GELS Handbook

Fundamentals, Properties and Applications

Volume 2: Applications of Hydrogels in Regenerative Medicine

Mohammad Reza Abidian, Umut Atakan Gurkan
and **Faramarz Edalat** *Volume Editors*

Utkan Demirci
Ali Khademhosseini
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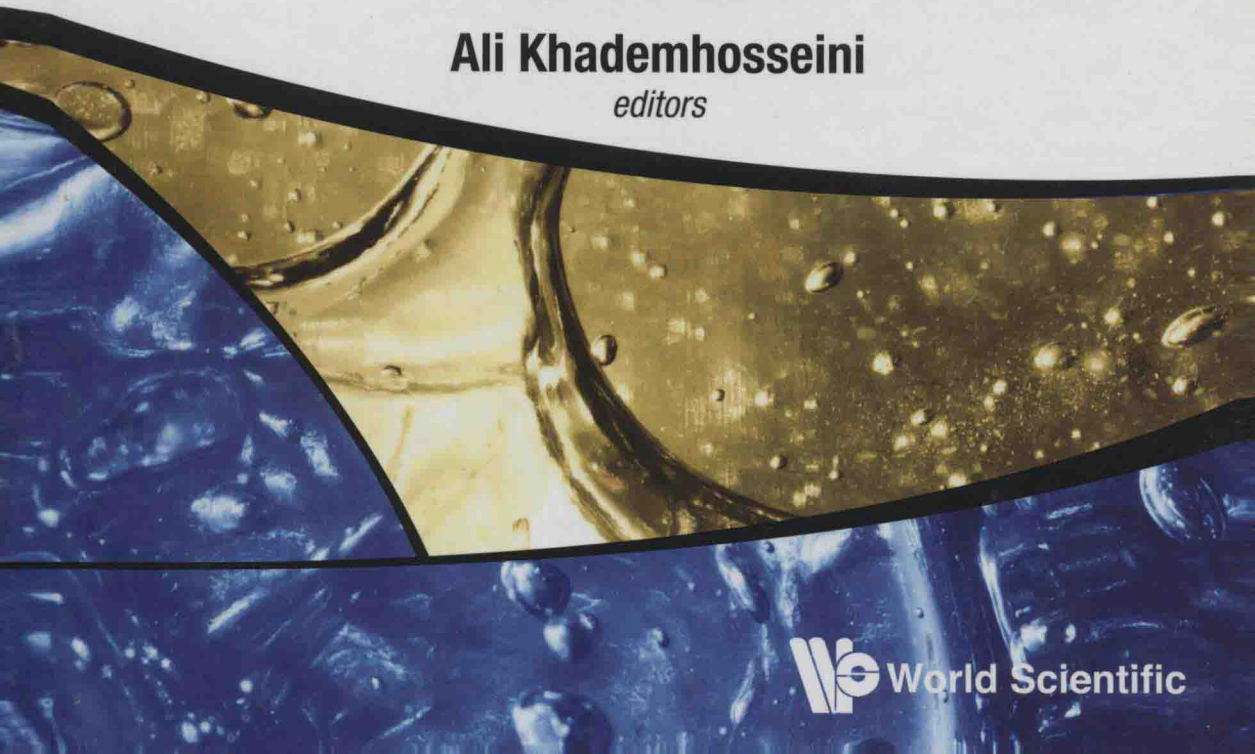

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Fundamentals, Properties and Applications

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Chapter 1

Hydrogels in Regenerative Medicine

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Hydrogels are soft, jelly-like, polymeric networks with very high water retention capacity. They are made from natural materials or synthetic polymers. They are very important for the biomedical field because of the similarity between their mechanical and chemical properties and that of the extracellular medium, the microenvironment of a cell. They can be used in cell loaded form. High water content of the hydrogels is very suitable even without the cells in mimicking the hydrated tissues or viscous bodily fluids where they serve as a lubricant or a shape forming material. Actually cells do not like to adhere to very highly hydrophilic structures so they are very useful in applications where non-adherence is sought, like prevention of adhesion of tissues after a surgery. On the other hand, when we need the cells to attach to the hydrogels we make some chemical modifications on the material such as attaching cell adhesive arginine–glycine–aspartic acid (RGD) amino acid sequences. In this chapter the types, the sources of their starting materials, the methods of insolubilization, the network formation, the methods to use to control their properties, and their biomedical applications have been presented. Among the applications discussed are drug delivery systems, tissue engineering scaffolds, wound dressings, anti-adhesive membranes, and meshes.

1. Introduction to Hydrogels

Hydrogels are hydrophilic polymeric networks that absorb and retain significant amount of water. Hydrophilic side or pendant groups attached to the polymeric backbone give hydrogels the ability of water absorption and the various types of contacts between the chains of the network provide resistance to dissolution in water.[1,2] The first synthetic hydrogel for medical purpose was produced by Witchterle and Lim in 1954[3] and since then this technology has been employed in a wide range of applications as biomedical materials, pharmaceuticals, biosensors, drug carriers, and food additives.[4] Hydrogels can absorb water up to thousands of times of their dry weight. The ones that contain more than 95% water by weight are called as super absorbent which are also highly biocompatible because of their high water content. Water inside the hydrogel is needed for the diffusion of water soluble molecules and the polymeric network is there in order to hold the water inside the structure. The form of "gel" mentioned in this definition is not completely solid nor liquid but between these two states that provides excellent relaxation and mechanical properties to the scaffold.[5] This property is the reason for their compositional and mechanical similarity to the native soft tissues and the extracellular matrix (ECM).[6] The interfacial tension is also low and this decreases protein adsorption. Low protein adsorption decreases the inflammatory responses, an essential prerequisite for biomedical applications.[7] Hydrogels offer significant advantages for tissue engineering purposes, such as high biocompatibility, ease of modification, and injectability into voids at injury sites. It is also possible to fabricate smart or stimuli responsive hydrogels that respond to environmental stimuli like pH, temperature, exposure to light, and concentration of the metabolites. Responsibility makes them suitable for controlled release of the bioactive agents including drugs and growth factors. Hydrogels, however, have several disadvantages including high cost, difficulty of sterilization, non-adherent nature, and low mechanical properties. Crosslinking is also challenging if attempted after loading the gels with cells and drugs.[2,4]

Hydrogels can be classified into several categories depending on their various properties. Based on their charges they can be categorized into four groups: neutral, anionic, cationic, or amphoteric hydrogels. Based on their structure, the network can be completely amorphous or semicrystalline. Finally, depending on their network porosity, they may be macroporous, microporous, and non-porous.[8,9]

1.1. Formation of hydrogels

Formation of a gel, namely gelation, is the creation of a three-dimensional (3D) network structure via linking of macromolecular chains together. Gelation can occur by physical linking (physical gels) or by chemical linking (initiated by

chemical agents or by radiation). Depending on the functional groups attached to the main chains, hydrogels may have responsive property.

1.1.1. *Physical hydrogels*

In the physical hydrogels the network is held together by forces like ionic interactions, hydrophobic forces, H-bonding, and chain entanglements, and therefore, network formation is reversible.[2] In the recent years, physical hydrogels are preferred more than chemical ones because crosslinking agents are not needed to form the structure. This is important because crosslinking with chemical agents or radiation can alter the activities and properties of the proteins, enzymes, and the cells if crosslinking follows entrapment. Moreover, chemical agents are usually toxic due to their high reactivity and the excess agents have to be removed with additional washing steps.[10] Physical crosslinking, on the other hand, has disadvantages such as inhomogeneity due to clusters of molecular entanglements, and ionic or hydrophobic domains. Additionally, transient network defects can be observed because of chain loops or free chain ends. Another limitation of reversible hydrogels is the risk of disruption of the molecular interactions due to changes in environmental conditions like pH, temperature, ionic strength, and application of stress.[11]

1.1.2. *Chemical hydrogels*

Chemical hydrogels with covalently crosslinked networks are called "permanent" gels. They are usually produced by polymerization or parallel crosslinking through covalent bond formation. The crosslinks may either form during the polymerization reaction or afterwards.[12] Condensation and addition polymerizations are two main polymerization categories and vulcanization is another covalent interaction via sulfur bonds which generally occurs after the formation of the polymer.[6,10,13] Covalent bonds are much stronger than the physical ones and therefore gels formed with covalent bonds have higher stability.

Four molecular interaction types, namely ionic bonding, entanglement, interpenetrating networks (IPN) and covalent bonding, are involved in hydrogel formation (Fig. 1).

1.1.3. *Stimuli responsive hydrogels*

Stimuli responsive (or intelligent) hydrogels can experience significant property changes (e.g., volume change) in response to small changes in physiological or biological environment.[14] For example, a reversible hydrogel which was formed using ionic groups will dissolve if the ionic groups lose their charges with a change in the

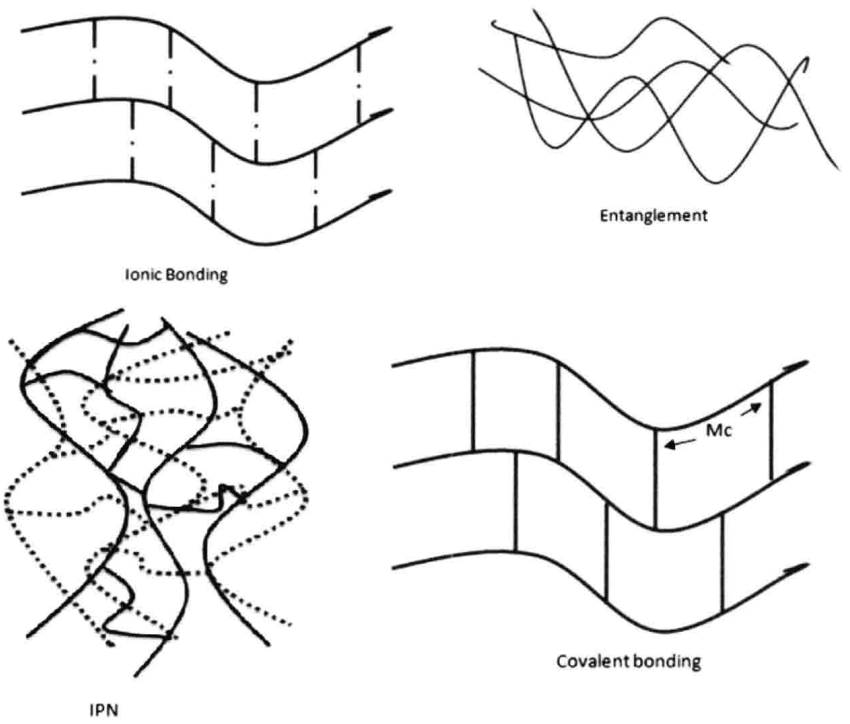

Fig. 1. Molecular interactions involved in the formation of hydrogel networks.

pH of the medium. pH-dependent swelling behavior of poly(acrylic acid) hydrogels obtained from crosslinking with poly(L-glutamic acid)-g-(HEMA is 2-hydroxyethyl methacrylate) (PGA-g-HEMA) was reported in the literature.[15] The hydrogels exhibited pH-dependent swelling behaviors where in acidic pH they had lower swelling ratio and in basic pH the swelling ratios reached 5 to 10 times more than those in acidic medium. Lower swelling ratio at acidic pH is due to the protonation of both acrylic acid and glutamic acid residues and higher swelling ratio is because of ionization of these groups above their pKa (pH > 4). In another study pH-sensitive hydrogels were developed for drug release by Vaghani and Patel.[16] They utilized chitosan and poly(N-vinyl pyrrolidone) (PVP) blends crosslinked with glutaraldehyde in order to obtain a semi-IPN structure. The hydrogels showed high swelling at acidic pH due to protonation of primary amino groups of chitosan and low swelling behavior was observed under alkaline conditions.[16] Light sensitive hydrogels may be another example for stimuli responsive gels which change conformation upon exposure to light. In their study,[17] they produced photoresponsive hydrogels in the design of microfluidic devices. They obtained hydrogels from poly(N-isopropylacrylamide) (PNIPAM) functionalized with chromophore spirobenzopyran. The hydrogels were fabricated into microchannels by *in situ* polymerization, and upon exposure to blue light shrinkage of the gels was observed, a response that may serve

as a useful tool in the control and fabrication of integrated microfluidic devices.[17] Photoresponsive hydrogels are widely used in drug delivery applications. Wells *et al.*[18] reported a hyaluronan-based hydrogel for controlled delivery of ophthalmic drugs upon ultraviolet (UV) exposure. They used two types of drugs with low and high molecular weights in order to see the pattern of delivery and concluded that the drug release could be manipulated with size of the drug and the exposure to UV radiation. One advantage of this system is that release rate can be decreased or totally turned off in case any unwanted reactions occur.[18]

1.2. Hydrogel types

ECM is the best model in the construction of the tissue engineering scaffold designs because the goal is to create an environment where the cells are able to attach and stretch as in ECM. Any scaffold should fulfill the basic requirements like biocompatibility, allowing cell ingrowth, proliferation, and differentiation.[19] Thus, the biomaterials to be used in hydrogel construction should be chosen by taking these requirements into consideration. The following are the materials used in scaffold formation grouped according to their sources.

1.2.1. *Natural materials*

Natural polymers are obtained from various sources including mammals, bacteria, plants, and crustaceans, and they are polysaccharides or polypeptides in nature.[20] Hydrogel networks based on natural materials offer significant benefits over synthetic materials due to their high biocompatibility and ECM-like chemistry, and as a result, they are in widespread use. Biodegradability is an inherent property of natural polymers and this is important for tissue engineering applications. Natural polymers are metabolized in the biological systems via hydrolysis or via enzymatic degradation and the typical products are amino acids or saccharide units if the macromolecules are polypeptides or polysaccharides, respectively.[20,21] The main concerns about the natural polymers and the resultant hydrogels are the risk of induction of immune responses, batch to batch variability, differences in the source, and limited availability. However, new techniques and processing methods for purification and production offer better materials for applications.[22] In the following sections, some information about the most commonly used natural polymers is provided.

1.2.1.1. Collagen

Collagen is the major constituent of the mammalian tissues like skin, cartilage, cornea, bone, and blood vessels and it is very popular in biomedical applications

due to its role in cell attachment, proliferation, anchorage, and differentiation (Fig. 2).[23] There are more than 27 different types of collagen identified so far[24] and all share the same basic structure of three left-handed polypeptide chains assembled into a right-handed triple helix.[25] Collagen can form fibers through self-aggregation and crosslinking and these fibers provide the mechanical strength and stability to the scaffolds. Collagen can be used as it is or often is stabilized by using physical and/or chemical methods in order to decrease the degradation rate which is important for many applications like drug delivery. Glutaraldehyde, carbodiimides, formaldehyde, chromium ions, and acyl azide are some of the chemical agents used to stabilize the collagen fibers. UV and gamma ray irradiation, and dehydrothermal treatments are examples for physical methods to crosslink the collagen matrix.[19,26] Collagen hydrogels have been used in many applications like corneal tissue repair,[27–29] cartilage defects,[30,31] cardiovascular tissue regeneration,[32,33] and meniscus repair.[34] They are semisolid and viscoelastic at rest but can be liquefied under stress. Injectable suspensions of collagen fibers or non-fibrillar collagen solutions carrying drugs or proteins can be applied directly to the defected site of the body.[35]

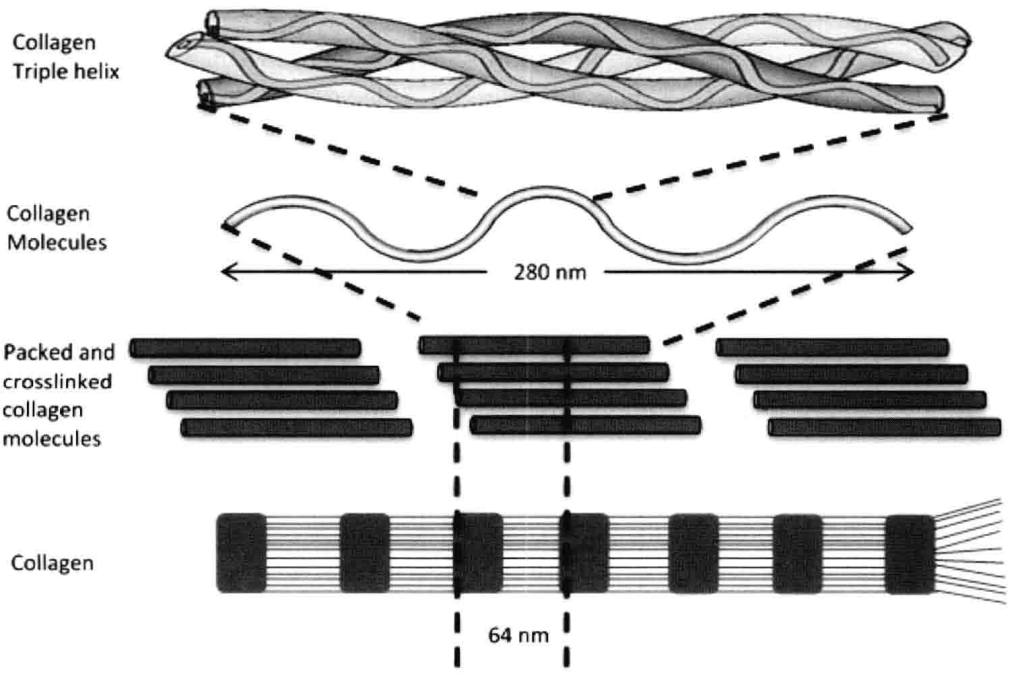

Fig. 2. Hierarchical organization of collagen fibers.

1.2.1.2. Hyaluronic acid (HA)

HA or hyaluronan is a linear, high molecular weight, and non-sulfated polysaccharide found in the ECM of many connective tissues like skin, vitreous humor, and umbilical cord.[19,36] It consists of the repeating disaccharide units of N-acetyl-D-glucosamine and D-glucuronic acid, that are linked with alternating β-1,3 and β-1,4 glycosidic bonds (Fig. 3(A)). HA is a glycosaminoglycan (GAG) of the body that is essential for matrix organization, cell signaling, mechanical integrity, and in wound repair. The hydrolysis of HA in the body is achieved rapidly by hyaluronidase and reactive oxygen species, within hours or days, the rate varying according to the tissue type.[37,38] Chemical modification of the HA may be done at three functional groups of the disaccharide: hydroxyl groups, the carboxylic acid of glucuronic acid, and the N-acetyl group of N-acetylglucuronic acid.[37] In recent years, HA has become a very popular biomaterial for tissue engineering and regenerative medicine since it can be modified to obtain the desired properties such as proper biological activity, hydrophobicity, and even crosslinkable groups. Injectable HA hydrogels loaded with drugs or bone morphogenic protein-2 (BMP-2) were used at the defect site to stimulate bone and cartilage formation.[39,40] Besides these applications HA is also widely used for cosmetic purposes like tissue augmentation in order to fill the wrinkles resulting from aging, sun exposure, and gravity, and it is one of the most popular non-surgical dermal filler approved by the Food and Drug Administration (FDA) (USA). One disadvantage of HA-based fillers is its short half-life and this could be solved by using different chemical crosslinkers.[41,42] HA can be bound to other polymers in order to obtain stimuli-responsive behaviors. In one study, researchers used PNIPAM to obtain a thermoresponsive injectable hydrogel that would both have biodegradability and also phase transition behavior with temperature change. This approach was shown to be promising for adipose tissue engineering as demonstrated in mouse *in vivo* studies where it was concluded that this hydrogel can be used for a variety of soft tissue engineering applications.[43]

1.2.1.3. Fibrin

Fibrin is another natural material used in the preparation of hydrogels. It is a biopolymer formed from the monomer fibrinogen and these two compounds are very critical for the homeostasis of the body. Fibrin results from the covalent crosslinks among the fibrinogen monomers and the fibrin network is quite resistant to protease degradation. Additional crosslinks can be introduced by using molecules like genipin to introduce more resistance and stability.[44] Fibrin can be purified from the blood of the patient; therefore, it does not pose any risk of infection or foreign body reaction. It is also widely used as a tissue adhesive in the medical field. Fibrin-based tissue adhesives mimic the last stages of the blood clotting scheme by utilizing

fibrinogen and thrombin that form a clot when mixed in the presence of calcium ions.[45] Fibrin sealants are used commonly following procedures like cardiovascular, vascular, neurological, abdominal, and thoracic surgeries.[46] Fibrin gels are also used together with other gels like HA-based ones in order to improve their mechanical properties to be used in different applications involving cartilage tissue repair.[47]

1.2.1.4. Chitosan

Chitosan is another naturally occurring polysaccharide obtained from shrimp or crab shells and consists of a series of linear copolymers of N-acetyl-D-glucosamine and β-1, 4-linked-D-glucosamine (Fig. 3(B)). Chitosan is derived from chitin which is composed mainly of only N-acetyl-D-glucosamine units and some of these units are deacetylated.[19] It is biocompatible, biodegradable, and has a pH-dependent cationic property. Chitin is stable and degrades upon heating instead of melting,[48] and this limits its processability. However, the main limitation of chitin is its poor solubility in many solvents. For chitosan, an acidic environment of around pH 6.2 is suitable for dissolution and upon neutralization (at pH over 6.2) it forms a gel.[49] Chitosan is one of the most widely used polysaccharides in biomedical applications such as, dermal tissue repair,[50] cell encapsulation,[51] bone reconstruction,[52–55] cartilage tissue engineering,[56] and drug delivery.[57,58]

1.2.1.5. Agarose

Agarose is a polysaccharide derived from red algae and seaweed, and has a structure of alternating 1,3-linked D-galactose and 1,4-linked 3,6-anhydro-L-galactose residues (Fig. 3(C)). Agarose is highly soluble in water at temperatures around 65°C and gels upon cooling in the temperature range 37–40°C. At lower temperatures it is stable and does not liquefy again until heated to 65°C. The gelation temperature is dependent on the degree of substitution of hydroxymethyl groups on the side chains.[59,60] Agarose hydrogels have been used in different applications including cartilage repair and bone regeneration. In their study Khanarian *et al.*[61] produced hydrogel-ceramic composite scaffold made up of agarose and hydroxyapatite (HAp) in order to achieve calcified cartilage formation. Their studies showed that biomimetic ceramic-hydrogel composite is a promising scaffold for osteochondral interface regeneration. For bone regeneration, Puértolas *et al.*[62] used biphasic calcium phosphate (BCP) and agarose in order to enhance the mechanical properties of the agarose-based hydrogels. They concluded that these ceramic-agarose systems offer advantages for bone tissue applications due to their biocompatibility, biodegradability, high mechanical properties, and affinity for proteins.

1.2.1.6. Alginate

Alginates are the second most abundant polysaccharides on earth after cellulose and they are produced by bacteria and seaweeds (Fig. 3(D)).[20] They are linear block copolymers of 1,4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. These residues in the blocks are either similar or alternating (MMMM, GGGG, or MGMG). L-guluronic acid blocks or the alternating blocks are stronger than D-mannuronic acid blocks due to diaxial linking among the residues. Thus, the physical and chemical properties vary with the M/G ratio and structure of the alternating regions. Alginate solution becomes a gel in the presence of divalent cations like calcium, lead, barium, and copper due to chelation of the carboxylic acid groups in the sugar. The interactions with these divalent cations are significantly affected by the M/G ratio.[63] Therefore, the viscosity of the alginate solution depends on the polymer concentration, molecular weight distribution and M/G ratio. Temperature is another factor that affects the crosslinking level of the structure; at lower temperatures more ordered gels are obtained because of slow diffusion of calcium ions. Covalent crosslinking of the alginate can also be achieved in the presence of diamines and dihydrazines; however, these methods are not so widely used as the chelation with the multivalent cations.[19,63] Alginate is widely used in tissue engineering applications due to its non-immunogenic and hydrophilic nature. It can be used as an injectable solution that forms a gel in the presence of multivalent cations at the defect site. Injectable alginate hydrogels are also used for controlled drug delivery

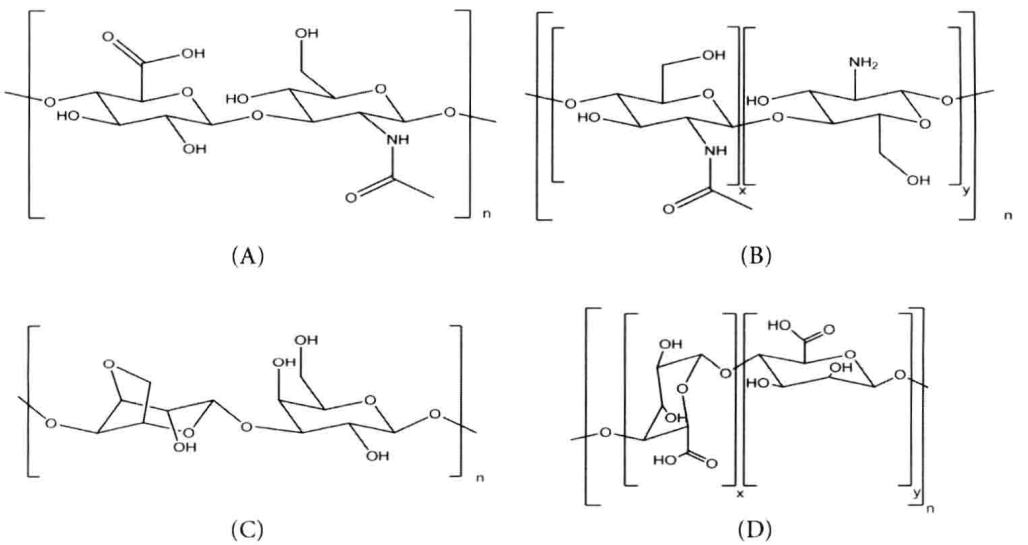

Fig. 3. Molecular structure of typical natural macromer units used in hydrogels for tissue engineering. (A) HA, (B) Chitosan, (C) Agarose, and (D) Alginate.

purposes.[64] For instance, these hydrogels were used in myocardial repair studies as a dual delivery system for hepatocyte growth factor (HGF) and insulin-like growth factor.[65] Alginate hydrogels are also widely used in the formation of interpenetrating polymer networks.[63] One of the limitations of the alginate hydrogels is their unregulated degradation because they are not degraded enzymatically. Moreover, chemical or biological modifications are needed to make alginate hydrogels suitable for cell attachment; otherwise cells do not adhere on them.[19,63]

1.3. Synthetic materials

Synthetic materials are attractive alternatives of the naturally originating compounds in building hydrogels because of their following advantages: (i) a large number of biodegradable synthetic polymers are biocompatible and do not induce any immunological responses; (ii) their mechanical properties and degradation rates can be altered by changing the production and process conditions and components; and (iii) their sources are not limited as the natural ones are.[66,67] However, sometimes harsh chemical methods are needed for their synthesis. The various additives and ingredients needed during the synthesis and the unreacted remnants could be detrimental to the cells and tissues.[1,19] Thus, it is very important to make sure no residues remain behind after polymerization before use in clinical applications.[19] A wide variety of synthetic polymers are used in the production of hydrogels. PHEMA, poly(vinyl alcohol) (PVA), poly(ethylene glycol) (PEG), and poly(N-vinylpyrrolidone) (PVP) are among the most frequently employed synthetic polymers.

1.3.1. *PHEMA*

PHEMA networks are obtained by free radical polymerization of monomer of 2-hydroxyethyl methacrylate.[19] PHEMA is used in various biological and medical applications due to its high swelling capacity, non-degradability, and biocompatibility.[68] It is one of the most widely known synthetic polymers used in hydrogel production (Fig. 4(A)). PHEMA is the earliest hydrogel material that is used in biomedical applications such as soft contact lenses, artificial cornea, and keratoprostheses.[69] PHEMA hydrogels are also extensively employed in drug delivery applications,[70] breast reconstruction,[71] cartilage repair,[72] nerve guide,[73] and wound dressings.[74]

1.3.2. *PVA*

PVA which is obtained from hydrolysis of poly(vinyl acetate) is another major synthetic material widely used in hydrogel preparations[19] (Fig. 4(B)). These