model of black rat. (C) Contrast images of the bleeding rat liver treated with HBCS-C and untreated every 30 s for 2 min. Figure republished from Shou *et al*. (2020) with permission from ACS, *ACS Biomaterials Science & Engineering*.

They achieved this by grafting hydroxy butyl and catechol groups onto the chitosan backbone. Catechol-hydroxy butyl chitosan achieved a fast thermosensitive sol-gel transition *via* hydrogen bond changes within and between molecular chains and hydrophobic interaction between hydroxy butyl groups. The interactions between the catechol amino functional group in the HBCS-C structure and the tissue allow the hydrogel to adhere firmly to the surface. The team also achieved hemostasis experiments on hepatic

hemorrhage in rats with this hydrogel. By injecting the hydrogel precursor solution into rats, the hydrogel could form *in situ* within 30 s and firmly bind to the bleeding tissue, resulting in a noticeable hemostatic effect. The work mentioned above not only illustrates a design method for polysaccharide-based hydrogels that can conform to irregular wound shapes and possess strong tissue adhesion properties but also demonstrates the importance of hydrogel material's tissue adhesion properties in wound hemostasis, offering new insights into the study of the agents.

Wound healing, specifically extensive full-thickness wounds, typically require a lengthy repair process (Zheng *et al*. 2020). Both acute and chronic wounds pose significant health challenges for patients and healthcare professionals. Therefore, creating wound dressings with fast healing properties is also difficult (Huang *et al*. 2023). Cao *et al*. (2021) developed a chitosan-based hydrogel wound dressing that is thermosensitive and has self-healing properties. The team added metal ions Fe^{3+} and Al^{3+} to the carboxymethyl chitosan solution system. These ions can enhance wound healing. They can bind with the carboxy groups in the chitosan structure, forming a dynamic cross-linked network. The hydrogel exhibited remarkable properties of self-healing, adaptability, and thermosensitivity. The researchers conducted *in vivo* wound healing experiments using a mouse skin wound model. They treated the wounds with gauze, commercial wound dressing, and self-repairing hydrogel. They compared the wound recovery time and found that the self-repairing hydrogel significantly accelerated healing compared to the other two materials. Moreover, given that conventional tissue adhesives cannot prevent wound infection and necessitate the use of additional antibiotics, it is imperative for the wound dressing to have efficient antimicrobial properties in clinical applications (Sánchez-Cid *et al*. 2022; Zhang *et al*. 2020).

Zhu *et al*. (2022) designed a novel supramolecular hydrogel using hydroxypropyl chitosan (HPCS) and PNIPAM. To begin, HPCS-CD was obtained by grafting β-cyclodextrin onto the HPCS backbone. Next, self-assembly occurred through supramolecular forces between HPCS-CD and adamantyl acrylate (ADA). Finally, a hydrogel was formed using ADA to undergo a free radical polymerization reaction with N-isopropyl acrylamide (NIPAM). Hydrogels have various functions, such as injectability, thermosensitivity, and self-healing. Injecting dipotassium glycyrrhizinate (DG) into the precursor solution resulted in the hydrogel acquiring antibacterial activity against *Staphylococcus aureus*. The team evaluated wound healing *in vivo* with a mouse whole-skin defect model. The study found that injecting DG-loaded hydrogels into mice resulted in effective tissue remodeling, collagen deposition, and reduced inflammation. These outcomes were better than those observed with commercial Tegaderm™ and 3M dressings. The study proposes a novel approach for treating wounds in emergencies. Zakerikhoob *et al*. (2021) used the mentioned method of hydrogel wound dressings and created a curcumin-containing PNIPAM-alginate Sodium (Algate-g-PNIPAM) hydrogel system. The hydrogel system has a commendable wound contraction effect and can reduce inflammation, promote collagen formation, and increase fibroblast count.

Drug Delivery

When drugs are delivered conventionally, only a tiny fraction of the drug can reach the intended treatment site in a biological target. On the other hand, the majority of the drug is distributed throughout the body (Chabria *et al*. 2021). Simultaneously, drug interactions and non-delivered target interactions can cause unmanageable side effects. Therefore, optimizing drug delivery, enhancing treatment effectiveness, and minimizing

side effects are pressing issues that require immediate attention (Chatterjee and Chi-leung Hui 2019). The gel-forming properties of thermosensitive injectable hydrogels with *in situ* gelation are crucial for drug delivery at the lesion (Chen *et al*. 2018; Chen *et al*. 2020; Duong *et al*. 2020). The hydrogel precursor solution, containing drugs, can be injected minimally invasively at the biological target site. The hydrogel can then rapidly form and immobilize with a temperature change (Myrick *et al*. 2019). The hydrogel, as described, can function as a concentrated drug depot, extending drug retention at the lesion site. This enhances drug utilization efficiency and reduces the need for frequent drug administration and toxic side effects (Yu *et al*. 2020; Ziminska *et al*. 2020).

Certain thermosensitive polysaccharide-based graft copolymers can undergo self-assembly in water to create micelles (Wang *et al*. 2020). These micelles have a hydrophobic core where hydrophobic drugs can be dissolved. The drug copolymers solubilize the aqueous solution and inject it into the body. Due to body temperature, the solution transforms into a gel through a phase transition. The mentioned delivery method can enhance both the hydrophobic drug loading and the hydrogel's network structure and can inhibit the passive diffusion release of the drug, ultimately decreasing the likelihood of burst release (Sun *et al*. 2019). Liu *et al*. (2017) synthesized Algate-g-PNIPAM, a graft copolymer of alginate and PNIPAM, using atom transfer radical polymerization (ATRP). The objective was to enhance the bioavailability and therapeutic efficacy of the doxorubicin (DOX) anticancer drug. The copolymer forms micelles at temperatures above the critical micelle temperature. These micelles can encapsulate DOX in their hydrophobic core. *In vitro* studies have confirmed that Algate-g-PNIPAM hydrogel can maintain DOX release, aiding in enhanced drug uptake by cells and decreased drug resistance, resulting in more efficient eradication of cancer cells.

Masanori's team suggested a fresh approach for subject-object recognition in thermosensitive injectable hydrogels (Okubo *et al*. 2020). The sol-gel transition was achieved by subject-object interactions between hydrophobically modified hydroxypropyl methylcellulose (HM-HPMC) and β-cyclodextrin (β-CD) at different temperatures. At low temperatures, β-CD and HM-HPMC formed inclusion complexes, resulting in the system being in the sol state. When the temperature reaches a level close to that of the human body, β-CD separates from the inclusion system, forming a gel state. Owing to these characteristics, they can be easily injected into the organism's lesion site at room temperature. A high-viscosity gel forms quickly at the injection site, enabling slow release of the drug and sustaining its effectiveness. The study demonstrated that the slow-released insulin from the HM-HPMC/β-CD hydrogel had a long-lasting hypoglycemic effect. The mean retention time (MRT) of insulin in the body increased by 1.6-fold compared to the direct drug delivery method. Furthermore, cyclodextrins played a role not only in the creation of hydrogels but also in the hydrophobic cavity, which is a good carrier for hydrophobic drugs.

Pourjavadi's team designed and synthesized an injectable hydrogel with a dual response to temperature and UV light through the host-guest interaction mechanism (Fig. 4) (Pourbadiei *et al*. 2023). The preparation process is complex. The copolymer P(NIPAM-AZO) was obtained by randomly copolymerizing the NIPAM and the photosensitive monomer azobenzene derivative (AZO) using free radical polymerization. Simultaneously, β-CD was used to alter double allyl starch (DAS) to accommodate the resulting DAS@SCD product, and ultimately, P(NIPAM-AZO) was combined with DAS@SCD to form the hydrogel precursor. The hydrogel underwent sol-gel phase transition under UV light and temperature stimulation using azobenzene derivatives with cyclodextrin host-

guest recognition and PNIPAM chain segments. Paclitaxel was loaded into the hydrogel through the β-CD hydrophobic cavity. The substance was released over 96 h under UV light at body temperature and a wavelength of 230 nm.

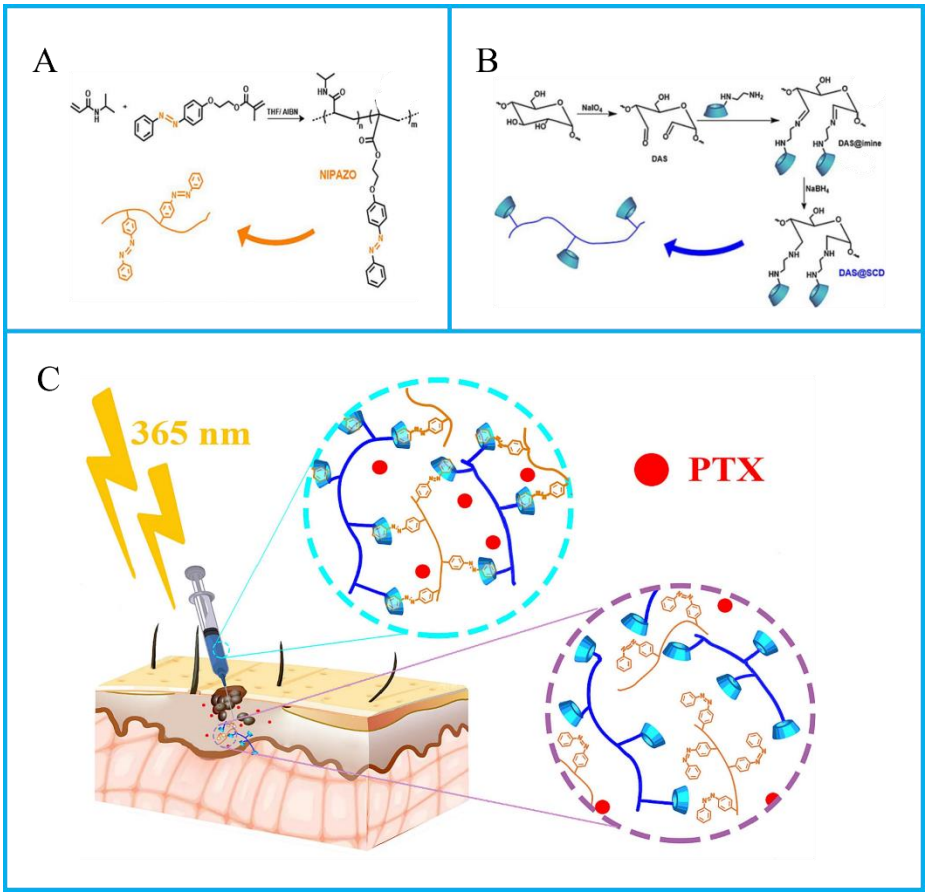

Fig. 4. The preparation process of DAS@SCD/NIPAZO hydrogel: (A) Synthesis of NIPAM-co-AZO monomer. (B) Synthesis of DAS@SCD. (C) A schematic view of PTX releases upon light irradiation. Figure republished from Pourbadiei *et al*. (2023) with permission from Elsevier, *Carbohydrate Polymers*.

Cartilage Repair

Bones are vital for human activities, as they are an essential body part. Osteoblasts and osteoclasts in healthy bone tissue typically maintain balance, but this equilibrium is disrupted by trauma, disease, and aging, resulting in irreversible bone damage (Chatterjee and Hui 2021; Chen *et al*. 2022b). Injectable polysaccharide-based thermosensitive hydrogels mimic the extracellular matrix structure and regenerate cartilage by filling hydrogel into bone tissue defects (Devi *et al*. 2021). This minimally invasive method forms a 3D scaffold that adapts to tissue defects. Injectable polysaccharide-based thermosensitive hydrogels are increasingly crucial for fast bone defect repair (Lin *et al*. 2021).

Hydrogels for bone tissue regeneration should have fast gelation and controlled degradation properties (Padmanabhan *et al*. 2023). Chenite *et al*. (2000) introduced a thermosensitive injectable hydrogel made of chitosan/polyol salt complexes. This hydrogel was used as a system for delivering growth factors *in vivo* and as matrices for chondrocytes. The degradation time can be controlled by adjusting the deacetylation degree of chitosan, ranging from days to weeks. In general, as the deacetylation degree decreases, the

degradation rate increases. However, the report failed to investigate the regulation of the gelation rate and the impact of high concentrations of polyol salts on hydrogel biocompatibility. Dang *et al*. (2011) manipulated the hydrogel formation and degradation time by adjusting the concentration of β-GP, and a higher dosage of β-GP notably reduced the hydrogel formation time. However, the researchers discovered that elevated β-GP concentrations were toxic to human HS68 cells and mouse embryonic fibroblasts. Hence, when preparing hydrogels, regulating the quantity of β-GP carefully is crucial. Polylysine (PL) is a lysine homopolymer with excellent solubility, thermal stability, and biocompatibility. Polylysine is also an antimicrobial agent with bactericidal activity against gram-positive and gram-negative bacteria. The PL chain has numerous hydrogen bond donors and acceptors, making it a suitable starting point for creating thermosensitive sol-gel phase change materials. Zheng *et al*. (2022a) developed a ternary thermosensitive injectable hydrogel composed of chitosan, PL, and glycerophosphate (GP) (SF/PCS/GP). The research team first modified chitosan with PL. Then, they blended it with GP to create the hydrogel's backbone material. In addition, the therapeutic effect can be optimized by loading bone marrow mesenchymal stem cells (BMSCs) and transforming growth factors (TGF-β1). The addition of PL enhanced the hydrogels' biocompatibility and antimicrobial properties. It also improved the gelation rate and mechanical properties by forming β-folded structures between PL and chitosan molecular chains through hydrogen bonding.

Hydrogel, as a bone tissue filler, should possess rapid gel performance, controllable degradation performance, and excellent mechanical properties (Wang *et al*. 2023). Additionally, its mechanical strength should align with that of the tissue organism. The neatness of the crosslinked network closely affects the mechanical properties of hydrogels (Saravanan *et al*. 2019). Verma's team introduced hydrothermally treated polyelectrolyte complexes (PEC) and gelatin into the chitosan-polygalacturonic acid (PgA)-β-glycerophosphate (β-GP) thermosensitive hydrogel system, based on previous work. The introduction of polyelectrolyte complexes creates a hydrogel with a nearly ideal crosslinked network structure (Wasupalli and Verma 2022). The mechanical properties are enhanced by this relatively homogeneous network structure, which effectively disperses the externally applied stresses. Moreover, the gelatin component infused within the hydrogel can enhance bioactivity and cell adhesion, promoting cell proliferation. The research team studied the mechanism of the mentioned improvement in the hydrogels' mechanical properties. The hydrogel's functions relative to cell adhesion and proliferation, and bone tissue growth, repair, and regeneration were systematically evaluated. The results showed that the hydrothermal treatment of polyelectrolyte complexes and polygalacturonic acid enhanced the connectivity of hydrogel pores and the neatness of PEC in the hydrogel, thereby enhancing the mechanical strength.

CURRENT CHALLENGES AND PROSPECTS

In recent decades, polysaccharide-based thermosensitive injectable hydrogel materials have combined biosafety and temperature-controlled *in situ* gel formation. This showcases the potential of hydrogels in biomedical field applications. Researchers have applied certain polysaccharide-based hydrogel products from *in vitro* to *in vivo*, from the lab to real clinical applications. The related research results and outcomes have gradually demonstrated a broad application prospect.

However, to broaden the application range of polysaccharide-based hydrogel products and speed up the promotion of their clinical applications, the development of

injectable polysaccharide-based hydrogels still faces the following constraints: (a) While polysaccharides possess non-toxicity and excellent biodegradability, the typical approach for designing injectable hydrogels based on polysaccharides often involves modifying the structure of the polysaccharide through physical or chemical means. Newly introduced moieties, compounds, and functional polymers may have tissue toxicity concerns. Therefore, it is essential to initially contemplate polysaccharide-based hydrogels' composition and design methods. Furthermore, the regulation methods for controlling the phase transition temperature of thermosensitive injectable hydrogels are relatively complex, and the range within which the phase transition temperature can be adjusted is limited. Hence, the upcoming research should prioritize the efficient and rapid control of phase transition temperature to meet the needs of practical applications. (b) The microenvironment of the lesion site of the organism is complex, and there are often subtle changes in pH, oxygen content, temperature, ionic strength, and microorganisms. In future research, developing injectable hydrogels with multiple stimulus sensitivity is significant. This will help them adapt to changes in the microenvironment of the organism's lesion tissue. (c) Currently, thermosensitive hydrogels have fulfilled the criteria for clinical applications by adjusting their mechanical properties through physical and chemical modifications. However, compared to other popular elastomers such as polyurethane, polydimethylsiloxane, and vulcanized elastomers, there remains a significant disparity in mechanical strength and stability. Therefore, the next development focus is on enhancing the mechanical strength of polysaccharide-based materials while maintaining their injectable properties and biosafety.

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