Recent Development of Polysaccharide-Derived Hydrogel: Properties, Stimuli-Responsiveness and Bioapplications

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To contribute through dedication, hard work and sincerity towards the overall growth of the organization; wherein she gets the opportunity of explore her academic, technical and scientific knowledge and prove her credentials as a reputed professional. She is currently working as a postdoctoral research associate in the University of Burdwan, West Bengal, India in the field of "**synthetic carbohydrate polymeric gel and their biomedical applications**" after completing her doctorate degree from Indian Institute of Science Education and Research (IISER) Kolkata, West Bengal, India on "**polymer synthesis from renewable sources and their antibacterial applications**". She is developing her research expertise on synthesis, characterization and biological applications of some injectable *in situ* forming polysaccharide-based gel; synthesis of polymers from renewable resources and amino acid-based monomers *via* controlled radical polymerization techniques; multistep organic synthesis; development of antibacterial system; protein immobilization through natural or synthetic polymer support. She has achieved University Gold Medal for her first class first position in Masters. She has been awarded as CAS Registry Innovator for innovation of novel substances as identified by Chemical Abstract Service (A Division of the American Chemical Society), Research Excellence Award by Institute of Scholars (InSc), Govt. of India, Best Poster Award in scientific international conference to celebrate 70th anniversary of India-Russia Diplomatic Relations. She is a professional reviewer and lifetime member of the InSc; regular member of American Chemical Society (ACS) and Royal Society of Chemistry (RSC). Currently she has joined as a peer reviewer in STAR Protocol, Elsevier after achieving a Certificate of Excellence in Certified Peer Review Course by Research Academy, Elsevier, UK and graduated from ACS Reviewer Lab.

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Abstract

Hydrogels are three-dimensional, hydrophilic, polymeric frameworks, constructed through diverse physical or chemical crosslinks. Recent research focuses on their physiological stimuli sensitivity enhancing bioapplications. Biopolymers with responsiveness to local environment like temperature, pH etc. form the foundation in this regard, around which all major natural processes are circulated. Polysaccharides, being most the abundant biopolymers and essential constituent of our daily food like cereals and fruits, are readily available in nature. Hence, among numerous biomacromolecules, polysaccharides are extensively used to prepare hydrogel recently. However, incorporation of certain properties to these gels through derivatization can trigger stimuli responsiveness suitable for various applications especially in biomedical field inspired from the mother nature. For example, in situ cross-linking ability may deliver an intermediate platform between solid scaffolds and saline injections through reversible or irreversible sol-gel transition. Easy injectibility provides patient's relief through reducing recovery time and inflectional hazards. Self recovery of gel within body results automatic inflammation and damage recovery of tissues. This review provides a detail impression on the synthesis of natural polysaccharide derived hydrogel network with structural classification to bulk, supramolecular, micro and nano gel. Several properties important from biomedical point such as in situ, injectable, self healing nature of these hydrogels and various physical, chemical, or biochemical stimuli-responsive characteristics are discussed further. Lastly, their major bioapplications are highlighted.

Keywords: Hydrogels, biopolymers, polysaccharides, stimuli, in situ, injectable, self healing, bioapplications

1. Introduction

Gels are extentisively studied self-organized materials with wide applications in day-to-day usable products of human life, such as toothpaste, soap, hair gel, shampoo, shaving gel, honey etc.[1] Depending on their solvent absorption efficacy; gels could be classified as organogels and

hydrogels.[2] Again, native wet hydrogels can be changed to aerogels, xerogel or other functional dry materials after the removal of water.[3] However, hydrogels have got enhanced attention for their structural tenability and excellent rheological property. Most of the bioapplications are associated with hydrogels as they can absorb water, the most preferred biological solvent and major constituent of body cells. Those gels are often described as soft and wet materials based on physical appearance where high-water absorption capacity and low stiffness are often preferred to introduce self healing nature resembling natural tissues, which leads to several biomedical applications like drug delivery, tissue engineering, would healing. Natural tissue possesses complicated structural feature through evolution over millions of years, whereas most synthetic hydrogels are amorphous.[4] Hence, inspiration from nature has generated great research interest to construct refined and aligned hydrogel structure with enhanced functionality and mechanical strength, as nature created materials have multi-layered and orientated structures.[5]

Particular attention has been paid to natural polysaccharides (e.g., hyaluronic acid (HA), alginate, chitosan, and cellulose) based hydrogels in this regard, due to their unique properties such as biodegradability, biocompatibility, non-toxicity, high cost-effectiveness, ready availability and reproducibility from abundant renewable resources. They are polyhydroxy aldehydes, ketones, alcohols, acids, or their polymers with acetal linkage and exhibit extremely attractive advantages compared with synthetic polymers due to structural diversity, rich stereochemistry, and their potential to play important roles in biology and disease controll.[1] Again, fabrications of hierarchical structure include sophisticated nature in gel materials.[6] This property of polysaccharides add a new dimension to encounter the superabsorbent hydrogel (SAH) with high ability to absorb, swell and retain aqueous solutions up to hundreds of times of their own dry weight without loss of physical structure.[7] Various natural polymers like starch,[8] cellulose,[9] chitosan,[10] xyloglucan,[11] konjac glucomannan[12] extracted from environmental sources such as raw bran,[13] rice husk [14] and tulips[15] and lead to the synthesis of eco-friendly SAHs recently *via* physical as well as chemical crosslinking method.

However, numerous bioapplications of gel like drug delivery, tissue engineering, cell immobilization, wound healing abilities can be limited due to poor interconnectivity, irregular pour size and lower flexibility. Functionalisation of polysaccharide precursor for gel formation can eliminate those limitations by incorporating porosity and *in situ* injectable, self healing properties. Porosity can be introduced by particle leaching method, where porogens with

controlled particle size are dispersed into pre-polymer solution. Fast gelling, injectable, and porous heparin-based hydrogel was demonstrated with pore interconnectivity by using gelatin microparticles as a porogen.[16] Thus porous and injectable hydrogel was constructed by using a gelatin-hydroxyphenyl propionic acid/carboxylmethyl cellulose tyramine (Gtn-HPA/CMC-Tyr) through horseradish peroxidase (HRP)-catalyzed oxidation with *in situ* pore size control by cellulose digestion.[17] Simply mixing with the gel precursor leads to effective immobilization of cells. Reversible sol-gel transition and several physiological stimuli like temperature, pH or enzyme responsiveness add another dimension in biomedical field to modern polysaccharide-based gel. Major biomedical fescilities observed from these properties are reduction of the risks insvolved in the surgical implantation of a conventional hydrogel such as pain and infection. Recently, research has been more focused on hydrogels responsive to physiological conditions like various stimuli along with reversible or irreversible sol-gel transition. Again, stimuli sensitive hydrogel was easy to load hydrophobic drugs, acting as efficient drug delivery system. Injectable hydrogels are able to form gel state after injection into the body *in situ*, are beneficial in biomedical applications as they can fill irregular defect sites.[18] A large amount of hydrogel together with bioactive molecules/cells can be delivered by applying these tools[19] with elimination of large scratch and reduction of recovery time, risk of infection, and pain to the patient.[20]

The past decade has witnessed a rapid progress in the field of polysaccharide chemistry for their wide applications. Multiple reviews discussed various approaches in developing specific polysaccharide-based materials and selective application site. Though several research groups have been demonstrated general synthesis such as extraction, purification or characterization[21] and applications of polysachharide composite materials like starch, cellulose, pectin, gum, alginate, chitin and chitosan[22] and derived gel,[23] very few reviews demonstrated overall idea about their *in situ*, injectable self healing properties along with stimuli responsiveness; and how their properties enhance biomedical applications.[24] In this review, we have tried to capture the comprehensive outline of latest advances in natural pollysaccharide-derived stimuli-responsive hydrogels with bulk, micro, nano and supramolecular architectures and their *in situ* injectable and self healing nature. We have cited some recent examples where stimuli-responsive *in situ* injectable polysaccharide gels incorporate major advantages in major bioapplications such as potential drug delivery, tissue engineering etc.

2. General Synthetic Strategies

Polysaccharides are composed of monosaccharides, the simplest monomeric units and are essential metabolic foundation in the body. Generally, monosaccharides, excess for cellular fuel purpose, are accumulated as polysaccharides. Some examples are as follows. In humans and animals, glycogen is the stored polysaccharide, found in liver and muscle cells. Plants stock up their polysaccharides in form of starch and cellulose, main structural component of the plant cell wall. These polysaccharide moities can be employed to synthesize gel network through simple structural modification. As for example seeds of the plant, *Artemisia sphaerocephala* Krasch (ASK), contain both water soluble (ASKP) and insoluble (ASKG) polysaccharides. [25] These can be extracted and modified to gel. A recent study showed that hydrogel was developed through binding of ASKP with ferric ions with potential application as an iron fortifier.[26] Multi-layered arrangement of polysaccharide-based hydrogel has been fabricated by reaction–diffusion processes like periodic precipitation[27] or dialysis method. Several common polysaccharides including curdlan (bacterial polysaccharide),[28,29] alginate,[30] and chitosan[31] based hydrogel were synthesized by these techniques. Another alternating process to design realistically was smooth recognition of chain condensation along with hydrogel integrity preservance. Several reports resulted the construction of an 'onion-like' multi-layered polysaccharide-based hydrogel in this regard through physical or chemical crosslinking pattern.[32,33] An alcohol-based chitosan gel can be introduced through putting in sodium hydroxide (NaOH) coagulation bath, where neutralization of protonated amino groups (NH_3^+) to free amino groups (NH_2) generate a physical hydrogel. Again, Duan *et al.* synthesized randomly shaped multi-layered chitosan hydrogels through loading of chitosan gel-core with chemical crosslinkers (terephthalaldehyde) in a layer-by-layer pattern in a repeted manner, followed by soaking.[34] Thus chemically crosslinked gel was generated through reaction between amino groups of chitosan chains and aldehyde groups. Similar strategy was also valid in other common polysaccharides, such as alginate and cellulose. Dai *et al.* reported an alginate-based multi-layer hydrogel by using a divalent cation crosslinker, Ca^{2+}.[35] Again, the generation of ordered structure in hydrogel can be activated through electrical signals [36] or *via* chronological maintenance especially in case of photocrosslinkable polysaccharides (methacrylate modified alginate and hyaluronan). On the other hand, fabricating three-dimensional (3D) scaffold through rapid-prototyping can be utilized in creating hydrogel scaffold materials. Lee *et al.* demonstrated a 3D freeform fabrication

technique to produce multi-layer hydrogel with hollow channels within.[37] Highly sophisticated structural pattern has been fabricated through ice-segregation-induced self-assembly technique, which is a cryogenic process, consisting of three stages: freezing, storage in the frozen state for a predetermined time, and defrosting. Now 3D printing followed by crosslinking was reported as another useful and modern method to construct polysaccharide hydrogel. Layer-by-layer repeatation of the process leads to a 3D multi-layer hydrogel. Collagen hydrogel precursor was printed and crosslinked afterward *via* nebulized sodium bicarbonate ($NaHCO_3$) solution, serving as crosslinker as well as the binder for the collagen layer. Decorative space was designed and printed with heated gelatin solution in some layers. A variety of gelation policies was adopted, including ionic crosslinking for alginate (with Ca^{2+} as physical crosslinker), UV crosslinking for gelatin (with photo initiator), enzyme crosslinking for fibrin (with thrombin as enzymatic crosslinkers), and Michael addition for thiolated HA (with chemical crosslinker poly(ethylene glycol) diacrylate). The alignment of polymer chain was dictated through accumulation of the spinning polymer solution jet on a rotating environment containing crosslinking agents. Through modulation of reaction time and effective gelation strategy, those alignments were regulated producing hydrogel matrix by crosslinking with oriented structure.[38] Electrospinning and wet spinning methods were very useful to fabricate hydrogel microfibers. Report showed that utilization of wet spinning method resulted oriented methacrylated sodium alginate hydrogel microfibers through dual crosslinked by both ultra violet (photo initiator) and calcium ion (Ca^{2+}).[39] Another effective technique was micropatterning to result organized structures, has been studied for numorous polysaccharide hydrogels. Continuous fabrication of alginate micro-ribbons with size-tuneable microstructures using coaxial microfluidic channels lead to gel through this technique, where the gelation nature is the ionic crosslinking of alginate by Ca^{2+}. Additionally, polysaccharide based composite gel has been prepared through coupling with commonly used carbonaceous materials such as activated carbon, carbon nanotubes, graphene, graphene oxide and biochar with chitosan or chitin.[40] Chitosan was dissolved in acetic acid at room temperature for 12 h and then stirred at 60 °C for 30 min to form gel followed by mixing with Fe (III) (as $FeCl_3$) and Fe (II) (as $FeSO_4$) under stirring condition for 2 hours (h). The final composite gel was prepared by dropwise addition into NaOH solution. Generally, gel materials can be majorly classified as bulk, supramolecular, micro and nano gel depending on their

structure, morphology and pour size (Figure 1). In the next section we aimed to discuss a detail outline for classification and synthetic strategies of common polysaccharide-based gel.

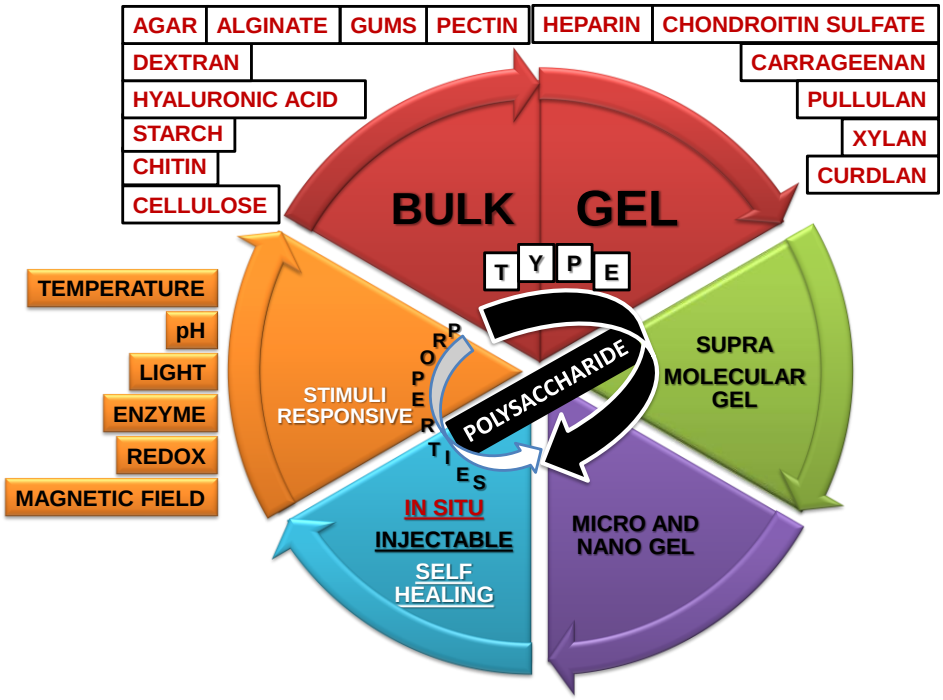

Figure 1. Major classification and properties of polysaccharide-based gel.

2.1. Bulk Gel

2.1.1. Cellulose Based Gel

Cellulose is attractive and inexpensive natural hydrophilic polysaccharide, which is most abundant on Earth and possesses high biocompatibility with low density. [41,42] Simple methyl derivative of cellulose was originated by partial substitution of hydroxyl groups (-OH) with methoxy groups (-CH_3O) with enhanced viscosity with temperature induced sol-gel transition property as a result of hydrophobic interaction. The gelation behavior has been organized by degree of substitution, molecular weight and its concentration. [43] Great functional alteration leads to gel networking structure. A wellknown cellulose derivative, carboxymethyl cellulose (CMC), has been extensively utilized to prepare hydrogels due to its water-solubility through numorous chemical modifications.[44] For example, hydrogels from thiol-modified CMC and polyethelene glycol (PEG)-tetra-norbornene through photopolymerization was reported by Lee *et al.*[45] However, applications of injectable hydrogels were restricted by ultra violet (UV)-triggered

reaction mechanism. *In situ* Schiff base reaction could be more feasible in this regard. Shen *et al.* engineered a CMC-based injectable hydrogel *via* this reaction, where 3,3′-dithiobis(propionohydrazide) is crosslinked to oxidized CMC.[46]

2.1.2. Chitin Based Gel

Chitin is a basic aminopolysaccharide obtained from crustaceans (shrimps and crabs). It is basically poly ($\beta(1\rightarrow4)$ *N*-acetyl-*D*-glucosamine) unit.[47] Being second most abundant after cellulose and common natural polymer with high viscosity, metal chelation capacity, polyelectrolyte tendency; chitin based hydrogels are very attractive materials in terms of applications.[48] Double crosslinked chitin hydrogels was reported recently by dissolving chitin in potassium hydroxide (KOH)/urea aqueous solution with freezing-thawing process through cross linking followed by coagulating in ethanol solution at low temperature.[49]

Chitosan was basically snthesized by *N*-deacetylation of chitin. They generally found in fungi and cell walls of algae, the exoskeletons of insects, mollusks and crustacean. [50,51] Easy physical and chemical modification of chitosan through reactive hydroxyl and amino groups resulted gelation. In contact with alkali, the amino functionality transformed to physically cross-linked hydrogel. Hydrogen-bonding played an important role to entangle macromolecular chains. Synthesis of chitosan hydrogels was trigerred by numorous chemical networking agents, including glutaraldehyde (GLA),[52] formaldehyde,[53] *N*, *N'*-methylenebisacrylamide (MBA),[54] genipin,[55] ethylene glycol diglycidyl ether (EGDGE) and epichlorohydrin (ECH).[56] Report showed that modification of chitosan with 1, 2-butene oxide and succinic anhydride (NSHBC) resulted gel as function of temperature ranging from 17 °C to 32 °C. Chemical modifications in acrylamide[57] or glycol chitosan obtaining *N*-hexanoyl glycol chitosan using hexanoic anhydride were able to form chitosan based gel.[58] Physical networking technique has been also taken to consideration in case of chitosan based gel formation. Ionic crosslinking was reported as a great approach in this regard. Anionic crosslinkers such as sodium tripolyphosphate,[59] sodium citrate, sulfosuccinic acid, and oxalic acid were used to prepare chitosan hydrogels.[60] Another method was freeze-thwing approach which led to combination of several polymers like starch, poly (vinyl alcohol) (PVA), poly (acrylic acid) (PAA) or alginate and capable of hydrogen bonding. [61] For example, synthesis of artificial bones composed of chitosan/PAA network using PVA and PAA by repeated freezing and thawing was reported. Another recent study exhibited that; a

novel chitosan hydrogel was prepared through dissolution in alkaline-urea aqueous solvent. The entire gelation process was observed through aggregation induced emission fluorescence.[62] A composite hydrogel of chitosan, heparin and poly (gamma-glutamic acid) for wound healing was reported by Zhang and co-workers *via* crosslinking by addition of acetic acid.[63]

2.1.3. Starch Based Gel

Starch, one of the largest biomasses on earth, is a natural, abundant, cheap, available, renewable, and biodegradable polymer. But native starch extracted from plants cannot tolerate the extreme processing conditions like temperature or acid base treatment leads to limited applications.[64] Hence to enhance or inhibit particular properties according different industrial requirements as well as bioapplications, several modifications regarding physical, chemical or enzymatic modifications by debranching enzymes (isoamylase or pullulanase) have been performed.[65] Smaller blocks were generated from linear short chains through highly debrancheing of starch (H-DBS) with less water holding capacity, which leads to stronger smooth, non-sticky and glossy hydrogel.[66] These gels have potentially used in the food and pharmaceutical industries[67]

2.1.4. Hyaluronic Acid Based Gel

Hyaluronic acid (HA) is another well known polysaccharides with large number of hydroxyl group, a non-sulfated glycosaminoglycan (GAG) and major constituent of skin extracellular matrix (ECM). In a work performed by Laurent, Gelotte, and Hellsing (1964), stability in aqueous solutions of HA was enhanced through crosslinking with 1, 2, 3, 4-diepoxybutane.[68] Similarly a stable and homogeneous hydrogel has been reported *via* mixing with butanediol-diglycidylether in sodium hydroxide solution followed by HA powder addition.[69] Hylase wound gel composed of emollients and sodium hyaluronate (2.5 %) was synthesized.[70] Fiorica *et al.* (2018) fabricated a hydrogel by crosslinking of a copolymer of HA (MW = 1.5×10^6 Da), (hyaluronic-(2-aminoethyl)-carbamate acid (HA-EDA)) with α-elastin.[71] Wu *et al.* (2017) utilized 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to provide crosslinking of HA with gelatin to synthesize hydrogel.[72] Initially, gelatin (GEL) and HA at different ratio (8:2, 5:5 and 2:8) were prepared followed by crosslinking with 0.1 % EDC. The crosslinking agent did not damage the porous structure of the hydrogel, essential for several biomedical applications. A hydrogel with improved mechanical properties by mixing HA -tyramine (HA-Tyr) with collagen

I-hydroxybenzoic acid derivative (COL-P) has been prepared, followed by crosslinking through blending with HRP and H_2O_2. Shi *et al.* (2018) reported HA (MW = 1.5×10^5 Da) modification through functionalization with pendant bisphosphonate (BP) groups.[73] HA based *in situ* injectable hydrogel could be formed through dynamic covalent bond between phenylboronic acid modified HA (HA-PBA) and PVA.[74] Another novel injectable DMEM (Dulbecco's Modified Eagle's Medium)-induced phenylboronic acid-modified HA self-crosslinking hydrogel was reported. Combination of the phenylboronic acid and a diol on HA resulted good self healing properties and tissue adhesion properties to the hydrogels through dynamically reversible phenylboronic acid esters.[75]

2.1.5. Dextran Based Gel

Dextran primarily composed of repeating $\alpha(1\rightarrow6)$ linked *D*-glucopyranose residues with less percent of $\alpha(1\rightarrow2)$, $\alpha(1\rightarrow3)$, or $\alpha(1\rightarrow4)$ linked side chains and major components of many bacteria.[76] Main groups, which can be modified through physical and chemical cross-linking leading to gelation, are hydroxyl groups per glucose unit. Physically crosslikned dextran gel could be prepared through derivatization with lactic acid oligomers while functionalized with bifunctional glutaraldehyde, isocyanates or by partial oxidation of hydroxyl groups to aldehydes followed by crosslinking with gelation, results chemically crosslinked gels.[77] Another interesting example of injectable biomimetic hydrogel was dextran-tyramine conjugated HA with high moduli, enhanced bovine chondrocyte viability, proliferation and matrix secretion.

2.1.6. Agar Based Gel

Agar is a complex polysaccharide mixture of linear agarose and branched agaropectin, extracted from marine red seaweeds.[78] The linear polymer composes of 1→3-linked-β-D-galactose (G) and 1→4-linked 3, 6-anhydro-α-L-galactose; whereas branched agaropectin linked with several substituent groups for example sulfate esters, methyl esters, pyruvate acid ketals.[79] Hydrogen bonding played vital role for linear agarose hydrogels which could be used for three-dimensional chondrocytes encapsulation.

2.1.7. Alginate Based Gel

Alginate is an unbranched anionic heteropolysaccharide derived from brown seaweeds and some bacteria, also found in outer wall of some brown algae such as kelps, composed of 1–4 glycosidically linked *β*-D-mannuronic (M) and *α*-L-guluronic (G) acids in varying composition and sequences.[80] Effective quantification of alginate hydrogel formation along with its mechanical strength could be dictated by external gelation process using calcium chloride ($CaCl_2$).[81] Hence divalent cations, such as Ca^{2+} and Ba^{2+}, played the mastered role as crosslinking agents to transform aqueous solutions of sodium alginate to gels. The predicted interaction strength order was reported as $Pb^{2+} > Cu^{2+} > Cd^{2+} > Ba^{2+} > Sr^{2+} > Ca^{2+}$.[82] Mechanistic elucidation revealed the gelation through ionic cross-linking of negatively charged carboxyl groups of the alginate chain and positively charged divalent metal ions.[83] It is important to note that, in order to obtain authentic information about molecular interactions, the knowledge of the initial values of the storage modulus in rheology was very important unlike the case of Alginate-Ca^{2+} gelation studies. A new custom-made rheometric setup was able to record the fast response from the very beginning, thus both the concentration and volume of the crosslinker could be controlled.[84] Again, injectable self crosslinking property was introduced through reaction between alginated dialdehyde and gelatin.[85]

2.1.8. Gums Based Gel

Gums are another class of naturally available polysaccharide derived from renewable sources. Capacity to hydration of these materials leads to form gel.[86] Common gums are generally classified as Gellan gum and Xanthan gum. Gellan gum is anionic exopolysaccharide, more precisely a linear tetramer composed of (1→4)-L-rhamnose-*α*(1→3)-D-glucose-1 *β*(1→4)-*D*-glucuronic acid-*β*(1→4)-D-glucose as repeating unit with one carboxylic side group; with high molecular weight, secreted by the bacteria *Sphingomonas paucimobilis.*[87] This polysaccharide resulted non-toxic, ionic and thermo responsive gels close to body temperature.[88] Gellan gum gel network was truly formed upon aggregation and ionic crosslinking through monovalent cations inspite of adopting ordered double helical architecture upon cooling. These monovalent cations broadcasted electrostatic repulsion amongst the carboxylate groups to induce gelation, but connection of two carboxylate groups was established by the divalent cations in addition to the screening effect. Thus, divalent cations formed stonger gels with higher viscosity than

monovalent cations. Additionally, this gellan gum based photocrosslinkable hydrogels *via* methacrylation and blending was also reported.[89]

Xanthan gum composed of five monosaccharides comprising two D-glucose, two D-mannose and one D-glucuronic acid units.[90] It is basically an extracellular heteropolysaccharide produced by the bacterium *Xanthomonas campestris.* Trisaccharide units of glucuronic acid replaced the alternating glucose units flanked by mannose entities. The backbone was protected from the external environment through covering with the side chains in helical secondary structure *via* hydrogen bonding. This structural complication leads to highly viscous gel even at lower concentration. Stability at various stimuli like pH, temperature, ion concentrations are some basic natures of the gel resulting pseudo-plastic property. Various medical applicative sides such as wound healing, drug carriers could be shown by gum-based gel. Carboxymethyl derivatization at the glucose residues of xanthan resulted microcapsule entrapment.[91] Thus, the injectable property or encapsulation could trigger this method. Running of intra-articular xanthan injection has the ability to protect the articular cartilage in osteoarthritic rabbit models.

2.1.9. Pectin Based Gel

Extraction from plant cell walls results pectin, a water-soluble polysaccharide, composed of α-D-galacturonate residues linked by (1→4) glycosidic bond, and Rhamnogalacturonan I (RGeI) and Rhamnogalacturonan II (RGeII). Monosaccharides such as D-xylose, D-glucose, L-rhamnose, L-arabinose or D-galactose are the major constituents[92] with partly methoxylated or amidated galacturonic acid (GalA) as a main building block. Classic egg-box model explained the gelation process which was regulated by several intrinsic and extrinsic factors such as pH, temperature, ion strength, molecular weight, Ca-binding blocks distribution, the degree of methoxylation. Egg-box dimers could be constructed from two antiparallel polyuronate chains with Ca^{2+} and further aggregated laterally to form multimers.[93]

2.1.10. Heparin Based Gel

Heparin is a highly sulfated linear glycosaminoglycan with alternating units of β-(1→4) linked uronic acids (mainly D-glucuronic, L-iduronic or L-2-sulfated iduronic) and glucosamine residues (mainly D-*N*-acetyl glucosamine and *O*- and *N*-sulfated glucosamine).[94] Presence of carboxyl and sulfate reactive groups result high negative charge leads to the electrostatic

interaction with proteins and chemical modifications. Numorous capacity of cellular signaling and growth could be regulated *via* enzymes like proteases and chemokines. An enzymatically crosslinked injectable heparin and dextran-based hydrogel exhibited higher storage modulus (~48 kPa), chondro compatibilty and cartilage matrix secretion. [95]

2.1.11. Chondroitin Sulfate Based Gel

Polyelectrolyte chondroitin sulfate, renowned anionic polysaccharides composed of disaccharide units consisting *β*-(1→4) D-glucuronic acid and *β*-(1→3) *N*-acetyl galactosamine with sulfate group glycosaminoglycan. Being major matrix components of cartilage, the presence of chondroitin sulfate empowered constricted strength of the scaffold through proteoglycan secretion. Combination of chondroitin sulfate with other synthetic or natural polymers like PEG through its reactive hydroxyl and carboxyl functional groups lead to gel formtion.[96] For example, injectable biomimetic hydrogels was generated from collagen type II (Col II) and activated chondroitin sulfate under physiological conditions without addition of any catalysts or crosslinker.[97]

2.1.12. Carrageenan Based Gel

Carrageenan is a linear hydrophilic polysaccharide composed of sulfated disaccharides with (1→3)-linked *β*-D-galactose and (1→4)-linked *α*-D-galactose units, which could be altered into the 3, 6-anhydro derivative depending on the extraction situation and starting materials. Due to structural resemblance to glycosaminoglycans, a large scientific attention has been paid to carrageenans based gel. *κ*-Carrageenan (kappa) extracted from *Kappaphycus cottonii* results strong rigid gels. On the contrary, elastic, dry, soft gels were prepared by the iota (*ι*-type) in the presence of calcium ions. Rigidity of this hydrogels can be monitored by changing potassium concentration. Another interesting property possessed by this polysaccharide-based gel is temperature triggered sol-gel transformation along with ionic gelation, since carrageenan can show upper critical solution temperature.[98]

2.1.13. Pullulan Based Gel

Pullulan, a component of the cell wall in the yeast-like fungus *Aureobasidium pullulans*, composed of linear maltotriose oligosaccharide connected through *α*(1→4) and *α*(1→6)

glycosidic bonds. Chemical functionalization or mixing with other organic or inorganic materials could transform highly water-soluble pullulan to gel with enhanced stability. Carboxymethylated pullulan conjugated with heparin and hydroxyapatite/pullulan/dextran composite has been developed with tissue regenerative ability.[99]

2.1.14. Xylan Based Gel

Xylan is a natural, biodegradable polysaccharide composed of arabinose, 4-*O*-methyl-glucuronic acid and xylose in a ratio of 1:2:11 respectively.[100] Low molecular weight and high degree of side chain substitution could not lower water solubility of xylan. Hence, hydrogel network was formed through crosslinking from hydrophilic xylan polymer.[101] Modification of carboxylic groups present in glucuronic acid residues could be used for transfoming gel. *In situ* injectable xylan-tyramine gel through enzymatic crosslinking using HRP and H_2O_2 has been synthesized.[102]

2.1.15. Curdlan Based Gel

Curdlan, composed of (1 → 3)-linked *β*-D-glucose, is a crystalline polysaccharide with high molecular weight over 1,000,000. Its unique gelation ability caused by heating or neutralization of its alkaline solution is well known. A novel curdlan hydrogel was recently reported through chemical cross-linking. This gel exhibited high compression ability and exceptional shape recovery capacity. Variable cross-linker such as ethylene glycol diglycidyl ether (EGDGE, C2), 1, 4-butane diol diglycidyl ether (BDDGE, C4), and 1, 6-hexane diol diglycidyl ether (HDDGE, C6) were recently utilized for this type of gel synthesis.[103]

2.2. Supramolecular Gel

Supramolecular hydrogels are generally formed through noncovalent interactions. Though it is similar as polymer hydrogel, different physical and chemical properties have to be taken in consideration in terms of three-dimensional entanglement, thermal stability and reversibility. Unlike to the typical molecular gels, thermal stability at lower temperature is an essential characteristic for supramolecular gel.[104] Again completely reversible sol-gel transition of these gels fascile desired biomedical applications harmonizing to exsisting polymer driven soft materials.[105] Generally polysaccharide based supramolecular gel can be prepared through host-guest interaction by accommodating organic/inorganic guest molecules, where cyclodextrins

(CDs), cyclic oligosaccharides extracted through enzymatic hydrolysis of starch and was reported to act as a potential host. The polar hydrophilic external surface and hydrophobic internal cavity are the major characteristics of CDs. Again, it contains numorous hydroxyl groups with variable reactivity. CDs can be classified into three categories namely α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), and γ-cyclodextrin (γ-CD). They can be differentiated by the number of glucose subunits. Some reports regarding supramolecular polysaccharide gels are demonstrated here. A supramolecular polymer coassembly, composed of Fmoc-tetrapeptide and light-responsive arylazopyrazole (AAP), was mixed with β-CD vesicles (CDVs) to result supramolucar gel by using host–guest chemistry.[106] Several iclusion complex based supramolecular polysaccharide gels were reported. For example, development of inclusion complex between PEG grafted dextran and α-CDs could originate a supramolecular hydrogel.[107] Another report showed star-shaped poly-*N*-isopropylacrylamide (PNIPAM) polymer with a β-CD molecule forming supramolecular self-assembled architectures through mixing with adamantly terminated eight-arm PEG polymer. Inclusion complexation between the β-CD molecules and the adamantyl groups played the major role here.[108] Hence inclusion triggered supramolecular architecture resulted from α-CD conjugated curdlan with photoirradiated gel–sol transition at 365 nm. Mixing of α-CD and PEG-terminated poly(amino amine) dendrimer bearing NIR-active platinum (Pt) nanoparticles in the core resulted self healing supramolecular network.[109] Poly(acrylic acid) functionalized cyclodextrins (pAA-CDs) (host) and pAA modified with ferrocene (pAA-Fc) (known for its redox-responsive properties) (guest) fabricated an interesting system upon addition of oxidant sodium hypochlorite (NaClO).[110] Again mono-carboxylated PEG modified chitosan was combined with α-CD resulting thermo-responsive supramolecular hydrogel leading to supramolecular gel.[111]. Hence it is already established that supramolecular gels could respond to various chemical (pH change, ionic, etc.) and physical (light, sonication, mechanical force, etc.) stimuli along with reversible phase transitions resulting advantageous bioapplications. For example, hydroxypropyl methyl cellulose (HPMC)-based pH-triggered *in situ* gel containing HP-β-CD-drug inclusion complex exhinited a novel nasal delivery of Paliperidone (PLPD).[112] Simply mixing of β-cyclodextrin-modified chitosan (CS−CD) with $AgNO_3$ under basic condition leads to a stable supramolecular hydrogel resulting high antibacterial and wound healing capacity (Figure 2).[113]

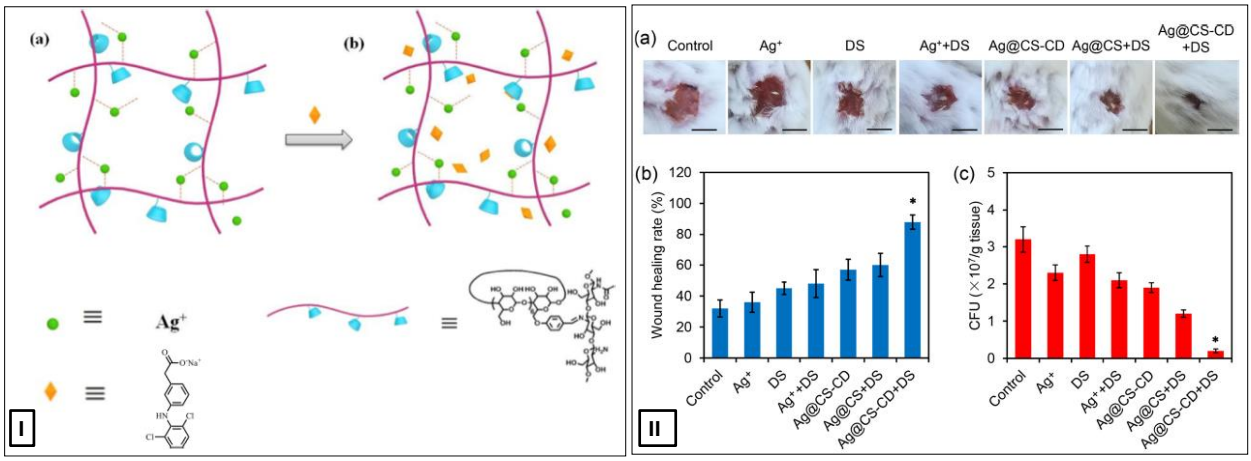

Figure 2. (I) (a) Illustration of the Prepared Hydrogels through Supramolecular Complexation (b) Illustration of the Supramolecular Hydrogels Loading Anionic Drugs. (II) In vivo antibacterial and wound healing capacity of the hydrogels in the mouse wound-infection model. (a) Images of wounds on mouse back in different treatments after 6 days of treatment. Scale bar = 0.5 cm. The calculated areas of each wound were 0.56, 0.5, 0.33, 0.25, 0.17, and 0.08 cm^2 from light to right. (b) Wound healing rate of different groups. (c) Bacterial numbers of different groups in wound tissues evaluated by colony forming unit (cfu) assays. Reproduced with permission from ref 113. Copyright 2021 American Chemical Society.

High attention has been paid in low molecular weight gelators (LMWGs) over recent dates in this regard, due to multi-stimuli responsive properties, which can lead to higher flexibility for the creation of smart materials.[114] The unique properties of LMWGs like reversible gel formation in different solvents arise from its lower molecular weight less than 2000 D. The resulting gels processed through non-covalent driving force including hydrogen bonding, hydrophobic interactions, π- π stacking, and van der Waals interactions, hence termed as physical gels or supramolecular gels. Several common monosaccharides and oligosaccharide units like D-glucose, D-glucosamine, *N*-acetyl-D-glucosamine, D-lactose, D-maltose based LMWGs are exceptionally good in this regard due to high biocompatatability, biodegradability (Figure 3). For example, severals sugar building blocks starting from D-glucose has been synthesized through functionalization with triazole, alditol. Similarly, derivatization of glyconamide at annomeric position or C-2/3 position from methyl glycosides leads to glycocluster formation and results LMWGs which leads to supramolecular assembly. Enzyme-responsive supramolecular hydrogel has also been reported using LMWGs.[115] Glucoside-introduced supramolecular hydrogel in

response to a protein is wellknown. Lactose containing amphipathic ureas forms LMWHGs, aimed at site-specific drug release in the small intestine.[116,117]

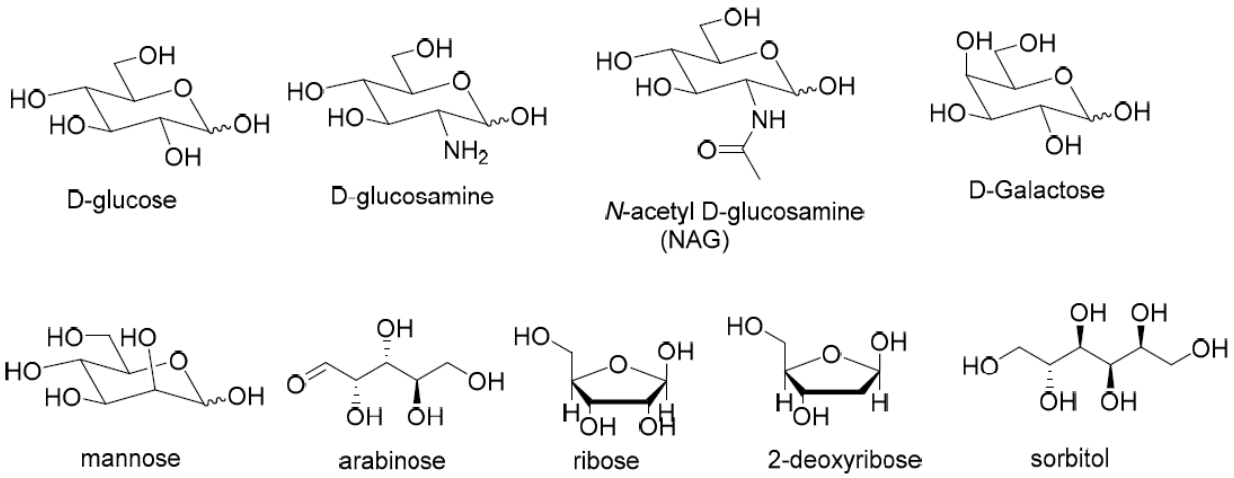

Figure 3. Structures of the sugar starting materials used often for designing low molecular weight gelators (LMWGs). Reproduced with permission from ref 105. Copyright 2021 MDPI.

2.3. Microgel and Nanogel

Depending on the gel particle dimension, the classification of micro and nanogel was made and today's biomedical field is basically ruled by those materials. Microgels are the hydrogels on a microscopic scale and nanogels are on a submicron scale. Now the basic question arises what are the properties which make them so evolitionary to modernize medical procedures. Basically those materials are more interesting compared to their bulk analogue due to smaller particle size,[118] superior encapsulation efficacy of many therapeutics, such as proteins, genes, drugs and contrast agents, enhanced colloidal stability,[119] inertness which fascilitates drug delivery and gene therapy. Again, they respond faster to their surroundings and effectively circulate in the blood to arrive at target sites after injection. Their high interfacial area per unit mass leads to higher exchange rate.[120] These exceptional characteristics add a new dimension to polysaccharide-based gel research field. A detail consideration about polysaccharide micro and nanogels are discussed in this regard.

Polysaccharide based microgels are physically cross-linked polymer of colloidal size between 1 and 1000 nm and leads to soft and porous architecture. This physical entrapment by cross-linking into a polysaccharide-based hydrogel network might happen *via* hydrazide

aldehyde interaction; afterward, this hydrazide-functionalized microgel transformed to covalently crosslinked bulk hydrogel.[121] Generally they are distinct particles with colloidal dispersions ability and good swelling capacity depending on cross-linking density, synthetic process, initial monomer concentration, composition and solvents. General synthetic methods used generally are anionic copolymerization, emulsion polymerization in presence or absence of sufactant, precipitation method, inverse microemulsion polymerization, cross-linking of neighboring polymer chains (Figure 4).

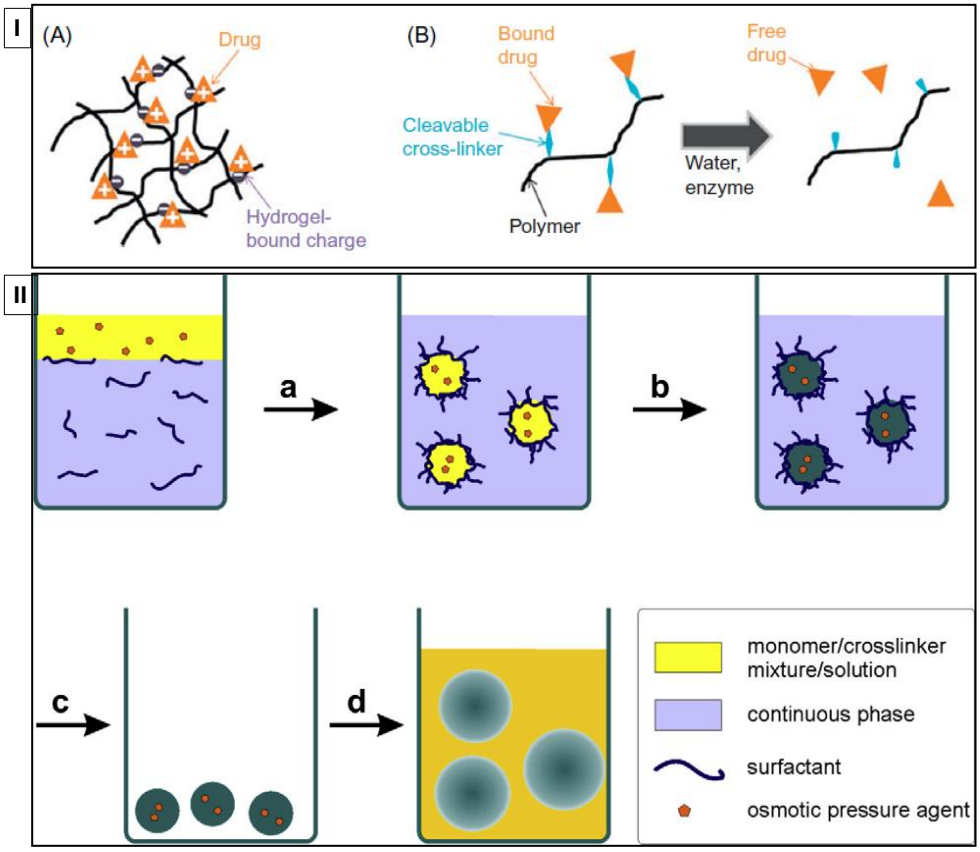

Figure 4. (I) Physical (A) and chemical (B) strategies for enhancing the interaction between a loaded drug and a polymeric gel to slow drug release. Reproduced with permission from ref 121. Copyright 2019 Elsevier. (II) Schematic representation of microgel preparation by radical crosslinking polymerization in (inverse) miniemulsion: (a) emulsification and homogenization, (b) polymerization, (c) removal of excess surfactant by washing/dialysis and subsequent freeze-drying and (d) redispersion of microgels in a good solvent for the network-forming polymer by swelling. Reproduced with permission from ref 122. Copyright 2012 Elsevier.

Thus, several wellknown polysaccharides like dextran, gelatin or chitosan formed microgel materials by using those methods.[122] Chitosan can form microgel through self assembly or derivatization. Dual stimuli (temperature and pH) responsive microgel was reported through copolymerization of ionizable chitosan with poly(*N*-isopropylacrylamide).[123] UV-crosslinkable and injectable chitosan based microgel has been synthesized by Wang *et al*.[124] Reversible binding of lectin metalloprotein, conA to glucose and mannose with high affinity resulted microgel.[125]

On the other hand, polysaccharide based nanogels are basically physically or chemically crosslinked nanosized polymer particles and can be prepared through nanoemulsions and nanosuspensions. Synthetic apporoach of several polysaccharide nanogels are as follows. Monodisperse dextran nanogels were synthesized through the self-assembly of amphiphilic poly (D-/L-lactide)-grafted dextran.[126] Combination of azobenzene and dextran was reported by Patnaik *et al*. and lead to azodextran-based nanogels by a self-assembly physical technique.[127] Aguirre *et al*. demonstrated emulsion polymerization process followed by electrostatic interaction to produce cationic and biodegradable polyvinyl chloride (PVCL) & polydiethylaminoethyl methacrylate (PDEAEMA) based core-shell nanogels utilising dextran-based macro-cross-linkers. Positively charged core-shell nanogels could interact with the negatively charged siRNA after loading and exhibited a charge reversal in zeta potential values. Another dextran based cationic nanogels combination with (2-(methacryloyloxy)-ethyl) trimethyalammonium chloride are also reported. Thus, stimuli responsiveness can be introduced including thermo-responsive PVCL and pH-responsive PDEAEMA, and dually thermo- and pH-responsive PDEAEMA/PVCL-based core-shell nanogels. These syntheses are basically driven by utilizing different biocompatible and biodegradable dextran-methacrylates as macro-cross-linkers. Modern studies show that bio-orthogonal and reversible reaction play important role to synthesize multistimuli-responsive dextran based nanogels. These reactions mainly fascilitated the nanogel preparation through formation of a polyhydrazone network by the cross-linking of nanodroplets obtained from functionalized dextran with *N*-reactive carbonyls. These systems would be oxido reductive stress and pH sensitive, as disulfide groups are exhibits reducing environment responsiveness.[128] Nanogels possessing dextran and oligolactide (OLA) chains connected through disulfide bonds (Dex-g-SS-OLA) were reported as an efficient drug delivery syntem. Galactose (Gal) based nanogel was reported leading to receptor-mediated endocytosis.

Another report shows, colloidal chitin nanogels has been prepared in calcium chloride solution with saturated methanol.[129] Nanofibrous microsphere with high cellular affinity resulted from chitin in NaOH/urea.[130] Chondroitin sulfate-nisin nanogel with variable morphology and different loading capacity was reported with electrostatic complexation.[131] Chondroitin sulfate based nanogels with enhanced solubility was prepared through direct crosslinking.[132] The major component for synthesizing these nanogels was *N*-diethylamino-4- hydroxymethylcoumarin (CM) and functional modification HA could enhance the selectivity towards cancer cells.[133] According to sevelar reports, HA is an extensively used polysaccharide for nanogel preparation with biomedical applications. HA based nanogels with good immunocompatibility and hemocompatibility could be prepared *via* radical copolymerization, emulsion and precipitation polymerization through functionalization with thiolated hydrophobic molecules.[134] Fluorescent HA-iodixanol nanogels (HAI-NGs) were synthesized by Zhu *et al*. and used for targeted X-ray computed tomography (CT) imaging and chemotherapy.[135] Synthesis of injectable nanocomposite temperature responsive gel from andhydroxypropyl methylcellulose (HPMC) as a matrix with nano-sized inorganic filler and biphasic calcium phosphate (BCP) has been reported.[136] Wu *et al*. demonstrated injectable nanogels with poly(NIPAM), poly(3-acrylamidophenylboronic acid) using maleic acid–dextran as a crosslinker with monodisperse property.[137] Another NIPAM and polysaccharide based hybrid nanogel was reported through chemically cross-linking with alginate used for as efficient anticancer drug delivery. Doxorubicin loaded DNA aptamer linked myristilated chitosan nanogel, Chitosan/albumin hybrid nanomaterials have also been explored by renowned research groups with anticancer drug delivery applications.[138] Some reports include formation of nanocomposite gel from analogous bulk structure. For example a novel chitosan-based thermosensitive hydrogel using a sol-gel method has been synthesized and by adding silica/calcium phosphate (SiCaP) nanoparticles it was transformed to nanocomposite hydrogels including chitosan and β-glycerophosphate (Ch-β) as a matrix.[139] Modification of an injectable thermoresponsive hydroxypropyl guar-graft-poly(*N*-vinylcaprolactam) (HPG-g-PNVCL) copolymer with nano-hydroxyapatite covalently crosslinked *via* divinyl sulfone (DVS) lead to HPG-g-PNVCL/n-HA/DVS as an efficient nanocomposite thermogel acting as a biocompatible scaffold for osteoblastic cell growth.[140] Glucose responsive nanogel based on electrostatic interaction between chitosan and alginate was reported with potential bioutilizations.[141] Modern research indicates the development of a new concept for

brachytherapy based on intrinsically radiolabeled gold-palladium (AuPd) alloy nanoparticles, followed by functionalization with carbohydrate-ester based liquid. Thus, the system was transformed to biodegradable ingectable nanogel allowing lower administration through small-gauge needles. Dispersion of nanoparticles in ethanol along with water insoluble carbohydrate esters resulted "nanogels" (Figure 5).[142]

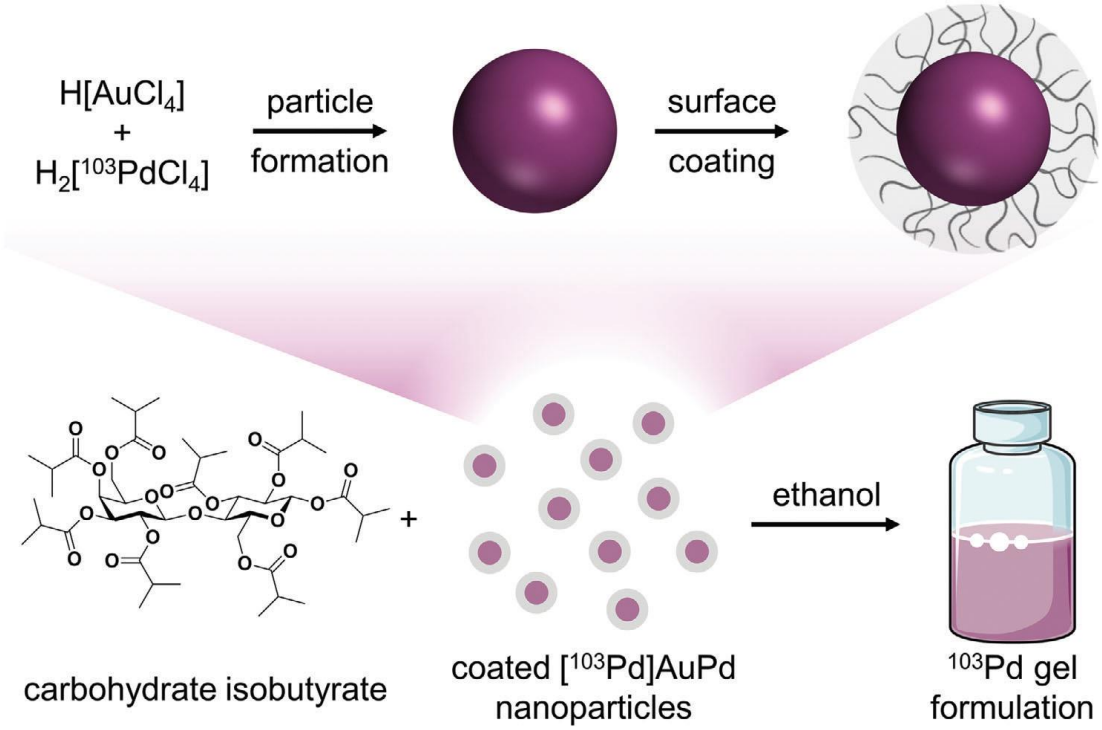

Figure 5. Preparation of the ^{103}Pd-nanogel formulation for immobilization of ^{103}Pd-containing AuPdNPs. [^{103}Pd] AuPdNPs were prepared from their chloride salts using trisodium citrate. Surface coating of the particles was carried out with thiol-terminated PNIPAAm. The liquid ^{103}Pd-nanogel formulation was prepared by adding the [^{103}Pd] AuPdNPs directly to a premixed solution of LOIB or SAIB in ethanol. Reproduced with permission from ref 142. Copyright 2021 Wiley.

As per the last section, these materials have been used in several biomedical applications. Hence sterilization is very important in this regard. A novel approach to get purified nanogel is autoclave method at high temperature and pressure. Montanari *et al*. reported the synthesis of gellan- and HA-cholesterol derivatives followed by dispersion in aqueous solutions, and then, sterilization through autoclave leads to pure polymeric nanogels.[143] An interaction between

hydrophobic cholesterol moieties and hydrophilic polysaccharide chains under autoclave condition (temperature (121 ^{o}C) and pressure (1.1 bar) for 20 min) was explored.

3. Properties

Texture and properties of hydrogel scaffold always play important roles in terms of application hence attracted increased interest in recent years. The major characteristics like *in situ*, injectable and self healing properties attracted scientist's attention while capturing the biomedical viewpoint. Recently modification through fictionalization of naturally occurring polysaccharides could introduce multiple properties in gel making it more feasible to several bioapplications. For example functionalization of dextran and cellulose to hydrazide-modified carboxymethyldextran (CMDX–ADH) with aldehyde-modified dextran (DX–CHO) or carboxymethylcellulose (CMC–CHO) followed by simple mixing lead to *in situ* crosslinking injectable gel with adhesion prevention.[144] The following section of this review focuses on the process of incorporation of these properties to polysaccharide based gels and how one property affects the others and their superiority majorly affects biomedical field in comparison to traditional synthetic gel.

3.1. *In Situ* Gelling

In situ is a Latin phrase, can be translated literally as 'In process'. Now a days, *in situ* gelling hydrogels are extensively studied due to their sustained release capacity of protein-based therapeutics majorly and reduced drug administration time. The major motivation to develop this property into gel is introducing efficient and realistic embeded platforms unlike traditional hydrogels, specially require for surgical interventions to provide medicine applications. Generally, *in situ* gels are administered into the body in sol form and transferred to gel *in situ* within body system. Controlled drug devery nature was generated by its special 'sol gel transition' which resulted lower dose drug requirement with zero accumulation, zero side effects and lower wastage of drug. Increased residence timing not only lower down the dose frequency and drug toxicity, but drug administration to unconscious patients also become much easier today. Thus, the non persistent chatacter with high localization ability irrespective of size, shape or irregularities include several advantages in research field. On the other hand, bioadhesiveness accompanied with *in situ* gelling propertis fascilitated drug targeting through mucus membranes.[145] Synthesis was triggered without any need of toxic chemical cross-linker, physico-

chemical dispensation or physical stimuli like pH change or heat. Simple click reactions like shiff base, azine alkyne reactions; several physical interactive forces like electrostatic, vanderwaals or steric basically fascilitated *in situ* gel formation. The reversibility was introduced through these interactions mainly, where sol-gel transition could be regulated by shear. This shear thinning properties included potential cell delivery properties.

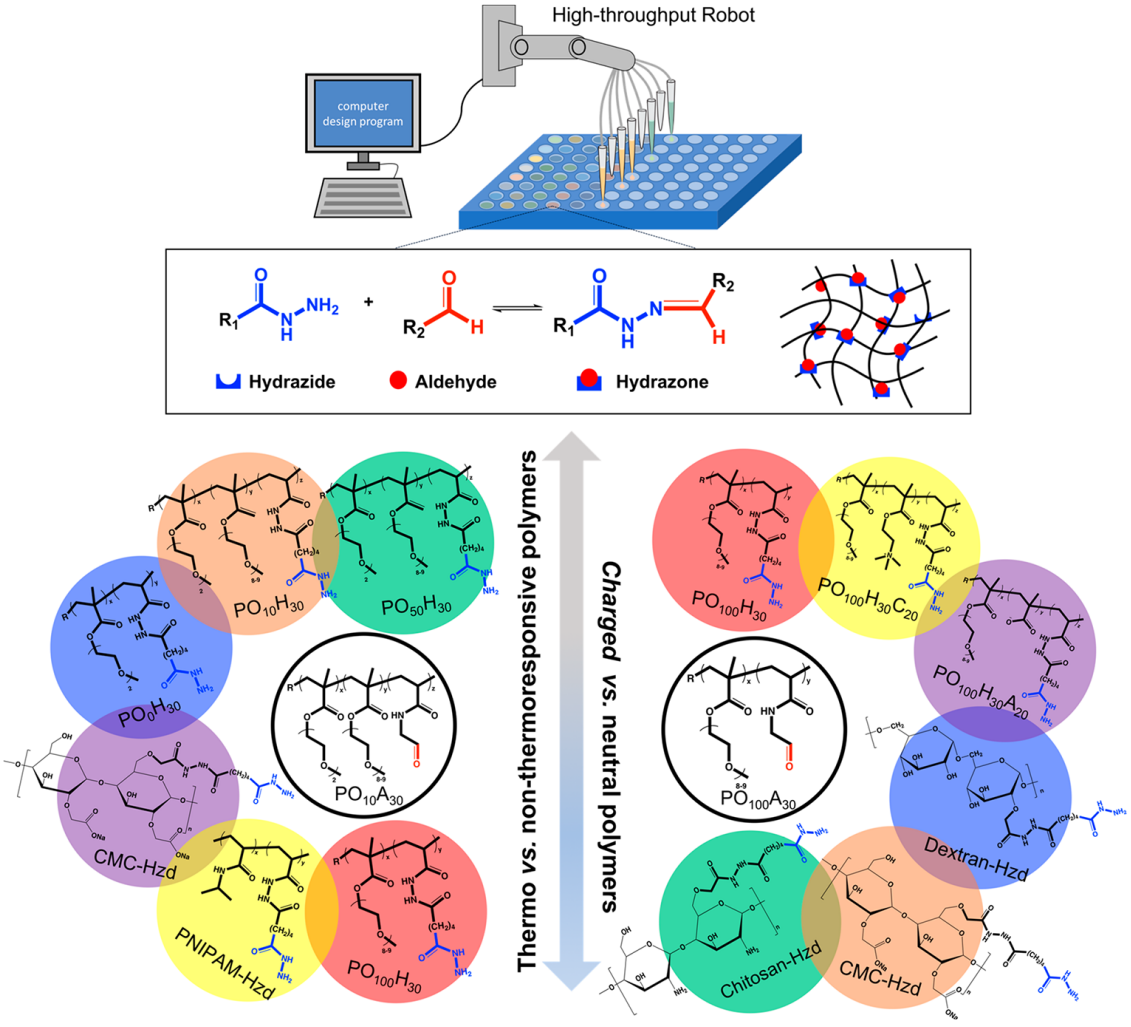

Figure 6. Schematic of the high-throughput robotic fabrication approach and the structures of the hydrazide and aldehyde-functionalized polymer precursors used for hydrogel preparation. Reproduced with permission from ref 148. Copyright 2020 American Chemical Society.

In recent years, natural polysaccharides based *in situ* hydrogels attracted great attention in research due to their promising properties. Numerous physiological trigger such as temperature,

pH or ion concentration majorly need for *in situ* gel formation within body system. Most common polysaccharides like gellan gum, guar gum, xanthum gum, xyloglucan, pectin, alginic acid, cellulose, chitosan and their derivatives were reported as *in situ* gelling scaffold. Wang *et al*. synthesized a novel polysaccharide-based hydrogel, which was manufactured by *in situ* crosslinking of starch-based nanoparticles and polyvinylamine. This report shows, starch was functionalized with cholesterol and aldehyde group to utilize as a potential platform for doxorubicin release. [146] Again, simple blending of arboxymethyl hexanoyl chitosan/HA produced *in situ* gel with injectable property.[147] Another study describes proper optimization of hydrazide functionalized dextran, chitosan or carboxymethyl cellulose with thrmoresponsive PNIPAM and poly(oligo(ethylylene glycol) methyl ether methacrylate (POEGMA) functional copolymers generated several *in situ* smart hydrogel materials by using high-throughput technique (Figure 6).[148] One pot triple network hydrogel of chitosan and HA (HA-triazole/CS-Cu(II) gel) was synthesized by triazole linkage, metal-coordination, and chitosan-HA polyion complexation using Cu(I) azidealkyne click chemistry (CuAAC).[149] By regulating the ratios of glucono-D-lactone (GDL) and pyridoxal 5′ - phosphate (PLP) in alginate solution mixtures (ASMs) containing paliperidone palmitate (PPP), $CaCO_3$, GDL and PLP; an *in situ* polysaccharide gel with clinically acceptable injectability and gelation time has been reported recently.[150]

3.2. Injectable Properties

A hydrogel possesses injectable properties when various hydrogel components, introduced through a syringe to a specific site in vivo, lead to formation of three-dimensional scaffolds under physiological condition. All common polysaccharides like chitosan, pullulan, starch, cellulose, gellan gum, cyclodextrin (CD), HA, alginate, chondroitin sulfate, pectin and heparin are widely utilized for the synthesis of injectable hydrogels because of ready availability, biocompatibility and biodegradability and acting as as injectable carriers for cells. This system can overcome difficulties with conventional hydrogel-based drug delivery structures in the clinic with a single injection, resulting patient's relief. Cells, nanoparticles, drugs, proteins, or other biomolecules can be blended with the precursor polysaccharide solution earlier to injection.[151] Thus functionalized polysaccharide and other ingradients injected as liquids and transformed further to solid gel where the triggered component was entrapped through covalent cross-linking

and delivered at the targated side in vivo *via* reversible or irreversible sol gel transition under physiological condition. All *in situ* forming gel can show injectable property. Several physical cross-linking interactions between the chains of pre-gel polymers triggered by physiological stimuli like particular pH, temperature or ionic strength to drive the phase transition. Some major physical interactions responsible for introducing injectable properties are electrostatic, hydrophobic, stereochemical, supramolecular.[152] Some literature regarding synthesis and utilization of polysaccharide based *in situ* gels are as follows. Functionalization of chitosan with carboxymethyl group lead to generate several injectable gels. Another *in situ* injectable gel with potential would healing was reported by polyelectrolyte complexation of carboxymethyl chitosan (CMC) and alginate.[153] Enzymatic crosslinking reaction resulted chitosan/PEG hydrogel through injection *via* HRP.[154] Functiona modification resulted chitosan-hydroxyphenyl propionic acid and PEG-tyramine (PT) followed by gel formation. Several biomedical applications like excellent hemostatic ability with tunable physicochemical and tissue adhesive properties were originated from this biocompatible injectable gel (Figure 7). Again, dual-crosslinked alginate/CMC based hydrogels containing *in situ* synthesized calcium phosphate (CaP) particles were reported through combined interaction of physical crosslinking and ionic crosslinking of alginate with divalent cation (Ca^{2+}). Cation of amino groups on CMC and anion of carboxyl groups on alginate fascilitated electrostatic interaction *in situ*, majorly results injectable ability and drug-encapsulated efficacy.[155] Schiff's base reactions are extensively used to generate *in situ* injectable gel with stimuli responsive reversibility. Injection of aldehyde xanthan (Xan-CHO) solutions and carboxymethyl chitosan (NOCC) solutions forming *in situ* gel by physical interactions (mostly hydrogen bonding) followed by chemical crosslinking *via* this reaction was demonstrated.[156] Liu *et al*. synthesized an injectable hydrogel based on chondroitin sulfate multiple aldehyde (CSMA) and *N*-succinyl-chitosan (SC) with variable CSMA to SC ratio *via* Schiff's base reaction between the aldehyde groups on CSMA and amino groups on SC under physiological condition.[157] Shear and thermo-responsiveness could be introduced to sodium alginate through grafting by a thermo-responsive copolymer of NIPAM, enriched with the hydrophobic *N*-tert-butylacrylamide (NtBAM) monomers and provided excellent hydrogel injectability with instant gelation capacity at physiological temperature.[158] Nanocomposite chitosan/ Chitosan-modified halloysite nanotubes (mHNTs) hydrogel were developed *via* sol-gel transition with lower gelation time, temperature and higher mechanical strength with bone tissue

engineering applications. For that synthesis mHNTs were synthesized first. Then, icariin (IC) as a bone inducer was loaded into mHNTs (IC@mHNTs), transforming to a sustained drug release system.[159] Injectable chitin hydrogel system through dynamic acylhydrazone crosslinking catalyzed by 4-amino-DL-phenylalanine (Phe-NH_2) with potential self healing nature was reported recently.[160] Thiolated polysachharides like HA (HA-SH) or collagen (COL-SH) has been utilized to generate injectable gel through solubilization of the thiolated polymers in an aqueous medium, mixing the polymer solution with stem cells and initiators, injection to the targated part and generation of gel by crosslinking under the physiological conditions. Degradation was monitored through using dithiothreitol (DTT) and mercapto ethanol as reducing agents to disrupt the disulfide bond.[161]

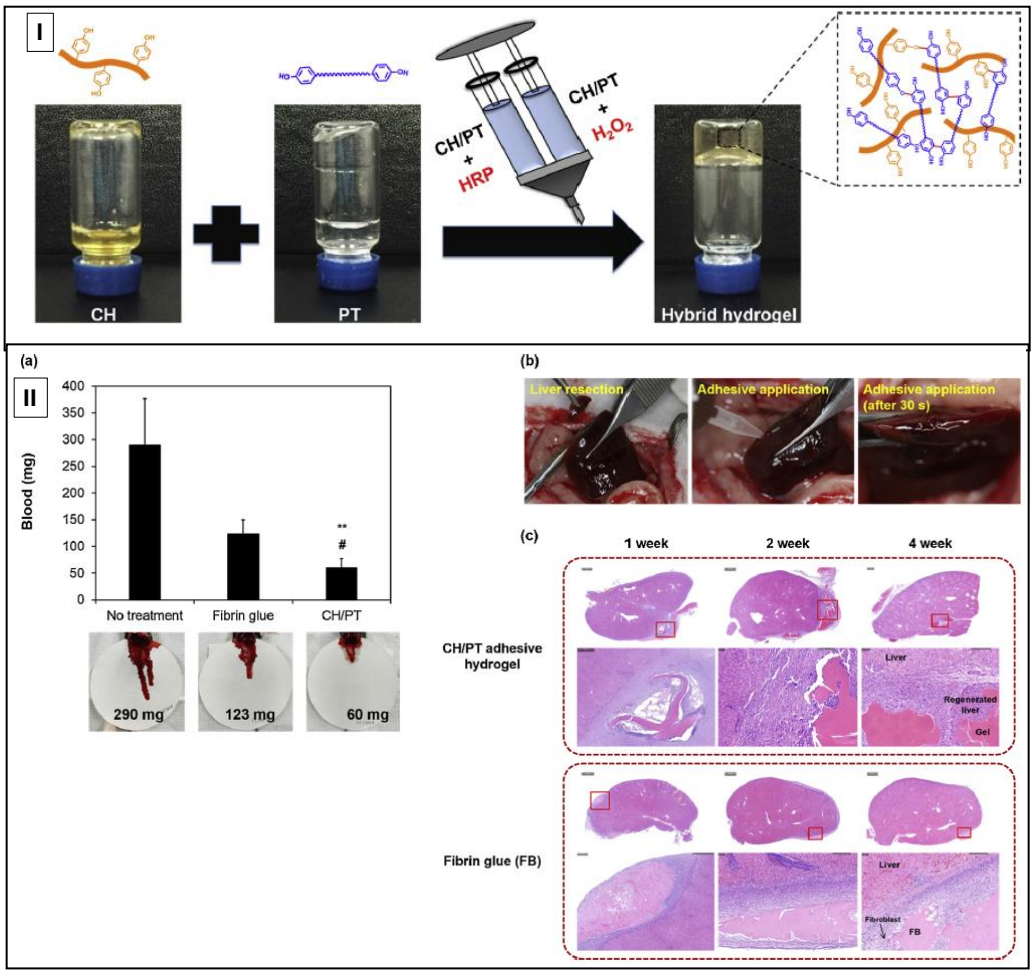

Figure 7. (I) Schematic illustration of HRP/H_2O_2 mediated *in situ* forming CH/PT hybrid hydrogels. (II) *In vivo* hemostatic and biocompatible performance of CH/PT hydrogel. (a)

Hemostatic effect of CH/PT hydrogels (linear PEG 10 kDa) evaluated by the total blood loss from a damaged mouse liver model (n = 3). (b) Images of the resected rat liver before and after application of adhesives (CH/PT hydrogel or fibrin glue). (c) Histological analysis of explants stained with H&E stain after 1, 2 and 4 weeks of adhesive application. (*) indicates a significant difference from the control group, including no treatment (**P < 0.01) and fibrin glue (#P < 0.05). Reproduced with permission from ref 154. Copyright 2021 Elsevier.

3.3. Self Healing Nature

In situ injectable hydrogels lead to in vivo cell protection during operation through preventing mechanical collapses unlike traditional gel materials, but the major problem is that their time-dependent sol-gel transition can not be precisely managed. This problem can be solved through introducing independent self-recovery ability i.c automatic damage control property with external trigger.[162] Those hydrogels are called smart self healing materials. The concept of self healing trough dynamic/reversible linkages is basically imitated from the living nature.[163] For example structural integrity of target tissues can be well regulated by self healing properties resulting potential biomedical applications. Interestingly numorous naturally occurring polysaccharides posess self-repair property originated from either physical or chemical interactions.[164] Physical non covalent mechanism involves hydrogen bonding, hydrophobic, host-guest, ionic interactions, metal-ligand coordination, and p-p stacking, diffusion or swelling.[165] Chemical reactions based on ‘click chemistry’ were majorly reported to introduce self healing nature with reversible chemical bond formation ability. Diels-Alder reaction, imine, acylhydrazone, boronate-catechol complexation, dynamic reshuffling radical reactions and disulfide bond formation along with chemical mechanisms such as ionic or enzymatic crosslinking photo polymerization played important role in this regard. Some specific reports are discussed here. Dynamic Schiff base reaction between amino groups of *N*-carboxyethyl chitosan (CEC) and aldehyde groups of dibenzaldehyde-terminated poly(ethylene glycol) (PEGDA) leads to the self healing hydrogel. [166] A series of pH responsive self healing pectin/chitosan hydrogels can be preaped *via* the Diels-Alder reaction resulting anti-inflammation, antineoplastic activity, nontoxicity, and biospecific degradation (Figure 8).[167] Another example of Schiff base reaction trigerred self healing property is as follows. An injectable self healing hydrogels with chitosan and konjac glucomannan was designed *via* oxidized konjac glucomannan with chitosan through

reversible imine linkages. This biocompatible hydrogels also exhibited adhesive, wound recovery and antibacterial properties against *Staphylococcus aureus* and *Escherichia coli* along with self healing nature.[168] Oxidized dextran has been introduced as a crosslinker *via* dianamic imine bond formation and lead to gelatin based self healing hydrogel with bio-functionality and used for endothelial progenitor cells (EPCs) delivery and healing wounds.[169] Two different types of hydrogels, oxidized hydroxypropyl cellulose/chitosan (Ox-HPC-Chitosan) and hydroxypropyl dextran/chitosan (Ox-HPDChitosan) were synthesized through cross-linking by imine bonds with high self healing capacity.[170]

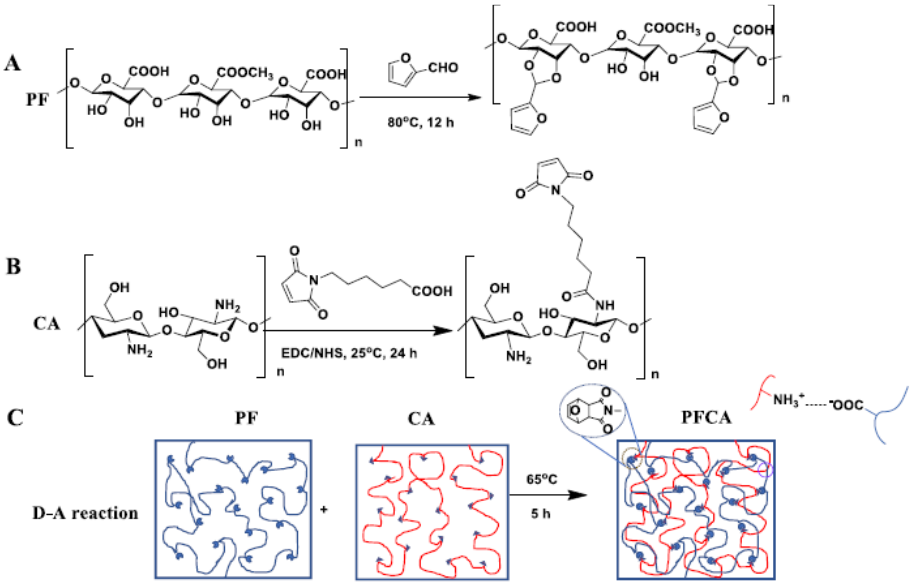

Figure 8. The reaction schematics of (A) synthesis of furan-modified pectin; (B) maleimide-modified chitosan; (C) Fabrication of hybrid hydrogels *via* D-A reaction. Reproduced with permission from ref 167. Copyright 2021 Elsevier.

Biocompatible and biodegradable carboxymethyl chitosan (CMCTS) has been transformed to self repairing injectable hydrogel *in situ* using oxidized hyaluronic acid (OHA) as a crosslinking agent.[171] Their self healing property was demonstrated through injection into soft tissues in experimental animal site. The initial capillary congestion and small inflammation to injection site has been reduced with time automatically as presented by Figure 9. Reversible guest-host interactions between *β*-cyclodextrin (CD) and adamantane (AD) on modified gelatins

was reported as a first approach to afford a self healing material based on a functionalized extracellular collagen protein with wide clinical prospect.[172] Simultaneous multi-functional properties like high stretchability, shear-thinning, pH- and glucose responsiveness, adhesive and re-shaping abilities could be introduced to alginate-boronic acid hydrogel along with self healing nature designated the scientific progress in this field.

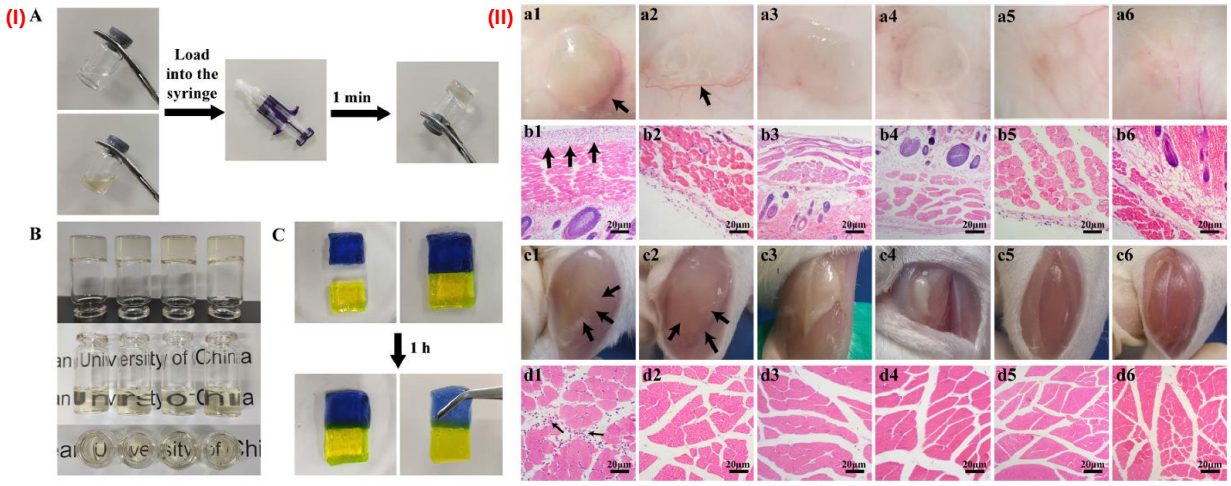

Figure 9. (I) A: OHA and CMCTS were mixed in equal volume by a double mixer to form a hydrogel. B: From left to right were images showing the transparency of CMCTS-OHA1, CMCTS-OHA2, CMCTS-OHA3 and CMCTS-OHA4 hydrogels. C: Macroscopic photographs of the self-healing process of CMCTS-OHA3 hydrogel. (II) The biocompatibility and biodegradability of the CMCTS-OHA hydrogel a: Macroscopic observation of skin at 1 w (1), 2 w (2), 3 w (3), 4 w (4), 6 w (5) and 8 w (6). b: H&E stained images of subcutis tissue at the injection sites. c: Macroscopic observation of muscles. d: H&E stained images of muscles at the injection sites. Reproduced with permission from ref 171. Copyright 2021 Elsevier.

4. Stimuli Responsiveness

When the properties of a hydrogel, such as the swelling nature, structure, mechanical strength can be altered in response to various stimuli such as temperature, pH, redox, enzymes or ionic strength; it can be termed as a ‘stimuli responsive’, ‘environmentally sensitive’ or ‘smart’ hydrogel. These stimuli can efficiently adjust hydrogel scaffold-based delivery system leading to the controlled delivery of therapeutics in a reversible or irreversible pattern.[173] The release profile of a therapeutic has been organized through modification of such stimulus-responsive

properties. Depending on nature of disease, general synthesis of responsive hydrogel system utilized stimuli responsive polymers, also designated as ‘smart’ polymers. Several natural polysaccharides like alginate (enzyme, ionic concentration), chitosan (pH, enzyme) etc. can rapidly respond to stimuli and thier structure with altered sol-gel property. Hence modern literarute shows that HA, chitosan, agarose, carboxymethyl cellulose, dextran, and methylcellulose has been majorly utilized for developing stimuli responsive hydrogel, where a series of synthetic oligomers (based on the thermosensitive polymer like PNIPAM) and functionalized polysaccharides with hydrazide or aldehyde functional groups blended by hydrazone-cross-linking reaction *in situ* through double-barreled syringe hydrogels resulting stimuli responsive nature. Variable ratios of oligomer or polymer precursors with different reactivity were mixed with covalently cross-linked hydrogel networks without any intermediate grafting chemistry to include stimuli responsiveness.[174] Report shows that stimuli sensitive reversible supramolecular polysaccharide-based gel has been tuned by pH responsive glusamine based LMWG. Again, photoresponsive functional groups such as azobenzene was introduced with polysaccharides to afford interesting light sensitive gelators. Recently numorous sensitive injectable polysaccharide hydrogel systems have been reported that are either single, dual or multi- stimuli responsive nature to provide biomedical applications. Each stimuli impression has been explained in details in the following section.

4.1. Temperature Sensitivity

Temperature is one of the most common and widely used stimuli, majorly utilized to develop sensitive injectable hydrogels-based delivery platform. Specific polymers with characteristic lower viscosity at ambient temperature and a higher viscosity as a result of certain temperature enhancement were reported to synthesize thermosensitive *in situ* forming gels. Hence, these gels were liquid at lower temperatures, known as sol form and converted to gel form on above a certain temperature. The system must be transformed to semi-solid state at body temperature for feasible bioapplications such that `sol-gel transition temperature' must be related to physiological condition.[175] Generally biomedical applications are inspired by those polymers having critical temperatures, such as a low critical solution temperature (LCST) (i.e., it is soluble at lower temperature) and an upper criticalsolution temperature (UCST) (i.e., it is soluble at higher temperature); hence show potential phase transion. If they posess a LCST between room

temperature and body temperature, gelation happens through thermally induced self-assembly of polymer chains under physiological condition (Figure 10). This thermal self-assembly *via* non-covalent interactions such as hydrophobic interactions can result at critical gelation temperature. This section demonstrated the role of polysaccharide-based gel to physiological stimuli responsiveness to enhance bioapplicability. They could be conjugated with a thermosensitive synthetic polymer like PNIPAM, glycerophosphate (GP), pluronics in this regard and resulted an *in situ* injectable system.[176] For example HA is not thermosensitive but has been transformed to thermosensitive polymers *via* modification with PNIPAM and pluronics to form thermosensitive polymers. Temperature sensitive hydrogel was prepared from another nonthermosensitive polysaccharide, chitosan, by the addition of glycerophosphate (GP) with high temperature and triggered sol-gel transition property.[177] Cellulose, another well known polysaccharide, has been transferred to thermoresponsive gel through functional modification, such as addition of alkyl groups.[178] Several other polysaccharides such as agarose, amylose, amylopectin, carrageenans, and gellant can also be transferred to thermoresponsive gel. They can undergo a sol-gel transition between room temperature and body temperature. Injectable hydrogels with thermo resonsiveness has been fabricated from HA, corn silk extract (CSE) and nanosilver with potential wound healing capacity.[179] Another report exhibits preparation of thermosensitive gel scaffold from chitosan (CS), nano-hydroxyapatite and collagen (Col), with *in situ* sol-gel phase transition property under physiological condition. These biocompatible substrates undergo proliferation of rat bone marrow stem cells (rBMSCs) in vitro.[180] A novel thermo-induced physical gel can be developed by combination of chitosan and β-glycerol phosphate disodium salt (β-GP).[181] Functionalization of polysaccharides through molecular engineering result stimuli responsiveness regulated by nature of induced moiety. For example post modification of alginate by conjugating temperature-responsive poly (ε-caprolactone-co-lactide)-*b*-poly(ethylene glycol)-*b*-poly(ε-caprolactone-co-lactide) and Ophosphorylethanolamine as phosphorylation functional groups resulted temperature responsivity.[182] Again, glycidyl methacrylate-modified hydroxypropyl chitin was transformed to photo crosslinkable hydrogel with reversible thermoresponsive property even at low concentration (2 wt% in PBS).[183] A series of novel thermosensitive hydrogels containing chitosan (CS) and acid-soluble collagen (ASC) were demonstrated in the presence of α, β-glycerophosphate. Their efficacy to imitate extracellular microenvironment for tissue regeneration was explored.[184] A new class of polysaccharide-based

thermogelling hydrogels with promising properties has been trigerred from *N*-acyl glycol chitosans (AGCs). Their high physical stability and fast gelation kinetics lead to various biomedical applications such as injectable drug delivery, cell encapsulation, and 3D cell culture.[185] Blending with HA enhanced their binding affinity and physical property. For example mixing of hexanoyl glycol chitosan (HGC) as a thermogelling AGC with acetylated HA (AcHA) leads to thermo triggered gel with irreversible sol gel transition.[186]

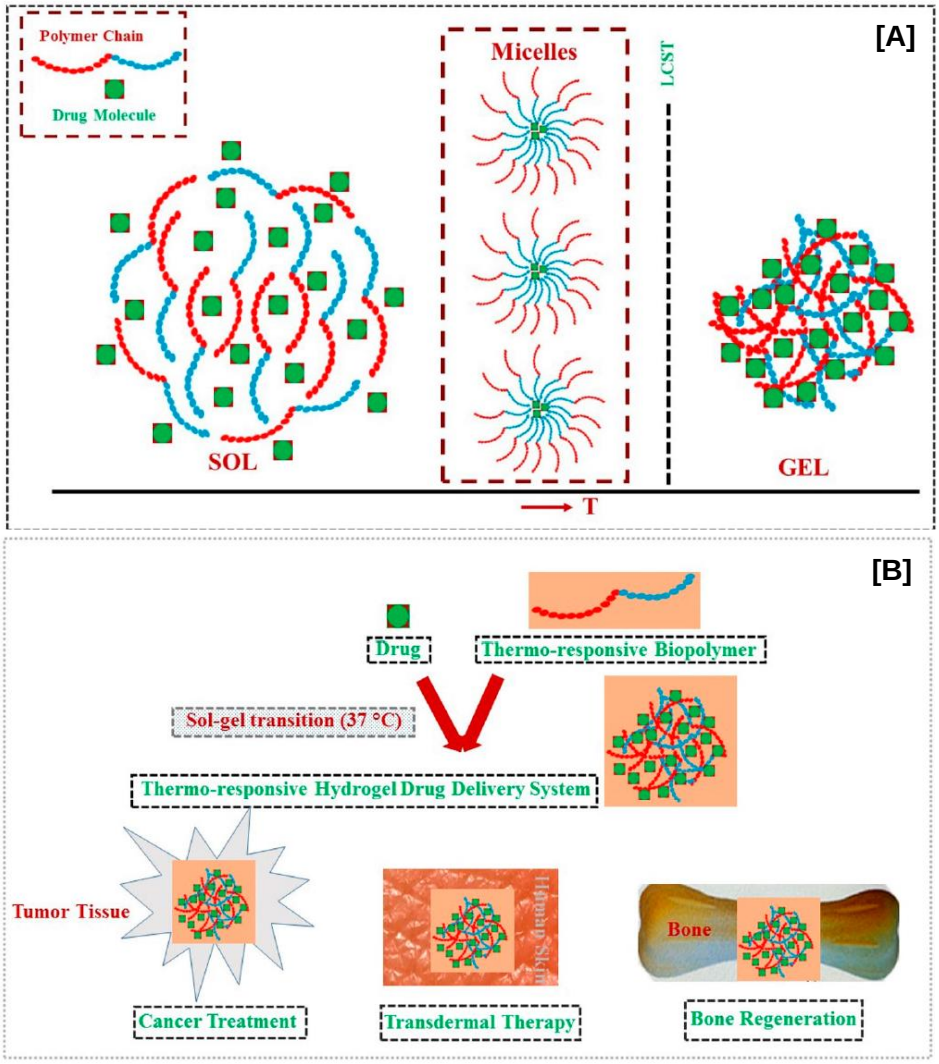

Figure 10. [A] The sol–gel transition of LCST-type thermo-responsive polymer-based drug delivery system (Schematic presentation). Formulation over LCST changes to hydrogel from the solution state. LCST-type thermo-responsive polymer in solution forms micelles at low concentration and that further aggregates at high polymer concentration to form gel at a temperature (≥LCST). [B] The formation of drug-loaded biopolymer-based thermo-responsive

hydrogel system *via in situ* gel formation and its bio-medical applications including cancer treatment, transdermal, and bone regeneration. Reproduced with permission from ref 176. Copyright 2021 MDPI.

4.2. pH Sensitivity

Another widely used stimulus for the preparation of an injectable hydrogel is pH. This stimulus can play an important role in preparation and purification of hydrogel device formation, swelling, therapeutic release and degradation rate of gel matrix. If ionization or de-ionization under physiological condition regulates the phase transition of a polymer system, they can be used to prepare pH sensitive gel. In polysaccharide chemistry, pH sensitivity could be introduced to gel system along with *in situ* injectable properties with enhanced applications in biomedicine field. Major polysaccharides used for this purpose are chitosan, alginate and cellulose through derivatization or blending. The detail discussion is as follows.

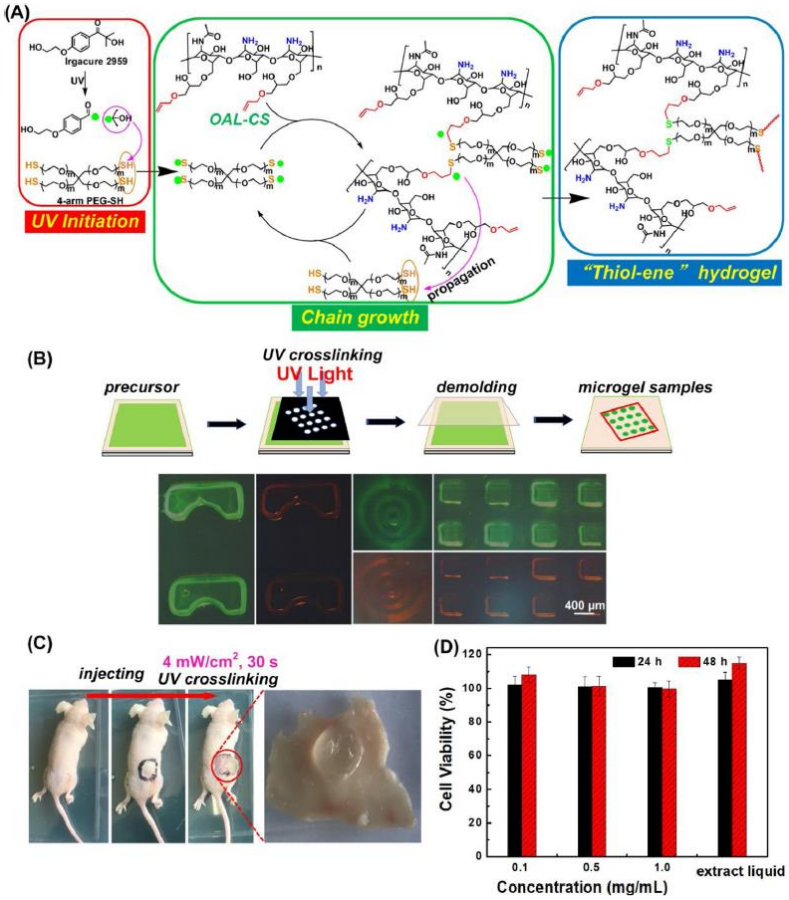

Figure 11. UV crosslinking of OAL-CS and 4-arm PEG-SH *via* UV trigged "thiol-ene" click chemistry to form patterned microgels and *in-situ* forming hydrogel *in vivo*. (A) Schematic

mechanisms of “thiol-ene” click chemistry in which enabled the rapid efficient hydrogel formation; (B) patterned OAL-CS microgels with spatiotemporal designability; (C) *in-situ* hydrogel forming *in vivo*; (D) cell viability of OAL-CS solution and the extractive liquid of hydrogel. Reproduced with permission from ref 187. Copyright 2021 Elsevier.

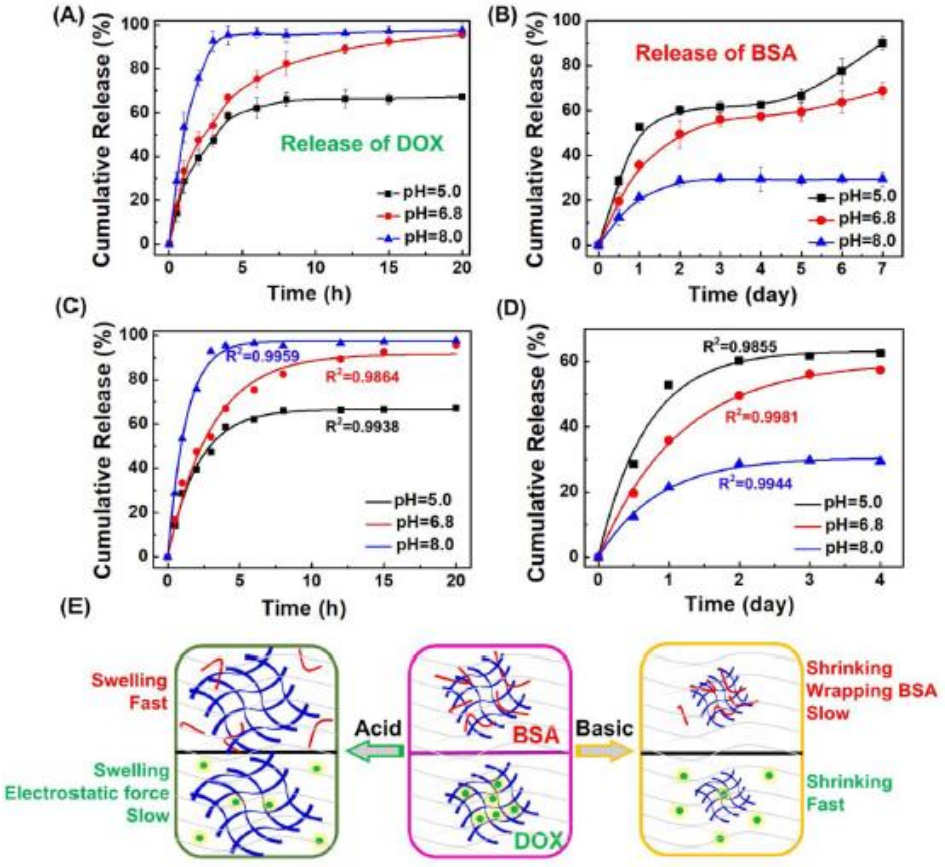

Figure 12. *In vitro* drug release of small molecular drug DOX and macromolecular drug BSA from OAL-CS hydrogel (Mallyl: MSH = 10:1) in PBS at pH = 5.0, 6.8 and 8.0, respectively. (A) The release kinetics of DOX from the OAL-CS hydrogel with different pH values after a regular interval of time at 37 ^{o}C; (B) the release kinetics of BSA from the OAL-CS hydrogel with different pH values after a regular interval of time at 37 ^{o}C; (C) the curve of the First-order kinetics model of DOX release; (C) the curve of the First-order kinetics model of BSA release; (E) schematic representation of the release of micromolecular DOX and macromolecular BSA under different pH conditions, in which the red lines represent BSA and the green dots represent drug DOX. Reproduced with permission from ref 187. Copyright 2021 Elsevier.

Chitosan is a pH-sensitive polysaccharide which can exhibit sol-gel transition by increasing the pH above its p*Ka* (about 6.3). Protonation of its amino groups in presence of mild acids enhanced solubility, hence transfered to sol form. Again, it is insoluble at a higher pH as repulsive electrostatic forces are neutralized. Basically, the gel formation was trigerred *via* electrostatic and hydrophobic interactions. Several reports exhibit that pH responsive chitosan-based hydrogels have been designed through crosslinking along with self healing nature. Glutaraldehyde or benzaldehyde modified PEG based synthetic polymers were reported as crosslinkers in this regard. A pH-responsive UV-crosslinkable chitosan based patterned hydrogel on silicone surface has been fabricated *via* 'thiol-ene' click chemistry followed by fabrication by micro molding.[187] Their *in situ* gel forming capacity with a mouse model and cytocompatability was futher explored (Figure 11). Again, active release behavior of doxorubicin (DOX) and bovine serum albumin (BSA) in different pH medium was demonstrated by Figure 12.

Alginate is another well known polysaccharide system to develop pH sensation within gel system. Presence of carboxylic groups in alginate structure grants to this system a potential sensitivity to external pH stimuli. Non ionized carboxylic functionality (COOH) leads to insalubility at pH below its pKa (pH < 3.4). Whereas at higher pH (> 4.4) alginate polymer chain can be expanded through electrostatic repulsion of these negative charges due to the ionization of carboxylic group (COO^-) resulting swelling of the hydrophilic matrix which is maximum around pH 7.4 specially in the range of physiological condition of gastrointestinal tract. Hence, pH-responsiveness of alginate biopolymer based gel triggerred the development of oral colon-specific drug delivery platform.[188] Thus targeted drug delivery to the colon with more effective drug administration and sustain release ability could overcome severe adverse effects as a result of long term release.[189] Another pH-responsive hydrogel composed of chitosan, aminated-nanowhisker (WN), and aminated-graphene (GN) was reported through reversible imine linkages.[190] Again, chitosan-hydroxyapatite-based pH-responsive hydrogel with $NaHCO_3$ as the gelling agent with injectable sol-gel property are explored.[191]

pH responsiveness can also result from cellulose based injectable hydrogel through functional alterations. Modified carboxymethyl cellulose (CMC) including hydrazide modified carboxymethyl cellulose (CMC-NH_2) and oxidized carboxymethyl cellulose (CMC−CHO) with varying degrees of oxidation were reported. An injectable hydrogel composite system with pH responsiveness was finally tuned through mixing of CMC-NH_2 and CMC−CHO polymer

suspensions containing pH-responsive poly (ethylene oxide)-block-poly (2-(diisopropylamino) ethyl methacrylate) (PEO-*b*-PDPA) copolymers as micelle cores through Schiff base reaction. The copolymer was developed through atom transfer radical polymerization (ATRP). A series of pH responsive novel natural hydrogels based on starch and l-asparticacid was also reported.[192]

4.3. Other Stimuli Responsiveness

Enzymes and light are two important stimuli, which can be utilized to prepare injectable polysaccharide hydrogel and target their bioapplications. Most polysaccharides exhibited bioenzyme triggered degradation. Hyaluronidase and matrixmetalloproteinases (MMPs) were reported as two important enzymes in this regard, which cleaved the glycosidic bonds of the polysaccharides hence showed enzyme responsiveness. MMPs triggered the cancer tissue site as they were related to endopeptidases structure and function.[193] Light-sensitive property can be introduced to polysaccharide gel through incorporation of orho-nitrobenzenes, a well know light responsive compound as demonstrated in Figure 13.[194] Here, a self-assembled nanogel was formed from light-responsive pullulan functionalized with cholesterol (Ls-CHP). Srain sensitivity with chemical and electromechanical responses were considered as some other external stimuli for polysaccharide gel originated from high surface area and large amount of hydroxyl groups in the surface in cellulose nano materials-based gel (CNMs).[195]

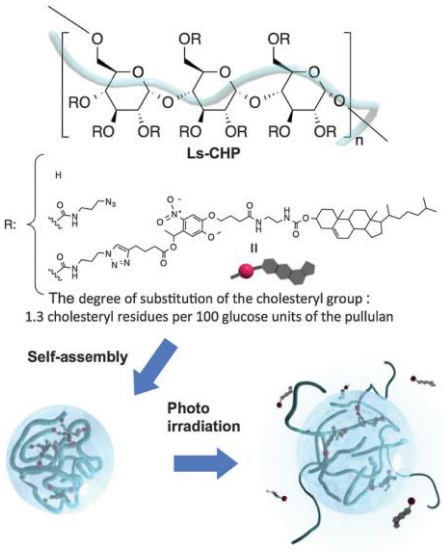

Figure 13. Chemical structure and illustration of a light-sensitive cholesteryl pullulan (Ls-CHP) nanogel. Reproduced with permission from ref 194. Copyright 2016 Royal Society of Chemistry.

4.4. Multistimuli Responsiveness

This section basically focuses those polysaccharide-based gels which are sensitive to more than one external stimulus at a time. Sometimes multiple stimuli responsiveness introduced extra fascility from biomedicinal viewpoint. Some reports are discussed here. A novel injectable hydrogel system, synthesized from chitosan/HA/*β*-sodium glycerophosphate, exhibited dual sensitivity at body temperature and pH. These properties triggerred potential drug release and adhesion to cancer cell.[196] Another novel thermosensitive and pH-sensitive hydroxypropyl chitin/tannic acid/ferric ion (HPCH/TA/Fe) composite hydrogel was demonstrated by a simple assembly with effective would healing capacity.[197] Another report shows that temperature and pH responsive wheat starch/methyl-3-aminocrotonate hydrogel has been synthesized through copolymerization with methyl-3-aminocrotonate *via* free radical polymerization.[198] Multistimuli responsive polysaccharide based gel has been fabricated by grafting with synthetic polymeric materials. A series of temperature responsive diblock copolymers based on poly(ethylene glycol) methyl ether (mPEG) and *ε*-caprolactone (CL) were synthesized followed by grafting onto chitosan, a pH responsive biopolymer resulting dual responsiveness.[199] Cellulose hydrogel obtained by simply mixing aqueous solutions of cellulose acetoacetate (CAA) and cystamine dihydrochloride (CYS) at room temperature and exhibited both redox and pH trigger sol-gel transition due to facile incorporation of enamine and disulfide bonds in the same system.[200] Another redox and pH dual responsive self healing HA based gel was accounted with excellence protein and cell delivery applications. Functional modification of HA precursor resulted redox and pH dual-responsive injectable hydrogels through dynamic acylhydrazone and disulfide linkages. The reversible sol-gel transition behavior of hydrogels could be replicated multiple times by adjusting DTT/H_2O_2 or HCl/TEA exhibiting redox and pH sensation respectively.[201] The redox and pH responsive bahiviour was demonstrated by Figure 14 and 15 respectively. Triple external stimuli like temperature, pH and magnetic field could trigger cyclodextrin based innovative gel prepared through one-pot synthesis. Combination of silica with magnetic ferric oxide followed by connection to cyclodextrin lead to the novel system. Here potential release of drugs (DOX, CUR) was regulated at acidic pH (= 5.0) and high physiological temperature (T = 40 ^oC), in presence of external magnetic field.[202] Another tri external stimuli responsive novel injectable alginate-amino caproic acid (Alg-ACA) derived thixogel system was demonstrated with stress, temperature and ultrasonication sensation.[203] Interestingly pH is most important

stimuli in case of polysaccharide driven multistimuli-responsive drug delivey system as drug release can be controlled easily by changing pH. But other stimuli mainly regulate the synthesis of this delivery platform. For example, Hao *et al*. incorporated photostimulation into the preparation of drug delivery system, where azobenzene has been introduced into cyclodextrins. The resultant dual pH-photo responsive nature of this system was explored. pH sensitivity basically triggered the DOX release and clearer cytotoxic effect of the system on HeLa cells than free DOX are further investigated using their photo responsive property.[204] Dual response of temperature and H_2O_2 in cyclodextrin based system was reported by Guo and his co workers. The model drug, DOX, was released through the interaction of the host and guest. The enhanced rate of release was reported with increasing temperature or adding H_2O_2.[205] Crosslinking of the *β*-cyclodextrin functionalized chitosan (CD-CS) with disulfide bond implanted crosslinker (Ad-SSAd) febricated pH/reduction dual stimuli responsive supramolecular nanogel.[206]

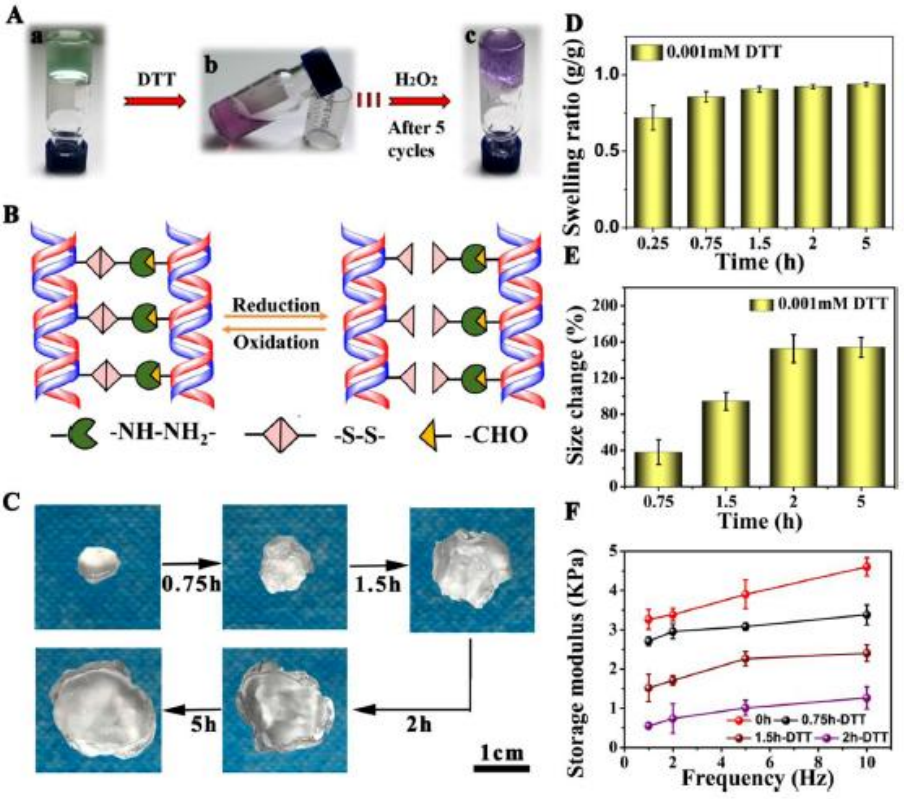

Figure 14. (A) Redox stimuli-responsive sol-gel transition of Gel-1.5, (a) original hydrogel stained by methylene blue, (b) hydrogel decomposition after adding DTT, (c) the dynamic recombination of hydrogel after adding H_2O_2. (B) Mechanism explanation of dynamic

recombination in redox stimuli-responsive microenvironments. (C) The morphological change of Gel-1.5 hydrogel at different time in 0.001 mM DTT solution. The (D) swelling ratio, (E) size change and (F) storage modulus of Gel- 1.5 in 0.001 mM DTT solution. Reproduced with permission from ref 201. Copyright 2020 Elsevier.

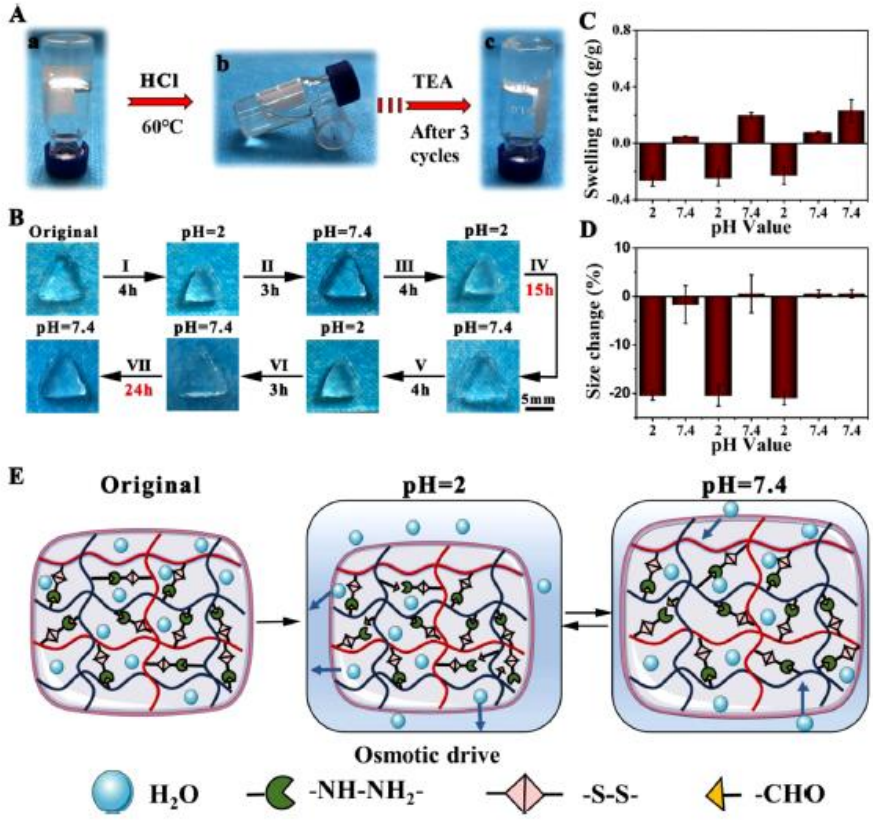

Figure 15. (A) pH stimuli-responsive sol-gel transition of Gel-1.5, (a) original hydrogel (b) hydrogel decomposition after adding HCl and (c) the dynamic recombination of hydrogel after adding TEA. (B) The images of Gel-1.5 put into pH 2 and pH 7.4 repeatedly. The (C) swelling ratio and (D) size change of Gel-1.5 with pH fluctuated repeatedly between 2 and 7.4. (E) Mechanism explanation of acid-switchable shape-recovery of hydrogel. Reproduced with permission from ref 201. Copyright 2020 Elsevier.

5. A Brief Overview of Bioapplications

This section demonstrates a brief overview about the major bioapplications of polysaccharide-based stimuli responsive gel having *in situ* injectable or self healing properties on the basis of recent literature. Those can be widely utilized in chemoimmunotherapy, cancer and diabatis treatment. Alginate based *in situ* crosslinked gel exhibited good cell immobilization capacity.

Stimuli responsiveness and *in situ* gel forming capacity majorly affect drug delivery to the targated cell with potential therapeutic applications. Alginate-HA based *in situ* gel was reported through ionic-crosslinking of alginate (ALG), HA and multivalent ions and extensively used for therapeutic purpose like antibacterial capacity as demonstrated in Figure 16.[207] Here, the morphologies of the hydrogel were gradually sacrificed through degradation and vanished in 8 weeks after injection. The biocompatibility of the hydrogel was exhibited by H&E staining without damaging tissues of the major organs (including heart, liver, spleen, lung and kidney). Antibacterial efficacy of this system was proved against Gram-positive *S. mutans* and Gram-negative *P. gingivalis* oral pathogenic bacteria where Fe^{3+}-EDTA complex solution was reported as the major antimicrobial constutents in the gel. Fluorescent staining test further confirmed the results obtained from colony counting method.

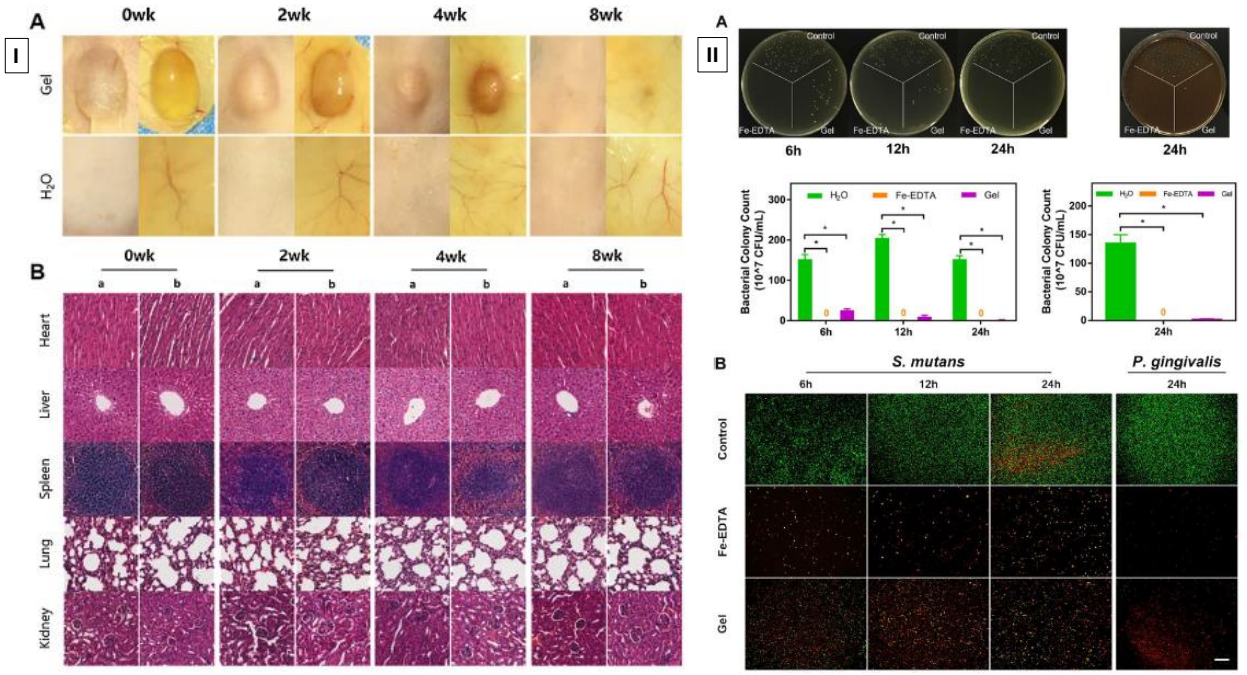

Figure 16. (I) In vivo formation, degradation and tissue biocompatibility of the ALG-HA hydrogel. A) Images of DI water control group and the implanted hydrogel were taken at 0 h, 2wk, 4wk and 8wk post-injection. Images of left sides were taken directly on the back of the mice, and images of right sides were taken on the inside of the dorsal skin peeled from the same mice. B) H&E staining images (in 5× magnification) were observed in main organs (heart, kidney, liver, lung and spleen) after subcutaneous injection of 200 µl DI water (a) and the ALG-

HA hydrogel (b) at different time points. (II) In vitro antimicrobial properties of the ALG-HA hydrogel. A) Images and colony counting calculated from the images of *S. mutans* (left panel) and *P. gingivalis* (right panel) from diluted bacterial suspension with treatment of DI water (H_2O), Fe^{3+}-EDTA solution (Fe-EDTA) and the ALG-HA hydrogel (Gel). B) Live/dead staining of *S. mutans* (left panel) and *P. gingivalis* (right panel) from diluted bacterial suspension with treatment of DI water (H2O), Fe^{3+}-EDTA solution (Fe-EDTA) and the ALG-HA hydrogel (Gel). Scale bar: 100 μm. Reproduced with permission from ref 207. Copyright 2021 Elsevier.

An injectable hydrogel by dissolving MoS_2/Bi_2S_3-PEG (MBP), doxorubicin (DOX) and agar into water was efficiently designed without compromising their photoacoustic and computed tomography imaging capacity for photothermal and chemotherapy application of tumor treatment.[208] Chitosan was injected into the tumor cell in sol form at room temperature and routine gel formation is triggered after warming to body temperature in presence of *β*-glycerophosphate (*β*-GP), utilized in hyperthermia and chemotherapy of colon cancer.[209] Thermosensitive Pluronic F127 hydrogel implanted with *N,N,N*-trimethyl chitosan, can result a potential application in brain tumor treatment.[210] A novel injectable, temperature sensitive, controlled drug delivery system based on chitosan-co-polyNIPAM injectable hydrogel extensively utilized for controlled delivery of loxoprofen sodiumas, a model drug.[211] Recently tissue engineering and bioprinting applications of *in situ* injectable hydrogels with self healing nature are widely investigated. Several criteria have to be fulfilled to act as a scaffold i.e a temporary supporting structures for growing tissues. The scaffold must be biodegradable, biocompatible, and reproducible; should permit cell adhesion, promote cell growth and allow the retention of differentiated cell function.[212] Polysaccharides, being natural biopolymers, are extensively investigated in this regard as they can meet all the above-mentioned properties. These injectable hydrogels embaded in soft tissue including the whole organs, tendons, ligaments, skin, fat, muscles, blood vessels or a hard tissue, such as bone and cartilage. Self crosslinking injectable hydrogel system based on HA-adipic dihydrazide and the oligopeptide G_4RGDS-grafted oxidized pectin can be used as scaffold for cartilage tissue engineering.[213] An efficient tissue engineering application was demonstrated by thermo-sensitive NIPAAm-*g*-chitosan (NC) hydrogels with thiol modification for introduction of disulfide cross-linking strategy by covalently conjugating *N*-acetyl-cysteine (NAC) with carbodiimide chemistry to

strengthen mechanical properties.[214] Numorous common polysaccharides such as alginate, cellulose, chitin, HA, chitosan can result a highly hydrated gel framework with extremely porous structure, which can mimick the environment for cellular outgrowth. Accommodation of living cells as well as gases, nutrients result potential wound dressing and wound healing applications.[215]

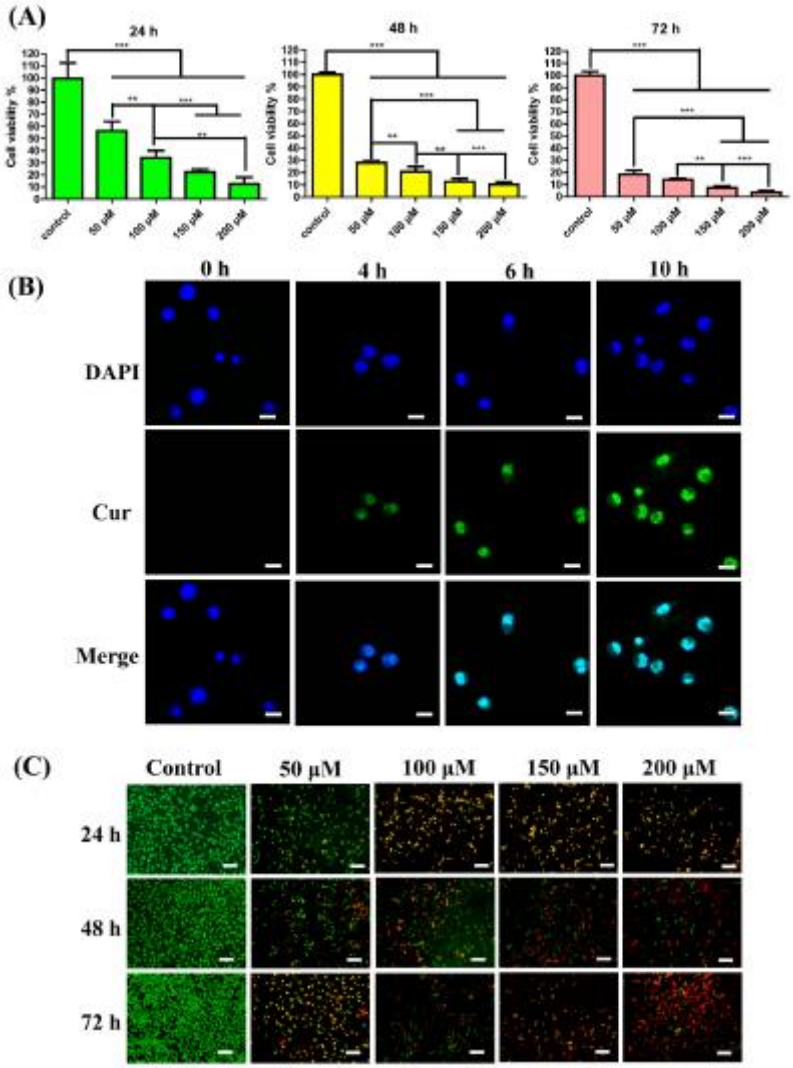

Figure 17. CLSM images of MCF-7 cells treatment with 200 μM CSSH/Cur@Lip-Cos-PC Gel after 0 h, 4 h, 6 h, and 10 h, scale bar is 10 μm (A). Cell viability of MCF-7 cells treatment with control, 50 μM, 100 μM, 150 μM and 200 μM CSSH/Cur@Lip-Cos-PC Gel after 24 h, 48 h and 72 h (B). The fluorescence images of AO-EB staining of MCF-7 cells treatment with control, 50 μM, 100 μM, 150 μM and 200 μM CSSH/Cur@Lip-Cos-PC Gel after 24 h, 48 h and 72 h, scale

bar is 100 μm (C). (*p < 0.05, **p < 0.01, ***p < 0.001, one-way analysis of variance, Graphpad prism 5.0.). Reproduced with permission from ref 219. Copyright 2021 Elsevier.

A mussel-inspired injectable hydrogel, prepared from catechol- and methacrylate-modified chitosan/gelatin, with high biocompatibility, inherent antimicrobial activity, and good adhesion to wet tissues, was reported recently, with potential application on tissue surfaces. This gel resulted a promising surgical and wound dressing applications *in situ* within seconds of body contact by a biocompatible and multifunctional redox initiator (H_2O_2-ascorbic acid) with faster wound healing.[216] Again biosensing applications of several polysaccharides like chitosan, HA or cellulose based gels were also reported and triggered by stimuli sensitivity and *in situ* injectable capacity. For example, hyaluronan tyramine- bisphosphonate derivative was reported recently with a novel aspect of using radiotracers. They could be used for dual-labelling with two radionuclides (^{99m}Tc and ^{131}I) concurrently for in vitro and in vivo tracking. This dual-radiolabelled hyaluronan derivative has been transformed to injectable gel through non-covalent crosslinking by hydroxyapatites.[217] Several excellent properties of the gel including high mechanical strength, easy biodegradability and self healing nature made the gel as an ideal biomaterial.[218] Injectable thiolated chitosan hydrogel was used to fix curcumin (Cur) loaded liposomes (Cur@Lip) after chronological coating with positive chitooligosaccharides (Cur@Lip-Cos) and negative phospholipids (Cur@Lip- Cos-PC).[219] Confined immobilization and sustained release of Cur to inhibit MCF-7 (tumor cell) growth were reported recently as two major biomedical utilizations from that system. The tumor cell proliferation was diminished with increasing concentration of Cur in gel observed through confocal laser microscope (CLSM) as demonstrated in Figure 17. The green fluorescent of DAPI resulting from living cells almost disappeared at concentration of 200 μM at 72 h.

6. **Conclusions and Future Perspective**

The practical applications of polysaccharide gels in diverse fields including controlled drug delivery and release, biosensing, cell encapsulation, tissue engineering is discovered by considering advantages of their natural availability, biocompatibility and biodegradability. Recently researchers are increasingly utilizing polysaccharide-based gel, responsive to various environmental stimuli like pH, temperature, ionic strength, redox state, light, etc. Those stimuli

mainly affect the sol-gel transion process; swelling capacity and mechanical strength which can directly trigger biomedical applications. If the gel is injectable to body temperature in sol form and transformed to gel at targated site *in situ* under physiological condition, they can fascile bioapplications. Hence, recent progress to include those properties to polysaccharide gels is greatly encouraged with respect to synthesis and biomedical applications. This technology can be designated as 'smart' technology, including artificial and tunable tissue culture scaffolds. In this review article, we have demonstrated a large percentage of relevant literature reports exhibiting development of polysaccharide-based hydrogels and how their stimuli responsiveness with *in situ* injectable self healing capacity trigger numorous biomedical applications.

Though scientists are making considerable advancement in this field, there are still vacancies for more development. Greater understanding can be built about their synthesis through functional modification, how several chemical reactions help to include those properties and open an economical pathway for therapeutic applications.

Conflict of Interest

The authors declare no conflict of interest

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