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Posted Date: 14 September 2023

doi: 10.20944/preprints202309.0901.v1

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Review

Natural Polymer-Based Hydrogels: From Polymer to Biomedical Application

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Abstract: Hydrogels prepared from natural polymer have attracted extensive attentions in biomedical fields such as drug delivery, wound healing, and regenerative medicine due to their good biocompatibility, degradability and flexibility. This review outlines the commonly used natural polymer in hydrogel preparation, including cellulose, chitosan, collagen/gelatin, alginate, hyaluronic acid and starch. The polymeric structure and process/synthesis of natural polymers are illustrated, and natural polymer-based hydrogels including the hydrogel formation and properties are elaborated. Subsequently, the biomedical application of hydrogels based on natural polymer in drug delivery, tissue regeneration, wound healing and other biomedical field is summarized. Finally, the future perspectives of natural polymers and hydrogels based on them are discussed. For natural polymer, novel technologies such as enzymatic and biological methods are developed to improve the structural properties and the development of new natural based polymers or natural polymer derivatives with high performance is still very important and challenging. For natural polymer-based hydrogels, novel hydrogel materials, like double-network hydrogel, multifunctional composite hydrogels and hydrogel microrobots are designed to meet the advanced requirements in biomedical application, and new strategies such as dual-crosslinking, microfluidic chip, micropatterning and 3D/4D bioprinting, have been explored to fabricate advanced hydrogel materials with designed properties for biomedical application. Overall, natural polymeric hydrogels have attracted increasing interests in biomedical application, and the development of novel natural polymer-based materials and new strategies/methods for hydrogel fabrication is badly desirable and still challenging.

Keywords: hydrogel; natural polymer; drug delivery; tissue engineering; wound healing

1. Introduction

Hydrogels are three-dimensional networks formed by hydrophilic polymers through chemical cross-linking (covalent bond, ionic bond) or physical cross-linking (hydrogen bond, van der Waals force, physical entanglement). They can swell in water and retain a certain shape while absorbing a large quantity of water, attracting increased interests in numerous areas since they were first reported by Wichterle and Lim in the 1960s [1]. Natural polymers, which are formed from photosynthesis or biochemical reaction in natural world or extracted from natural products, are ideal skeletons for the fabrication of hydrogels because of their diversified properties such as the biocompatibility, biodegradability and environmental-friendlier. Hydrogels based on natural polymers such as alginate, starch, cellulose, chitosan, gelatin, collagen, hyaluronic acid and son on show good degradability, biocompatibility, nontoxic degradation products, good flexibility similar to natural tissue and natural abundance [2], which endowed them a widespread application in medicinal fields, for instance, the carriers for drug delivery, the wound dressing for wound healing, the substrates for cell culture, the scaffolds for tissue regeneration and so on [3,4].

Biocompatibility is one of the most significant characteristics of hydrogels for biomedical application, referring to the ability of a material contacted with bodily organs with minimum damage to the surrounding tissues and without triggering undesirable immune responses, but performing its

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desired function in a medical treatment [5]. Hydrogels based on natural polymers usually have excellent biocompatibility owing to the intrinsic properties of natural polymers. However, for most of the natural polymeric hydrogels, the limited toughness and poor mechanical properties become the major obstacles to their application. Several efforts were made to improve the mechanical properties of hydrogels, including chemical crosslinking, physical crosslinking and mixing with other synthetic/semi-synthetic polymers or inorganic particles.

Biodegradability is another essential property of hydrogel materials for biomedical application, such as tissue regeneration, as cells need space to grow, migrate, and proliferate [6]. It refers to the capacity of a substance to break down after interactions with biological elements [7]. Most of the hydrogels prepared by natural polymer can be decomposed under the action of enzyme, allowing cells to spread and reshape their surroundings. The rate of biodegradation is another required factor, which depends on properties such as molecular weight, the amorphous or crystalline and hydrophilic/hydrophobic behaviors of polymers [8].

This review outlines the current progress of natural polymer-based hydrogels in biomedical field (Figure 1). The most widely used natural polymers such as collagen, gelatin, alginate, cellulose, chitosan and hyaluronic acid, are described, and natural polymer-based hydrogels including hydrogels prepared from natural polymers, natural/synthetic polymer and natural/inorganic particles are summarized. Then, the biomedicine application of natural polymer-based hydrogels in drug delivery, wound healing and regenerative medicine are summarized and discussed, respectively. Finally, the future perspectives and conclusion are proposed.

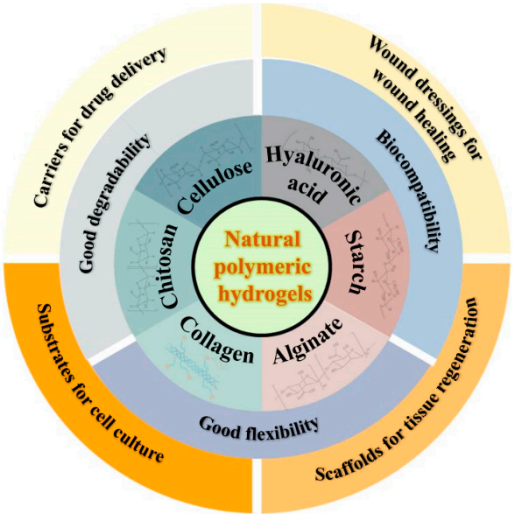

Figure 1. Current progress of natural polymeric hydrogels.

2. Natural polymer-based hydrogels

Natural polymers derived from materials in natural world, such as polysaccharides and proteins, have good biocompatibility and biodegradability, making them ideal choice for the fabrication of hydrogels. Polysaccharides used for hydrogel fabrication include cellulose, chitosan, dextran, alginate, hyaluronic acid as well as their derivatives. There are abundant hydroxyl groups and/or other functional groups (amino, carboxyl groups and so on) on the chains of polysaccharides, offering versatile opportunities to prepare polymer-based hydrogels via chemical or physical crosslinking. Proteins including collagen, gelatin and fibrin are essentially polymers of amino acids. It is thought that proteins can form fibrils in nanometer width and micrometer length via intermolecular and/or intramolecular force (such as hydrogen bonds, electrostatic interactions and hydrophobic effects) and further form three-dimensional hydrogels via self-organization and entangling under appropriate conditions [9].

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2.1. Cellulose and cellulose-based hydrogels

Cellulose is the most abundant natural polymer compound on earth, consisting of β (1-4)-glycosidic-linked glucose units (Figure 2). Cellulose organizes in a rather intricate supramolecular structure formed by the intermolecular cohesion of cellulose molecules, which is an extended intra/inter-molecular network of hydrogen bonds. In the dissolution process of cellulose, the intramolecular hydrogen bonds are broken, and the supramolecular structure of cellulose is disintegrated, enhancing the activity of hydroxyl in cellulose, and making it easy to combine with other natural or synthetic polymers by reconstructing hydrogen bonds. Therefore, cellulose is one of the ideal candidates for hydrogels preparation, endowing cellulose composite hydrogels with specific performance, such as biodegradability, renewability, flexibility and high mechanical strength [10].

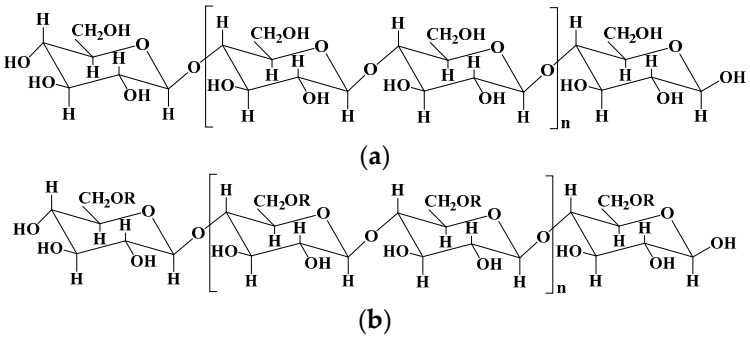

Figure 2. The chemical structures of cellulose (a), and some important derivatives of cellulose (b).

Methylcellulose: R=-H,-CH_3; Ethylcellulose: R=-H,-CH_2CH_3; Hydroxyethyl cellulose: R=-H,-CH_2CH_2OH; Carboxymethylcellulose: R=-H,-CH_2COOH; Hydroxypropyl cellulose: R=-H,-$CH_2CH(OH)CH_3$; Hydroxy propyl methyl cellulose: R=-H, -CH_3, $CH_2CH(OH)CH_3$.

Plant cellulose is the main source of cellulose, producing in a non-pure form as lignocellulose [11]. Cellulose can be purified using ecofriendly biological method and non-ecofriendly chemical method. Biological method depends on microbial enzymes with low productivity. While chemical method has many steps and types of equipment with high productivity. In addition, bacterial cellulose produced via certain types of bacteria has high purity and functionality, which is a major alternative source of plant cellulose [12]. Pellets or sheets of cellulose can be produced via certain types of Gram-negative and Gram-positive strains by cultivating in a high glucose containing media.

Nanocellulose (NC) is a mesoscopic material formed in the regeneration process of cellulose. NC including cellulose nanocrystals (CNCs) and cellulose nanofibrils (CNFs) has been widely used in the field of functional materials due to their unique morphology of nanostructures and many featuring advantages, like excellent mechanical properties, biodegradability, and environmental friendliness [13,14]. For example, CNFs prepared by TEMPO (2, 2, 6, 6-Tetramethylpiperidine-1-oxyl) mediated oxidized has plenty of hydroxyl groups and carboxyl groups on its surface, endowing CNFs with good stability and dispersibility without aggregation in water, and making it easy to combine with other polymers or nanoparticles to construct novel reinforced composite materials and hydrogels [15,16].

The fabrication of cellulose-based hydrogels is challenging because cellulose is hardly soluble in common solvents due to its highly extended hydrogen-bonded structure. One of the strategies to resolve this issue is the development of several solvent systems, for example dimethylacetamide, alkali/urea (or thiourea) aqueous systems, and ionic liquids [17,18]. Chemical modification is another effective method to resolve the poor solubility of cellulose. Hydroxyl and carboxyl groups are the most commonly used active groups introduced to the skeleton of cellulose, obtaining various cellulose derivatives including carboxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose and hydroxypropyl methyl cellulose [19,20]. Cellulose-based hydrogels can be formed via the crosslinking between these functional groups. For instance, the esterification of hydroxyl and carboxyl groups by carbodiimide condensing agents, Michael addition reaction between hydroxyl groups and carbon-carbon double bonds under alkaline conditions, epoxide and alkyl halide

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crosslinking under strong basic and high temperature conditions, and free radical polymerization [21,22].

Cellulose-gelatin hydrogels with high strength and pH-responsiveness are prepared using the cyclic freezing-thawing method [23]. The repeated freezing-thawing cycles played a vital role in the formation of the supramolecular network structure via physical cross-linking between cellulose and gelatin. The superior mechanical performance was contributed to the combination effect of hydrogen bond and the reinforcement of CNFs. A bacterial cellulose-based hydrogel with good mechanical properties and improved ionic conductivity was prepared for thermo-electrochemical cells in practical wearable electronics by introducing highly soluble urea and thermodiffusion effect of NaCl [24]. The bacterial cellulose hydrogel-based electrolyte has nanofiber-porous 3D network structure, and its nanochannels containing plentiful surface hydroxyl groups, which are favor to the transport of positive ion.

2.2. Chitosan and chitosan-based hydrogels

Chitosan is a natural polycationic polymer with hydrophilic properties, consisting of the repeating residues of D-glucosamine and N-acetyl-D-glucosamine (Figure 3) [25]. It is obtained by partial deacetylation of chitin, one of the most abundant polymers after cellulose extracted from the fungal cell walls and the exoskeleton of crustaceans/insect [26]. Generally, chitosan should have a deacetylation degree of 60, containing at least 60% of D-glucosamine for the deacetylated chitin [25]. Chitosan is an analogous of glucosaminoglycans, one of the main components found in extracellular matrix (ECM) of some living tissue, so chitosan can be used to mimic the ECM in regenerative medicine and has been widely studied in the areas of biotechnology[27,28].

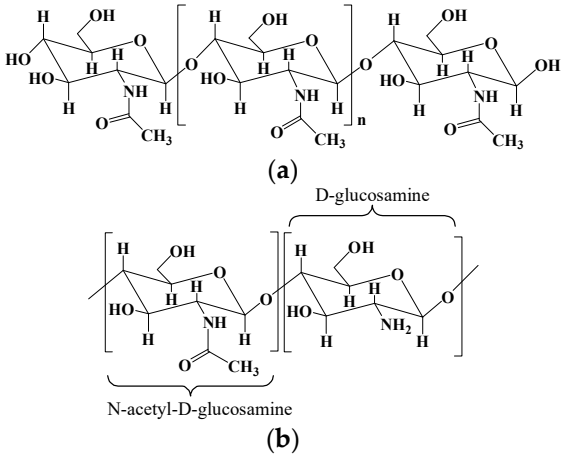

Figure 3. The chemical structures of chitin (a), and chitosan (b).

The two main sources of chitosan are animal source (crustaceans and fungal mycelia) with seasonal supplies and mushroom source with better reproducibility. Chitosan is derived from the partial deacetylation of chitin using chemical or biological approach. The chemical hydrolysis is performed under severe alkaline conditions (i.e. concentrated sodium hydroxide and sodium borohydride) and biological method is performed by particular bacterias and/or enzymes [29].

Chitosan-based hydrogels are formed by physical association or chemical cross-linking [30]. In physically cross-linked hydrogels, non-covalent interactions, namely electrostatic interactions, hydrophobic interactions and hydrogen bonding between polymer chains are used to fabricate the gel network. In chemically cross-linked hydrogels, cross-linker agents, secondary polymerizations, click chemistry or irradiation chemistry are used to form the chitosan based hydrogels. Many bifunctional/polyfunctional molecules with aldehyde, including glutaraldehyde, glycidyl ether, isocyanate, acrylate, azides and so on have been used to cross-link chitosan polymers and fabricate chitosan-based hydrogels, especially hydrogels with environmental sensitivity [31,32].

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Chitosan hydrogels with self-healing ability were prepared using natural vanillin as crosslinking agent [33]. The hydrogel network contained hybrid linkages of Schiff-base bonds and hydrogen bonds. The concentration of vanillin has an important effect on the self-healing ability of the hydrogel, because the self-healing ability mainly comes from the reconstruction of dynamic Schiff-base bond. Liu et al. reported a novel type of homogeneously structured polyelectrolyte complexes (PEC) hydrogel with electro-responsive performance and high mechanical strength based on chitosan and carboxymethylcellulose using the cyclic freezing-thawing method [34]. The PEC hydrogel undergoes a variety of programmable 3D shape transformations, such as helix, flower, V- and M-like shape and other intermediate variations owing to the asymmetric deformation of gel strips caused by the uneven osmotic stress on both sides of the hydrogel.

2.3. Collagen/gelatin and collagen/gelatin-based hydrogel

Collagen, as one of the most abundant renewable natural polymers along with cellulose and chitosan, has significant application in biomedical field [35,36]. It plays an important role in structural protein for most tissues, i.e. skin, bones, muscles, blood vessels, cartilages, and contains plentiful of functional groups, i.e. hydroxyl, amino, carboxyl, guanidyl and imidazoles, endowing collagen with many physical and chemical properties. Collagen is able to self-assemble into triple helical fibrous structure under physiological conditions (Figure 4), rendering collagen great tensile strength and durability [37]. Collagen has many excellent natural characteristics, such as hydrophilicity, biocompatibility, biodegradability, non-immunogenicity and mechanical durability, making it an essential component in the investigation of biomedical field [38]. For instance, in the engineered tissues, collagen can afford mechanical strength to other amorphous hydrogels and modulate the hydrogel to mimic native tissues, providing critical recognition sites for cellular migration and attachment and long-term structural support for tissues [39].

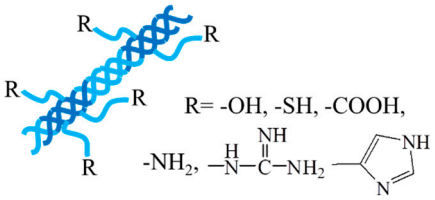

Figure 4. The triple helical fibrous structure of collagen.

Animal is the main source of collagen, and the animal-derived collagen can be obtained from porcine skin, bovine tendon, rat tail, or marine sources by extraction and purification using chemical or enzyme method. The commonly used agents for chemical treatment including neutral salt solution, alkali solution, dilute acetic acid, and hydrochloric acid. Besides, recombinant collagen produced by recombinant technology and biosynthesis is an alternative source of collagen, which expressed in yeast, Escherichia coli, mammalian cells, insect cells, tobacco plants, corn seeds, and so on [40].

Chemical modification of collagen is generally applied to render desired properties since the direct usage of collagen in specific application sometimes may lead to several problems like calcium deposition, high thrombogenicity, uncontrollable degradation rate, inadequate mechanical properties and so on. The chemical modification can be achieved through structural decoration by insertion of new functional groups and/or by combination of novel materials containing new functional groups [35,41]. Hydrogels with M2 macrophage-polarization and anti-inflammatory properties were prepared through enzymatic crosslinking of tyramine-grafted collagen and gallate dimer-grafted hyaluronic acid [42]. The hydrogels integrated with deferoxamine-loaded mesoporous polydopamine nanoparticles have enhanced mechanical strength, desirable tissue adhesion and injectability, showing an improved repair effect of diabetic wounds.

Gelatin is a denatured, water-soluble polypeptide derived from collagen being irreversibly hydrolyzed [43]. Hydrolysis of collagen dissociates the triple helix into three peptide chains and

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decomposes collagen to gelatin, making the material biocompatible and biodegradable for cell growth [44]. Gelatin aqueous solution at a concentration of 0.5-50 wt% is in a sol state above the melting temperature (31.7-34.2 °C) and forms a thermo-reversible gel after cooling [45]. However, the low melting temperature limits its application under physiological conditions. The addition of salt or other small soluble compounds could lead to structural reorganization of the gelatin hydrogels due to the change of the interactions between gelatin molecules, such as hydrogen bonding, hydrophobic forces, and electrostatic forces [46,47]. Gelatin methacrylate (GelMA) is a photo cross-linkable gelatin derivative prepared by modifying the reactive side groups of gelatin using glycidyl methacrylate, and has received attractive attentions in biological fields[48,49]. GelMA can be crosslinked to form hydrogel via photo-polymerization due to the presence of methacrylate, and the stiffness and porosity of GelMA hydrogel can be regulated by controlling the production parameters such as hydrogel concentration, degree of functionalization, UV intensity, and additive supplementation [50].

2.4. Alginate and alginate-based hydrogel

Alginate is a commonly available natural biopolymer, which is a linear anionic polysaccharide consisting of repeated residues of α-L glucuronate and β-D mannuronate (Figure 5) [51]. It provides biocompatibility, biodegradability, non-antigenicity, chelating ability as well as good stability for a long time [52]. Alginate can absorb large quantities of biological liquids and be purified to prevent immunogenicity, rendering alginate an ideal polymer for hydrogel preparation under mild conditions [53].

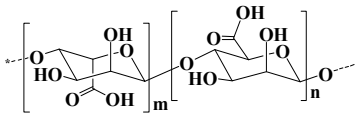

Figure 5. The chemical structure of alginate.

Alginate can be extracted from brown algae using alkali solutions (typically sodium hydroxide solution). The extraction liquid is filtered and then alginate is precipitated by adding sodium or calcium to the filtrate. In addition, bacterial alginate produced by bacterial biosynthesis from Azotobacter and Pseudomonas is another source of alginate, which provides alginate with more defined chemical structures and physical properties than that of seaweed-derived alginate [54].

Alginate hydrogels can maintain structural similarity to ECM in tissues and be administrated to play several vital roles, thus these hydrogels have attracted remarkable interest in biomedical application, such as wound repair, drug delivery and tissue regeneration [51]. Various approaches have been applied to prepare alginate-based hydrogels, including ionic cross-linking with divalent cations (i.e. Ba^{2+}, Fe^{3+}, Ca^{2+}) [55,56], covalent cross-linking with multi-functional molecules (i.e. poly(acrylamide-co-hydrazide, poly(ethylene glycol)-diamines)) [57,58], thermal gelation and in situ copolymerization of thermol-sensitive polymers (i.e. N-isopropylacrylamide (NIPAAm)) by UV irradiation [59]. In addition, various alginate derivatives including amphiphilic aglinate and cell-interactive alginate have also been used in the fabrication of hydrogels for biomedical application. Amphiphilic alginate derivatives obtained by coupling hydrophobic moieties, such as alkyl chains and hydrophobic polymers, to alginate skeleton can self-assemble to form hydrogels in aqueous solution, which have promising prospect in drug delivery systems [60].

Hydrogels prepared by cell-interactive alginate, which was synthesized by chemically coupling cell-adhesive peptides (i.e. RGD) as side-chains, are crucial for cell growth and tissue regeneration due to the promotion and regulation of cellular interactions [61]. Hong et al. developed a polymeric hydrogel based on alginate-boronic acid conjugate through borate ester cross-linking between the intrinsic cis-diol at the alginate backbones and boronic acid [62]. The prepared hydrogel demonstrated unprecedented multi-functionalities simultaneously, such as self-healing capacity, stretchability, shear-thinning, stimuli-sensitivity, adhesive and reshaping properties, which were

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owing to the reversible intermolecular/intramolecular interactions resulted by the dynamic complexation and dissociation of borate ester.

2.5. Hyaluronic acid and hyaluronic acid-based hydrogel

Hyaluronic acid is a natural polysaccharide found in the ECM with excellent biocompatibility, comprising of N-acetyl-glucosamine and D-glucuronic acid (Figure 6) residues [63]. As a main component of ECM, hyaluronic acid plays a significant role in many physiological functions including lubrication, water absorption and retention for tissue and ECM, structural and space-filling, and interacts with various cell receptors to coordinate cell communication and behavior [64]. It has non-toxicity, non-allergy, and biocompatibility, which has been widely used as biomedical materials including tissue engineering scaffold, wound dressings, and drug carriers [65,66].

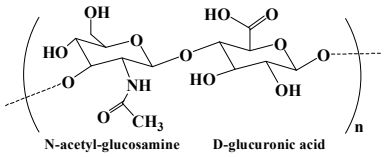

Figure 6. The chemical structure of hyaluronic acid.

Hyaluronic acid is mainly manufactured via extraction from animal tissues (including human umbilical cords, vitreous humor of cattle, bovine synovial fluid, and rooster combs), and from microbial/bacterial fermentation using pathogenic bacteria and non-pathogenic bacteria is another source of hyaluronic acid [67]. Recently, a novel enzymatic technology for hyaluronic acid manufacture has been developed, and hyaluronic acid with high molecular weight, defined chain length and low polydispersity can be enzymatic polymerized using UDP-sugar monomers [68].

Hyaluronic acid contains hydroxyl and carboxyl functional groups in the main chain, which can be chemically modified to obtain hyaluronic acid derivatives with unique biological and physicochemical properties. The carboxyl group can be modified to synthesize hyaluronic acid derivatives with ester (by alkyl halides, diazomethane and tosylate activation) and amide (using carbodiimides, carbonyldiimidazole) [65]. For example, adipic dihydrazide functionalized hyaluronic acid (HA-ADH) was obtained by coupling adipic dihydrazide to hyaluronic acid through the amidation between the carboxyl group of hyaluronic acid and the amine group of adipic dihydrazide. Hydrogels integrated with sanguinarine loaded gelatin microspheres were prepared based on HA-ADH and oxidized dextran through Schiff's base crosslinking for wound healing, exhibiting improved re-epithelialization and ECM remodeling, enhanced anti-bacterial activities and decreased inflammatory responses [69]. In addition, the vicinal diol in hyaluronic acid can be oxidized to aldehyde groups by sodium periodate to form aldehyde-hyaluronic acid, and chitosan-hyaluronic acid hydrogel was formed through Schiff's base crosslinking for the immobilization of insulin-like growth factor 1, which improved stem cell therapy for limb ischemia [70].

2.6. Starch and starch-based hydrogel

Starch is a natural polysaccharide composed of glucose repeating units through α-D-(1 - 4) and α-D-(1 - 6)-glycosidic linkage (Figure 7) [71,72]. It has a wide range of applications in the food, agriculture, biomedical, and pharmaceutical fields due to its low cost, renewability, biodegradability, and biocompatibility. Starch exhibits a granular appearance in nature, which is called starch granule, containing a small amount of proteins, fatty acids, and minerals [73]. Amylose and amylopectin represented the primary two types of polysaccharides in the starch structure. Amylose with linear structure is known for the double helix formation due to left-handed helical conformation, and can form supramolecular inclusion complexes with guest molecules [74]. Amylopectin has a more highly branched structure than that of amylose, and can form a helical structure and crystallized, playing an important role in stabilizing starch granules structure [75].

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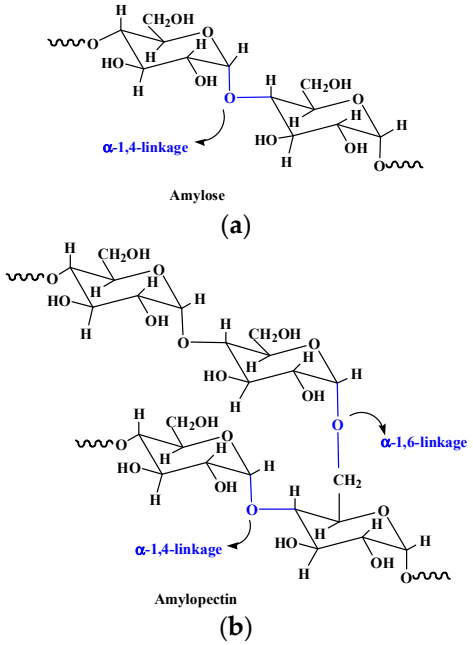

Figure 7. Chemical structures of amylose (a) and amylopectin (b) in starch.

Starch can be extracted from seeds (such as beans, peas, grasses, cereal grains, etc.), roots, tubers, stems, fruits, and all leaves. The component of amylose and amylopectin in starch varies from plant source. For instance, cornstarch contains about 28 wt% of amylose, and amylose in cassava starches is about 17 wt%, while amylose in waxy potato starches is only 8 wt% [76].

Starch-based hydrogels can be formed by environmentally and friendly physical methods including starch retrogradation and extrusion [73]. The formation of starch hydrogels by retrogradation is due to the rearrangement of amylose during the heating-cooling process. In addition, various chemical methods including etherification and grafting can be adopted to fabricate starch-based hydrogels [72]. The hydroxy groups in the starch structure can be substituted by various ether groups like carboxy-methyl to form etherified starches, and different vinyl monomers (such as acrylamide, acrylic acid and so on) can be grafted on starch in the grafting method [73]. Light curable starch-based hydrogels with Fe_3O_4 nanoparticles encapsulation were prepared by crosslinking of acrylic-glycol modified starch under blue light, exhibiting controllable release of quercetin, improved bioavailability, and enhanced mechanical properties [77].

Table 1. Natural polymer used for hydrogels formation.

Natural polymer	Chemical structures	Preparation and processing	Ref
Cellulose	Composed of β (1-4)-glycosidic-linked glucose units	1. Lignocelluloses purification by chemical treatment. 2. Biological method depending on microbial enzymes. 3. Bacterial cellulose produced by certain types of bacteria.	[12]
Chitosan	Poly-(β-1-4) N-acetyl-D-glucosamine	Derived from chitin by partial deacetylation though chemical or enzymatic hydrolysis.	[25,29]
Collagen	A helical fibrous protein formed by three peptide chains	1. Extracted and purified from various animal sources by chemical and enzyme treatment.	[40]

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		2. Recombinant collagen produced by recombinant technology and biosynthesis.	
Alginate	Consisting of α-L glucuronate and β-D mannuronate repeating units	1. Extraction from brown algae (Phaeophyceae) by treatment with aqueous alkali solutions. 2. Bacterial biosynthesis from Azotobacter and Pseudomonas.	[54]
Hyaluronic acid	Consisting of N-acetyl-glucosamine and D-glucuronic acid residues	1. Extraction from animal tissues. 2. Microbial fermentation using pathogenic bacteria and non-pathogenic bacteria. 3. Enzymatic polymerization of UDP-sugar monomers.	[67]
Starch	Composed of α-D-(1 - 4) and α-D-(1 - 6)-glycosidic- linked glucose units	Extracted from seeds, roots, tubers, stems, fruits, and all leaves.	[73]

3. Natural polymer-based hydrogels for biomedical application

Hydrogels based on natural polymers have emerged as promising alternatives for ECM in biomedical applications due to their unique integration of biodegradability, biocompatibility, mechanical property tunability, biomimicry and responsiveness, which could provide microenvironments for the preservation of cellular functions, promotion of cell health and encouragement of tissue formation [78]. A variety of hydrogels formulated using natural biopolymers have been fabricated as drug carriers, cell delivery systems, scaffolds for tissue engineering and so on, which provides versatile platforms for medicinal application.

3.1. Drug carriers for drug delivery

Hydrogels represent a class of drug delivery systems that have excellent drug delivery performances. The entangled polymer networks of the hydrogels could trap a large amount of water as well as hydrophilic small/macro-molecular drugs without dissolving, protecting the drug molecules from hostile environments, such as enzymes and low pH values[79]. In addition, smart hydrogels containing sensor moiety that can response to various physical (i.e. temperature, sound, electric and magnetic fields) and/or chemical stimuli (i.e. pH, oxygen concentration, enzyme, glucose and specific molecular recognition events) can undergo reversible gel-sol phase or volume phase transitions upon minute even second change in the environmental condition, making them ideal candidates for controlled drug release systems [80]. With the wide application of hydrogels in drug delivery, the demand for hydrogels is increasing, and hydrogels have been designed to possess various functions, such as conductivity, self-healing ability, and so on. Besides the hydrogels prepared by natural polymer only, multi-component hydrogel materials prepared by natural polymers integrated with semi-synthetic/synthetic polymers and/or inorganic particles often exhibit variety of functions. Several excellent reviews of intelligent hydrogel for drug delivery based on synthetic hydrophilic polymers have been reported previously [81] and hydrogels based on chitosan as drug carriers were also reviewed elsewhere [82]. In this part of review, the recent process of natural polymer-based hydrogels for drug delivery was outlined, including the composite hydrogels formed from natural polymer combined with synthetic polymer or inorganic nanoparticles.

Chitosan-based micellar hydrogels with self-healing and injectable property are developed from phenolic chitosan and a micellar crosslinker for stroke therapy [83]. Minocycline (a hydrophilic drug) and edaravone (a hydrophobic drug) are individually encapsulated in the hydrophilic network and hydrophobic micellar core of the hydrogel, and exhibit asynchronous releasing behaviour with a first-

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order rapid release for minocycline and a zero-order sustained release for edaravone. Rats treated with the dual-drug loaded hydrogel showed behavioral improvement with ≈84% recovery and balanced brain midline shift with ≈0.98 left/right hemibrain ratio. Leach et al. have developed a multi-domain peptide hydrogel for cyclic dinucleotide (CDN) delivery [84]. Compared to standard collagen hydrogel, this multi-domain hydrogel exhibited an eight-fold slower release rate of CDN and a six-fold improvement in survival of mice. A chitosan/poly (glutamic acid)/alginate polyelectrolyte complex hydrogel with homogeneous structure and excellent performance was prepared via a newly developed semi-dissolution/acidification/sol-gel transition method [85]. Piroxicam was in situ embedded in the hydrogel successfully and showed controlled colon-specific drug release, reducing the gastrointestinal irritation side effect of piroxicam. Mukhopadhyay and coworkers developed a precisely controlled host-guest interaction system to accomplish controlled stepwise release and capture of guest molecule (cyclodextrin) using coordinated polymer-based host with azobenzene side appendage and temperature as stimuli [86], exhibiting temperature responsive stepwise release of α-CD both in the solution and quasi-solid (hydrogel) states.

Magnetic nanoparticles with low toxicity and biocompatibility, like Fe3O4 particles are commonly introduced into drug delivery systems, rendering the drug carrier with targeted and controlled delivery character [87,88]. Novel magnetic hydrogel microrobots featured with sustained drug release, targeted movement, satisfactory antioxidant properties and biosafety are developed based on alpha-lipoic acid-conjugated gelatin methacryloyl for inner ear administration [89]. Pre-injection of the magnetic hydrogel microrobot into the middle ear of cisplatin-deafened mice can effectively prevent deafness. Kesavan et al. reported hybrid gel beads based on chitosan and Fe_3O_4 cross-linked polyethylene glycol for rifampicin (an anti-tuberculosis drug) delivery [87]. The magnetic gel beads showed pH and magnetic field responsive asset in the drug delivery application. $CaCO_3$/sodium alginate/Fe_3O_4 hydrogel-based capsule microrobots are prepared by a triaxial microfluidic chip for intravascular targeted drug delivery [90]. After intravenous injection, the hydrogel-based capsule microrobots can reach the destination in blood vessels along the predetermined trajectory through an external magnetic field.

Carbon quantum dots are another extensively investigated inorganic materials for drug delivery owing to their excellent properties, for instance, chemical inertness, great dispersibility, easy changeability of size and shape, high surface area with delocalized electrons, intensive fundamental fluorescence, unequaled excitation relevant emission, local functional groups at the edges and drug loading capacity by π–π interactions [91,92]. Javanbakht et al. reported a novel hydrogel nanocomposite films via incorporation of graphene quantum dot in to carboxymethyl cellulose hydrogel for doxorubicin delivery [93]. The nanocomposite hydrogel films showed a pH-sensitive and consecutive prolonged release of doxorubicin, and non-obvious cytotoxicity on K562 cells. Pooresmaeil et al. developed pH-sensitive bionanogels via chemical crosslinking of carbon dots and gelatin using dialdehyde carboxymethyl cellulose for both bioimaging and tumor-targeted co-drug delivery [94]. Curcumin and doxorubicin were loaded in the nanogels with drug entrapment efficiency of ~ 44.0% and 41.4%, respectively. The bionanogels showed pH-controlled release for both drugs and superior anticancer effect in comparison with free curcumin/doxorubicin.

Table 2. Natural polymer-based hydrogels as drug carriers for drug delivery.

Hydrogels	Drugs	Properties and function	Ref
Chitosan-based micellar hydrogels	Minocycline and edaravone	Self-healing and injectable property. First-order rapid release for minocycline and zero-order sustained release for edaravone. Behavioral improvement in stroke rats.	[83]
Multi-domain peptide hydrogels	Cyclic dinucleotide (CDN)	An eight-fold slower release rate of CDN and a six-fold improvement in survival of mice compared with standard collagen hydrogel.	[84]

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Chitosan/poly (glutamic acid)/alginate polyelectrolyte complex hydrogels	Piroxicam	Controlled colon-specific drug release, and reduced gastrointestinal irritation side effect of piroxicam.	[85]
Host-guest interaction hydrogel system	α-CD	Temperature responsive stepwise release of α-CD both in the solution and hydrogel states.	[86]
Magnetic hydrogel microrobots	Alpha-lipoic acid	Sustained drug release, targeted movement, satisfactory antioxidant properties and biosafety.	[89]
Hybrid gel beads based on chitosan and Fe_3O_4 cross-linked polyethylene glycol	Rifampicin	pH and magnetic field responsive asset in the drug delivery.	[87]
$CaCO_3$/sodium alginate/Fe_3O_4 hydrogel-based capsule microrobots	Indomethacin	Intravascular targeted drug delivery by following a predetermined trajectory in the blood vessel under magnetic drive.	[90]
Grapheme quantum dot/carboxymethyl cellulose-based hydrogel nanocomposite films	Doxorubicin	pH-sensitive and consecutive prolonged release of doxorubicin, and non-obvious cytotoxicity on K562 cells.	[93]
Carbon dots/gelatin/carboxymethyl cellulose based bionanogels	Curcumin and doxorubicin	pH-controlled release for both drugs and superior anticancer effect in comparison with free curcumin/doxorubicin.	[94]

3.2. Wound dressings for wound healing

In recent years, hydrogels have aroused great interest as wound dressing owing to the high content of water in the three-dimensional hydrophilic polymeric networks, which could afford a moist environment for the wound site, cooling the surface of lesion skin, alleviating the pain, helping wounds to heal faster [95,96]. Besides, hydrogels with porous and continuous networks are capable to absorb wound fluids, facilitate the exchange of nutrients and metabolites, adjust the oxygen concentration and protect the wound from infection [97]. Whereas, wound healing is a complicated process comprising several overlapping stages: hemostasis, inflammation, proliferation, re-epithelialization, and remodeling [98], which involves the systematic, coordinated and balanced activity of inflammation, blood vascular, connective tissue and epithelial cells.

Sodium alginate-polyacrylamide hydrogels were prepared for antibacterial study and wound healing using novel cross-linking strategy by divalent ion, such as copper, zinc, strontium and calcium [99]. The results indicated that hydrogels cross-linked by zinc had good antibacterial activities and could enhance wound healing by promoting fibroblasts migration, vascularization, collagen deposition and granulation tissue formation. Antibacterial hydrogels with conductive, adhesive and self-healing properties are prepared based on oxidized sodium alginate-grafted dopamine/carboxymethyl chitosan/Fe^{3+} via Schiff base and Fe^{3+} coordination bonds [100]. The hydrogels showed photothermal antibacterial properties under near-infrared irradiation and improved the wound repair in the infected skin wound in mice by reducing inflammation and increasing vascular regeneration. Yang and coworkers developed an intelligent responsive MXene-based hydrogel system as photo- and magnetic-responsive drug delivery carrier for deep chronic wound healing [101]. The system integrated a dual-network hydrogel composed of covalently cross-linked poly(N-isopropyl acrylamide) and ionically cross-linked alginate with MXene-wrapped Fe_3O_4@SiO_2 magnetic nanoparticles. The hydrogel system exhibited precisely control release of AgNPs when exposed to alternating magnetic field or near-infrared and presented desirable performance in chronic diabetes wound healing by eliminating bacteria attached and promoting M2 macrophages polarization and angiogenesis. A novel epsilon-polylysine modified cellulose/γ-PGA

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double-network hydrogel with good biocompatibility and antibacterial activity is designed for wound repair [102]. The hydrogel exhibits promoted wound healing effect in infected skin wound by improving collagen deposition, accelerating vascularization and enhancing cell proliferation.

Biomolecules including enzymes, proteins, pro-inflammatory factors and angiogenic markers have been reported to play a vital role in the successful healing of wound [103]. Thus, functionalized hydrogel formulations containing bioactive substances such as protein drugs, growth factors, live cells, which could up-regulated or down-regulated these markers were developed for wound care, especially for non-healing wounds such as diabetic wounds. Glucose-responsive hydrogels based on hyaluronic acid, phenylboronic acid, fulvic acid and EN106 are prepared via dynamic crosslinking of phenylboronate ester for improved diabetic wound repair [104]. Fulvic acid in the hydrogel servers as a crosslinking agent and provides antibacterial and anti-inflammatory abilities, and the sustained release of EN106, which is a FEM1b-FNIP1 axis inhibitor, can ameliorate oxidative stress and improve angiogenesis, thus resulting a promoting effect in the diabetic chronic wound healing. Composite hydrogels based on sodium alginate integrated with desferrioxamine (DFO) and bioglass have been designed for diabetic wound healing [105]. The results showed promoted expression of HIF-1α and VEGF and enhanced vascularization in the wound sites due to the synergistic effect of bioglass and DFO. Yuan and coworkers developed composite hydrogel based on epigallocatechin modified hyaluronic acid and tyramine-grafted human-like collagen, and integrated with deferoxamine-loaded mesoporous polydopamine nanoparticles for diabetic wounds repair [42]. The hydrogel exhibited prominent enhancement of angiogenesis, excellent anti-inflammatory and bacteriostatic effect, good antioxidant and hemostatic properties, biocompatibility, promoted the M2 macrophage polarization, and enhanced the diabetic wounds healing. Exosome-loaded hydrogels based on α-Lipoic acid modified chitosan via photo-crosslinking are designed for diabetic wound repair [106]. The hydrogels show strong adhesion, photo-induced self-healing and pH/H_2O_2/glucose responsiveness, and enables targeted exosome release to accelerate wound healing by regulating the wound environment, such as reducing oxidative stress, lowering blood glucose levels, and promoting angiogenesis.

Table 3. Natural polymer-based hydrogels as wound dressings for wound healing.

Hydrogels	Properties	Effects in the wound healing	Ref
Sodium alginate-polyacrylamide hydrogels	Excellent mechanical strength by zinc crosslinked hydrogel.	Antibacterial activities and promoted fibroblasts migration, vascularization, collagen deposition and granulation tissue formation.	[99]
Alginate/dopamine/carboxymethyl chitosan-based hydrogels	Antibacterial, conductive, adhesive and self-healing properties.	Photothermal antibacterial property, reduced inflammation and increased vascular regeneration.	[100]
Alginate/MXene-based hydrogel	Photo- and magnetic-responsive, and precisely control release of AgNPs.	Eliminatied bacteria attachment and promoted M2 macrophages polarization and angiogenesis.	[101]
Epsilon-polylysine modified cellulose/γ-PGA double-network hydrogel	Good biocompatibility and antibacterial activity.	Improved collagen deposition, accelerated vascularization and enhanced cell proliferation.	[102]
Hyaluronic acid-EN106 hydrogels	Glucose-responsive, antibacterial and anti-	Ameliorated oxidative stress and improved angiogenesis.	[104]

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	inflammatory abilities, and sustained release of EN106.		
Sodium alginate hydrogel containing desferrioxamine (DFO) and bioglass	Injectable and sustained release of DFO.	Promoted wound healing by increasing HIF-1α and VEGF expression and vascularization.	[105]
Composite hydrogels based on hyaluronic acid/collagen /deferoxamine-loaded polydopamine nanoparticles	Desirable mechanical property, improved tissue adhesive and injectable performance.	Exhibited prominent enhancement of angiogenesis, excellent anti-inflammatory and bacteriostatic effect, promoted the M2 polarization of macrophages, and enhanced the diabetic wounds healing.	[42]
Exosome-loaded hydrogels based on α-Lipoic acid modified chitosan	Strong adhesion, photo-induced self-healing and pH/H_2O_2/glucose responsiveness	Accelerate diabetic wound healing by regulating the wound environment, such as reducing oxidative stress, lowering blood glucose levels, and promoting angiogenesis.	[106]

3.3. Scaffolds for regenerative medicine

Hydrogels have aroused great interests as scaffolds for regenerative medicine due to their inherent ability to mimic the ECM, such as high content of water, the ability of cell engraftment at targeted sites for suspending cells, and the promotion of cell health [107]. The high permeability of hydrogel enabled the interchanges of oxygen, nutrients, and soluble metabolites, rendering them as ideal ECM substitutes for tissue engineering and regenerative medicine applications [108]. Natural polymers-based hydrogels offer a versatile platform for tissue engineering owing to their specific combinations of biocompatibility, biodegradability, biomimicry, responsiveness and mechanical tunability to mimic native ECM, providing spatial support, preserving cellular functions and encouraging tissue formation [109]. Collagen/alginate/fibrin based hydrogels were developed and investigated for soft tissue engineering [39]. The hydrogels have thermo-sensitivity and mechanical stiffness similar to native soft tissues, showing enhanced osteogenic potential of human mesenchymal stem cells and improved aggregation MIN6 β-cells with the indication of pseudo-islets formation, which provide a tunable microenvironment for promissing pancreas and musculoskeletal tissue engineering.

Composite hydrogels incorporating relevant inorganic fillers, such as bioactive ceramics, clays, grapheme oxide, have been reported to improve their physicochemical and biological performance in the application of tissue engineering [110]. Multifunctional composite hydrogels based on methacrylated poly(glutamic acid), methacrylated gelatin and fibroblast activating protein inhibitor (FAPi)–loaded MnO_2-coated calcium phosphate microspheres were prepared for osteoporotic bone defects treatment[111]. The hydrogels can effectively alleviate intracellular ROS, promote M2 macrophages polarization, and reduce inflammation in vitro experiments, and show promoted repair of osteoporotic bone defects by rescuing ROS microenvironment and guiding the immune response in bilateral OVX-induced osteoporotic rats. RGD-modified alginate-based osteoconductive hydrogels were prepared for mesenchymal stem cell (MSC) delivery in craniofacial bone tissue engineering, and hydroxyapatite microparticles were incorporated into MSC aggregates to induce osteogenesis [112]. The hydrogels showed tunable mechanical properties and biodegradability, resulting in complete bone regeneration around ailing dental implants with peri-implant bone loss in a rat model.

In general, cells encapsulated in three-dimensional are isotropic. However, many biological tissues, such as skeletal muscle, tendons, ligaments, and cardiac tissue, have an anisotropic cell

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arrangement. Furthermore, some physiological functions also require a cellular anisotropy, like uniaxial muscle contraction requires coaxial alignment of muscle fibers. Therefore, anisotropic cell encapsulation in 3D hydrogels is highly desirable and necessary for tissue regeneration [113]. GelMA-based hydrogel scaffolds containing anisotropic microchannels were developed by vertical 3D cryo-bioprinting technique, and live cells were encapsulated at high viability levels in desired cellular alignments to fabricate muscle-tendon unit and muscle-microvascular unit [114]. The hydrogel has improved robustness and versatility, and extend broad utilities in tissue engineering, especially those anisotropic in nature. Magneto-patterned cellular hydrogels were developed based on methacrylated hyaluronic acid via photo-crosslinking to generate heterogeneous tissues [115]. Diamagnetic objects, including living cells, polystyrene beads and drug delivery microcapsules can be pre-positioned in the 3D hydrogels. Engineered cartilage constructs similar to natural tissue were fabricated based on the patterned cellular hydrogels, with a high cellularity in the top region and a low cellularity in the bottom region. After long-term culture, the cartilage constructs retained these cell gradients and subsequently created gradients in the extracellular matrix content, which enables engineers to design and manufacture complex tissues.

Table 4. Natural polymer-based hydrogels as scaffolds for regenerative medicine.

Hydrogels	Properties	Functions	Ref
Collagen/alginate/fibrin-based hydrogels	Thermo-sensitivity and mechanical stiffness similar to native soft tissues	Enhanced osteogenic potential of human mesenchymal stem cells and improved aggregation MIN6 β-cells with the indication of pseudo-islets formation.	[39]
Gelatin/FAPi-loaded microspheres composite hydrogels	Antioxidant Properties	Promoted repair of osteoporotic bone defects by rescuing ROS microenvironment and guiding the immune response in bilateral OVX-induced osteoporotic rats.	[111]
RGD-modified alginate-based osteoconductive hydrogels	Tunable mechanical properties and biodegradability	Complete bone regeneration around ailing dental implants with peri-implant bone loss in a rat model.	[112]
GelMA-based hydrogel scaffolds containing anisotropic microchannels	Improved robustness and versatility	Encapsulated live cells at high viability levels in desired cellular alignments to fabricate muscle-tendon unit and muscle-microvascular unit	[114]
Magneto-patterned cellular hydrogels based on methacrylated hyaluronic acid	Pre-positioned diamagnetic objects in 3D hydrogels.	Fabricated cartilage constructs similar to natural tissue with gradient cellularity and maintained these cell gradients in the extracellular matrix content.	[115]

3.4. Other biomedicine applications

Drug discovery is of great importance in the chemotherapy of critical disease. Systematic and rapid analysis of the binding signals of small molecule drugs versus their target proteins is critical in the study of drug discovery and drug repositioning or repurposing [116]. Zhou and co-workers developed a 3D-dextran hydrogel chip for label-free and high-throughput detection of small

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molecule drugs [117]. They combined surface plasmon resonance imaging technology with protein microarray on the surface of hydrogel chip, resulting in good quality and uniformity binding signals for the detected drug molecules.

Cell-cell and cell-ECM interaction can influence cell behavior, for instance the response of cells to drug treatment [118]. Thus, the generation of 3D multicellular tumor spheroids (3D TSs) is of great importance for characterization of anticancer drugs because that 3D TSs can provide a more representative model than conventional 2D monolayer culture, and the tumor cell-ECM interaction in a 3D culture environment is more parallel to a tumor in vivo than that in a 2D environment. Karamikamkar et al. developed 3D hydrogel beads containing alginate and collagen as in vitro tumor spheroid models using a flow focusing process combined with ultrasound treatment [119]. Breast cancer cells MCF-7 were encapsulated in the hydrogel beads and retained cellular viability and proliferation ability to produce homogeneous TSs.

The physical microenvironment of stem cells significantly affects the maintenance of pluripotency, differentiation behavior and subsequent tissue morphogenesis [120]. Studies showed that in vivo stem cell niches are mechanically dynamic microenvironments that constantly evolve to coordinate tissue morphogenesis and homeostasis cell activity. Thus, ECM modeling/remodeling is indispensable in regulating stem cell behavior. Various types of scaffolds, especially hydrogel systems, have been widely used in the three-dimensional microenvironment of ECM in vivo due to their physicochemical similarities[121]. Magneto-responsive GelMA-based hydrogels were developed by incorporating Fe_3O_4@SiO_2 magnetic nanorods [122]. The pre-alignment of nanorods under a low magnetic field enables dynamically and reversibly modulated modulus of the hydrogel, and control of the hydrogel stiffness significantly influenced the differentiation of human-induced pluripotent stem cells by regulating the activation of mechano-sensitive signaling mediators. Dual-crosslinked alginate hydrogels with 3D micropatterns were prepared for growth factors immobilization and stem cells encapsulation by photo-crosslinking of various geometries and micropattern sizes on heparin substrates [123]. Stem cells within the 3D hydrogels showed spatially localized growth and differentiated responses to diverse growth factor patterns, indicating that the micropatterned hydrogel is a promising platform for the control of stem cell behavior in tissue engineering.

4. Future perspectives

Natural polymers purified from animal or plant sources have aroused great interests in recent years because of their intrinsic properties such as chemical stability, structural versatility, good flexibility and biocompatibility, displaying enormous potential in biomedical fields including drug delivery and tissue regeneration. Most natural polymers require complex purification or extraction processes, and the property of materials may be different according to their sources. Thus, various technologies have been explored to improve the structural properties. For example, enzymatic technology has been developed to produce hyaluronic acid with high molecular weight, defined chain length and low polydispersity, and biological technology such as recombinant systems have been used for the synthesis of collagen molecules [40,124]. In addition, natural polymer derivatives prepared by chemical modification combined the merits of natural polymers and synthesis polymers, and have been widely used in diverse biomedical areas. What's more, the development of new natural based polymers with advanced properties in structure, interaction with surrounding cells and tissues to achieve promoted in vivo performance in biomedical application is still very important and challenging.

Although hydrogels based on natural polymer are attractive in biomedical application due to their bioavailability, biodegradability, biocompatibility, non-toxicity, and immunogenicity, such materials suffer from several limitations involving poor mechanical strength. Hence, various strategies have been designed to obtain strength and toughness hydrogels, such as nanocomposite hydrogel, double network hydrogel and dual cross-linking hydrogel. Nanocomposite hydrogel uses micro- or nanofiller reinforcement technique to solve the limitation, and carbon materials, clays, inorganic nanoparticles, nanofibrillated cellulose and many other inorganic materials were applied

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to combine with polymeric gels for the fabrication of hydrogel nanocomposites with multiple network structures and high mechanical performance [125,126]. For example, chitosan-based hydrogel nanocomposite with multi-structural network was designed to enhance the mechanical strength by filling with grapheme oxide (GO) nanosheets [127]. A small amount of GO filling (≤0.30 wt%) can promote the formation of multiple network structures of the hydrogel composites due to the self-assembled GO-chitosan units, thereby enhancing the mechanical properties. Double network hydrogel based on two interpenetrating cross-linked polymer networks exhibits excellent mechanical properties due to the strong network entanglement, and has gained significant development since it was first proposed by Gong et al [128]. Dual-crosslinking hydrogel including non-covalent and covalent bonding provide another efficient way to enhance the mechanical strength of hydrogel. For example, Chen et al. developed a dual crosslinked hyaluronan hydrogels through the combination of electrostatic interaction and Schiff-based bonding [129]. The hydrogel is further photopolymerized to achieve a high modulus and stability, exhibiting rapid gelation and good injectability for stem cell protection with a cell viability of 92% after extrusion.

On the other hand, various novel hydrogels with designed properties have been developed for biomedical application. Intelligent hydrogels, also known as environmentally responsive hydrogels, are prepared by introducing environmental response moieties into polymer chains, which could respond to external and/or internal stimuli and function as sensors or effectors. Many chemical and/or physical stimuli (such as temperature, pH value, light, pressure, sound, electric and magnetic field, solvent components, ions and some special recognition molecules) can induce the responsiveness of intelligent hydrogels. Due to the differences between the physiological microenvironment of many lesion sites and normal tissues, intelligent hydrogels have great advantages for smart drug delivery, which can target drug delivery to the lesion sites, reduce the toxicity and side effects of drugs, and improve their bioavailability. Injectable hydrogels are another new type of hydrogel materials, generally cross-linked by dynamic bonds, which can be implanted into the human body without surgery. Biomolecules and/or cells can be encapsulated in hydrogels in situ by mixing them in polymeric solution before injection. By injection, cell-laden scaffolds can be implanted into the desired location, avoiding the pain and complexity of surgical implantation, and in situ cell fixation is also beneficial to fill irregular tissue defects. Chen et al. developed a multi-responsive hydrogel for logic diagnostic and therapeutic to orchestrate diabetic bone regeneration [130]. The regeneration of diabetic bone defects is seriously impaired by the fluctuation of glucose level, high concentration of active oxygen species, and overexpression of proteinases. The injectable multi-responsive hydrogel with a double network is consisted of phenylboronic-acid-crosslinked poly(vinylalcohol) and gelatin colloids, demonstrating a logic-based cargo release and regulation of macrophage polarization for better diabetic bone regeneration.

Furthermore, various new technologies have been explored to fabricate advanced hydrogel materials with designed properties for biomedical application. In recent years, with the widespread usage of 3D printing, this technology has been adopted to create cell-laden hydrogels with biomimetic structures for regenerative medicine and tissue engineering. This technique of 3D printing living cells is also known as 3D bioprinting. Due to the attractive features, such as high water content, highly porous and permeable that mimics ECM and helps cell attachment and migration, hydrogels are ideal candidates for bioinks as both cellular carriers and structural constituents [131]. The most frequently-used 3D bioprinting techniques include extrusion, injection, laser-assisted bioprinting, and stereolithography. Wang et al. designed an implantable gelatin-based hydrogel scaffold with porous structure loaded with immunoregulators for cancer vaccine delivery therapy using 3D printing technology [132]. Compared with conventional hydrogel, this 3D printed hydrogel scaffold with specific structure can recruit immune cells and inhibit tumor growth more efficiently. Zhang et al. developed a unique shinking printing technique to enhance the resolution of 3D printing [133]. They found that by immersing a 3D-printed patterned hydrogel with network formed by polyionic polymers into a polyionic solution with opposite net charges, shrinkage can occur rapidly due to complex condensation and water expulsion, resulting in reduced linear dimensions printed constructs compared to the original pattern. Zhang et al. developed a fast hydrogel stereolithography

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printing (FLOAT) method to create a centimeter-sized, multi-scale solid cell-laden hydrogel model within minutes [134]. The rapid 3D printing of large-size hydrogel models is significant to reduce the part deformation and cellular injury in conventional 3D printing methods. An inherent limitation of 3D printing is that the printed object is static and therefore inappropriate for dynamic remodeling in response to external stimuli. Thus, 4D printing has been created to represent a careful combination of additive manufacturing, smart materials, and appropriate geometry, which allows for animated structure, changing the shape, function or performance over time when subjected to specific external stimuli after fabrication [135,136]. Wang et al. developed a multi-responsive bilayer membrane comprising of a hydrogel layer and a micro-structured shape memory polymer layer using 4D printing technology [137]. The membrane allows active topography change via external heating and can tailor the mechanical property to match the demands at different stages in the bone repair process, improving the overall efficiency.

5. Conclusions

Natural polymer-based hydrogels are attractive in biomedical fields due to their unique and excellent properties. The polymeric structure and process/synthesis of commonly used natural polymer for hydrogel preparation and the natural polymer-based hydrogels including hydrogel formation and property are elaborated. The biomedical application of hydrogels based on natural polymer in drug delivery, tissue regeneration, wound healing and other biomedical fields like drug discovery, cell-cell and cell-ECM interaction and cell guiding is summarized. With the development of the biomedicine, hydrogels as well as polymers with high performance are badly desirable. Thus, new technologies such as enzymatic and biological methods are developed to improve the structural properties of natural polymer and to develop novel natural based polymers or natural polymer derivatives with high performance. In addition, novel hydrogel materials (such as hybrid and/or composite hydrogels, intelligent hydrogels with stimuli responsiveness, and injectable hydrogels) are designed to meet the advanced requirements in biomedical application, and new strategies (such as in situ crosslinking, dual-crosslinking and 3D/4D bioprinting) have been explored to fabricate advanced hydrogel materials with design properties for biomedical application.

Author Contributions: Conceptualization, Lingling Zhao and Hui Tan; Writing-Original Draft, Lingling Zhao; Visualization, Jiaying Zhang; Supervision, Hongze Liang; Resources, Xianwu Chen; Funding Acquisition, Lingling Zhao and Hui Tan.

Funding: This research was funded by Natural Science Foundation of Zhejiang Province, grant number LTGY23E030002; the Fundamental Research Funds for the Provincial Universities of Zhejiang, grant number SJLY2021002; the Key Laboratory of Diagnosis and Treatment of Digestive System Tumors of Zhejiang Province, grant number KFJJ-202204; Natural Science Foundation of Guangdong Province, grant number 2021A1515010720; Shenzhen Science and Technology Innovation Commission, grant number JCYJ20220818102804009.

Conflicts of Interest: The authors declare no conflict of interest.

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