GELS Handbook

Fundamentals, Properties and Applications

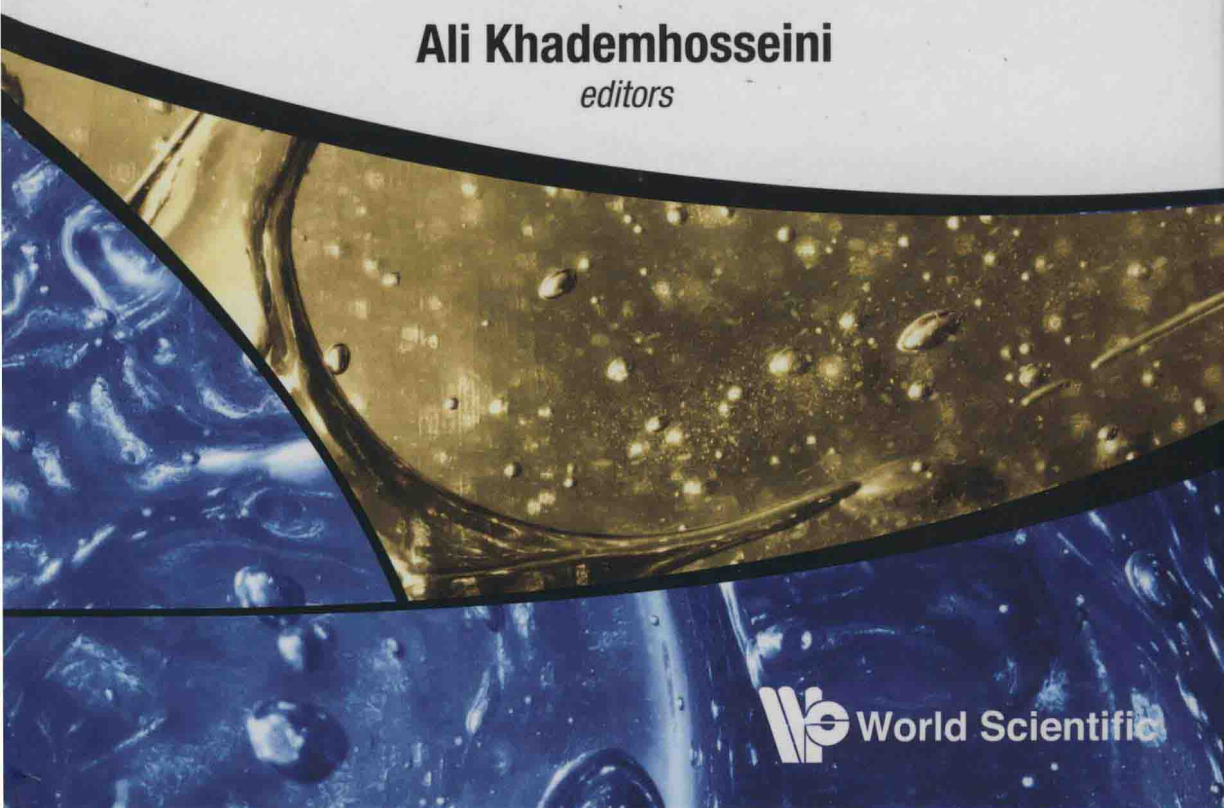
Volume 1: Fundamentals of Hydrogels

Qi Wen and **Yi Dong** *Volume Editors*

Utkan Demirci

Ali Khademhosseini

editors



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Chapter 1

Natural Hydrogels

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1. Introduction

Gels are ubiquitous materials that can be found all around our everyday lives. In fact most of our tissues and organs are gels. Most gels are made up of a network of polymers or colloids that is filled by a fluid.[1] At steady state when the gel is neither stressed nor strained, it will not flow.[2] That is, a gel's elastic modulus, G', is greater than its loss modulus, G''; a gel is thus a solid albeit a soft and squishy one. Even though the network of polymers or colloids that form a continuous 3D network in a gel can be very dilute (less than 1% by weight), this dilute network is enough to support shear stresses and thus gives the gel its solid-like material properties.[3]

1.1. Types and chemistry of natural hydrogels

A hydrogel is simply a gel where the fluid discontinuous phase is water whereas the solid continuous phase is still a network of polymers or colloids.[4] Hydrogels can be characterized by how their networks are held together. "Physical" hydrogels have their polymer network linked together by entanglements, ionic, hydrogen bonds or other forces. "Chemical" hydrogels, on the other hand, have their networks linked by permanent chemical bonds. In general, the links or connections of physical hydrogels are transient in nature and are therefore weaker than those of chemical hydrogels. These transient connections are also sometimes reversible compared to the covalent chemical connections.[5]

Natural hydrogels that are formed from protein such as collagen or fibrin or from polysaccharides such as agarose or alginate are all around us and have been

known for a long time. Synthetic hydrogels have to be polymerized in the laboratory and are first reported in 1954.[6] Such artificial hydrogels are highly customizable and can be tailored to specific applications by fine-tuning their mesh size,[7–9] polymer length,[10,11] water content,[12] mechanical and chemical properties, etc.[13–15] However, natural hydrogels have the strong advantage of being biocompatible[16–19] and biodegradable.[20–22]

1.2. Classify hydrogels based on their formation

Yet another way to classify hydrogels is by how they are synthesized. The hydrogel research field is quite diverse. Here we will briefly discuss two new very exciting developments. One, smart hydrogels are materials that change their properties in response to a change to external stimuli or inputs.[23,24] Some possible inputs are changes in pH,[25,26] temperature,[27,28] salt content,[29–31] chemical or biochemical contents, etc.[32,33] Possible outputs or changes in the hydrogel properties include changes in mechanical properties,[34,35] shapes, swelling, etc.[23,36] These "smart" properties can be exploited for many important industrial and biochemical applications. For example, drug-laden hydrogels microparticles can be allowed to circulate the blood stream to only release upon encountering particular temperature,[37] pH or biochemical situations.[38] Second, self-healing hydrogels are materials that can automatically "repair" themselves when defects such as cracks, changes in mechanical properties, etc. are encountered.[39–41] Typically, such healing occurs because of spontaneous formation of new bonds.[42] Intuitively, it is not hard to envision the utility of such self-healing materials in tissue engineering, organ regeneration, etc.[43,44]

Due to their high water content, hydrogels has wide ranging applications in a variety of areas. In a research setting, hydrogels have been used for cell culture and tissue scaffold bioengineering.[45–47] In the medical area, hydrogels have been used as drug delivery systems, wound dressing scaffolds, biosensors, etc.[48–50] Moreover, hydrogels are used widely in diapers[51,52] and contact lens.[53–55] As a rule of thumb, natural hydrogels are biocompatible and are rapidly finding more and more uses in the biomedical setting.

2. Hydrogel Synthesis

In this chapter, we describe methods that are most commonly used to synthesis hydrogels. In general, physical and radiation crosslink techniques are prefer in medical application compare with chemical crosslink and grafting techniques due to nontoxicity in preparation process.

2.1. Physical crosslink

2.1.1. *Heating or cooling process*

A sol-gel formation bases on temperature alteration of polymer solution is a process without using chemical crosslink agents. Phase transition occurs at a distinct temperature of polymer solution is called low critical solution temperature (LCST) and form a reversible hydrogels. Natural polymers such as agarose[56] or polyethylene glycol (PEG)–polylactic acid[57] dissolve in water as random coils, and then form helix structure if solution temperature is lower than LCST and reversible. Upon cooling the structure aggregates to form a rigid hydrogels (Fig. 1). A similar example is from Yoshioka group that dissolved gelatin in warm water at 37°C and form hydrogels at 27°C.[58]

2.1.2. *Ionic interaction*

Ionic interaction occurs between ionic polymer chains containing opposite charges with multivalent ions that plays a role as crosslinkers.[59] In the case of chitosan, ammonium groups with positively charges on chitosan form ionic bonds with Pt (II) which is commonly used as crosslinker (Fig. 2).[60]

2.1.3. *Complex coacervation*

The interaction between an anionic polymer solutions with a cationic polymer solution forms a complex coacervation gel (Fig. 3). Gotoh group developed alginate-chitosan

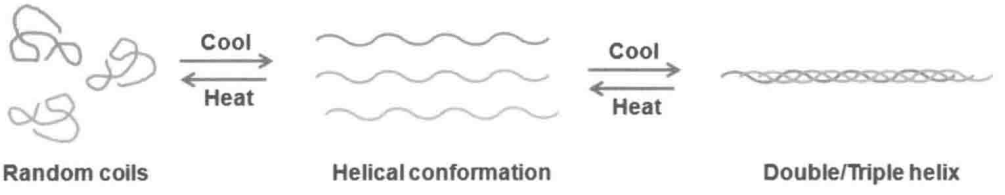

Fig. 1. Gel formation due to phase transition upon changing temperature.

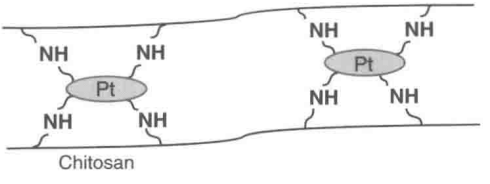

Fig. 2. Gelation based on ion interaction between charged ammonium groups of chitosan with divalent Platinum ions.

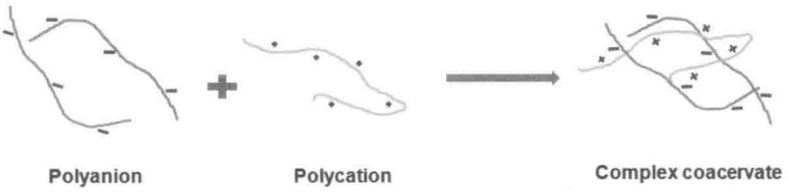

Fig. 3. Schematic of complex coacervation process.

hybrid gels by mixed anionic polysaccharide alginate acid, which contains carboxyl group, and cationic polysaccharide chitosan, which contains amino groups in neutral aqueous solution. The polyelectrolyte complexes were synthesized provide a good substance to absorb Cu (II), Co (II), and Cd (II) in drug delivery system.[61]

2.1.4. *Freeze-thaw process*

Freeze-thaw is one of the gelation techniques occur in mild condition without chemically crosslink agents. Polymeric solution is frozen and thawed to form cryogels. The method was first reported on aqueous poly(vinyl alcohol) solutions by Peppas group in 1975.[62] Continuously repetition of freeze-thaw process leads to form crystallites as a crosslink region. Crystallites degree has associated with aqueous solution concentration, freeze and thaw time.[63–65] Starch (amylose, amylopectin and their mixtures), agarose, hyaluronan (HA), maltodextrins (MDs) that are natural materials are able to form hydrogels upon freeze-thaw technique.[66,67]

2.2. Chemical crosslink

Chemical crosslink is the predominant method in hydrogel synthesis, in which natural or artificial crosslinkers form covalent bonds to functional groups on side chain of polymer (such as –OH, –COOH, and NH_2).[68–72] Mincheva *et al.* developed a network hydrogel net-chitosan-ɪ-PEG-$(COOH)_2$ by using PEG diacid as an agent to crosslink chitosan. The amide bonds formed between carboxyl groups of chitosan react with the amine groups of PEG diacid PEG-$(COOH)_2$ in low pH and high temperature aqueous solution (Fig. 4). The highly equilibrium degree of swelling of networks serves effectively as a matrix of the regenerating processes in artificial bone defects.[73]

Similarly, Wang group used genipin as a natural agent for crosslinking the lysine groups in the poly(l-lysine)-block-polyglycine (PLL-b-PGly) polypeptides to constitute genipin-crosslinked hydrogels in a short time.[74] The novel hydrogels were biocompatible on support cell attachment or proliferation. Additionally, PEG, Polypropylene glycol (PPG) and Isophorone diisocyanate (IPDI) were combined to form pre-polymer with polyurethane (PU) serving as crosslinker in

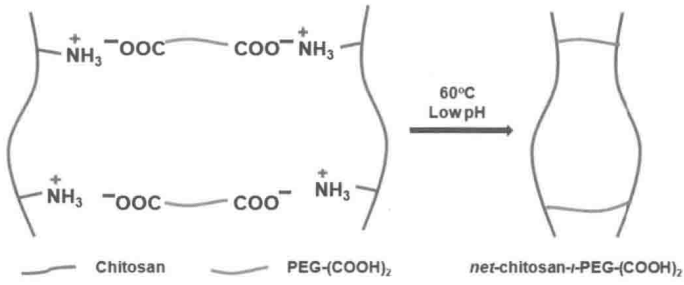

Fig. 4. Formation of covalently crosslinked network net-chitosan-ɪ- PEG-$(COOH)_2$.

Huang group.[75] PU chain not only provided –NCO groups to form covalently bond with $–NH_2$ and –OH groups on silk fibroin (SF) chains but also interacted to form hydrogen bonds makingmup a crosslinked network of silk fibroin-polyurethane (SF-PU) hydrogels. The permanent structures show good properties in swell-deswell and sensitive to pH, temperature and ionic strength.

2.3. *Grafting*

The polymerization of monomers or polymers on to the chains of a polymeric material is called grafting crosslink. The polymers are treated by high energy radiation or chemical reagents to expose functional groups on monomers or polymers as well as polymeric material. As a result, the process leads to branching and crosslink to synthesize stable hydrogels (Fig. 5).

Amin group developed bacterial cellulose/acrylic acid hydrogel by grafting bacterial cellulose on acrylic acids that were ionized radiation.[76] In another case, a conventional polyethylene was grafted with a chelate agent function was done by Tamada group. The amidoxime fibrous absorbent was obtained after crosslinking

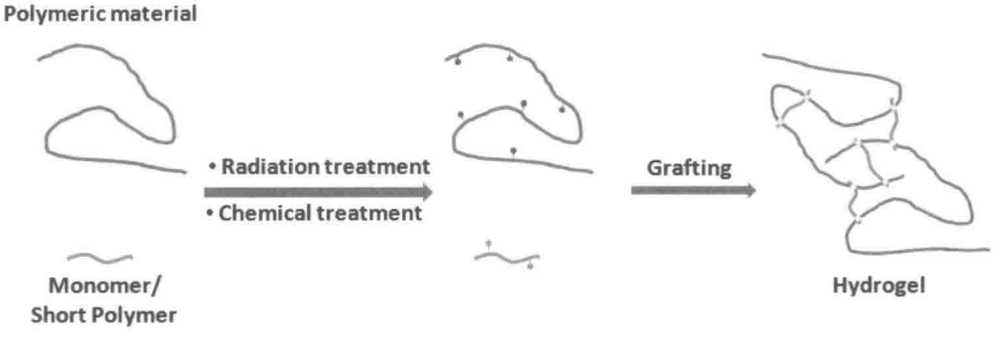

Fig. 5. Preformed monomers or short polymers graft on and crosslink preformed polymeric material.

process.[77] Similarly, Yazdani-Pedram and Retuert de la Torre introduced carboxylic groups onto chitosan by grafting process with poly(acrylic acid) to form a swelling network hygrogels.[78]

2.4. Crosslinking by ionizing radiation or radiation crosslinking

Radiation crosslinking technique has been widely used as an effective approach to modify the structure of natural polymers and induce the hydrogel formation. In principle, the hydrogel can be obtained when exposed interest polymer in solid or aqueous solution state under ionizing radiation with high energy such as gamma radiation.[79,80] As a result, there are two main events to be achieved (i) direct energy transfer to the polymer to generate a lot of free radicals called macroradicals which are important for the recombination of inter-molecules and (ii) the direct or indirect effect of water induces polymer chains mobility or radiolysis macromolecules products, respectively.[81]

2.4.1. *Radiation crosslinking between natural polymers*

The application of this technique has been recently reported for the hydrogels preparation of several natural polymers used in biomedical studies such as carboxymethyl-chitosan (CMCTS)/gelatin hydrogel, methylcellulose hydrogel, hydroxyethyl metacryate (HEMA)-agar and hydroxyethyl metacryate (HEMA)-gelatin hydrogel.[82–85] In brief, the mechanism for the formation of hydrogels was proposed to be

$$H_2O \xrightarrow{\text{Ionizing radiation}} {}^{\bullet}OH, {}^{\bullet}H, H_2O_2, H_3O^+, H_2, e^-_{aq}, \quad (1)$$

$$RH + {}^{\bullet}OH\ ({}^{\bullet}H) \leftrightarrow {}^{\bullet}R + H_2O\ (H_2), \quad (2)$$

$${}^{\bullet}R + {}^{\bullet}R \rightarrow R\text{–}R, \quad (3)$$

where RH represent as gelatin or carboxymethyl chitosan molecule.[84]

In the study on CMCTS/gelatin hydrogel, these sterilized protein/polysaccharide polymers were mixed at different weight ratio to form homogenous solution in the absence of additives (crosslinking reagents). By using a ^{60}Co facility, the polymer complex solution was then gamma-irradiated with 30 kGy absorbed dose. The crosslinked hydrogel products were used for implantation experiment or wound healing experiment. The CMCTS/gelatin CG4:6 hydrogel was optimally applied in clinical techniques due to the biodegradation behavior, swelling property and integration ability. More interestingly, the application of hydrogels to

mouse cutaneous wound model leaded to thicker granulation tissue in the skin recovery process.[84]

2.4.2. *Radiation crosslinking between synthetic polymers and natural polymers*

The same technique is currently applied on plenty of complex systems of water-soluble polymers crosslinking between synthetic and natural materials. Most of these radiated hydrogels are more benefit and effective than one synthesized by chemical crosslinkers. Even though the treatments by chemical reagents such as glutaraldehyde, D,L-glutaraldehyde or genipin[86–88] were shown to have high degree of crosslinked products, the chemical compounds could be highly toxic to the cells or mouse models *in vivo* which is the main limitation for biomedical applications. In contrast, hydrogels achieved from radiation crosslinking technique are considered biocompatible due to whole sample was prepared in a system of pure water. There is an interest example for the radiated hydrogel of mixture of polyvinyl pyrrolidone (PVP) and CMCTS. The final hydrogel membranes of PVP and CMCTS are reported to non-toxicity, biocompatibility and pH sensitivity. These blend hydrogels enhanced reported to have the potential ability of protein absorption and immobilization. The increasing CMCTS content to the blend system indicated the improvement in the surface property and the BSA adsorptive property.[89]

3. Application of Hydrogel

Hydrogels broadly exist in natural conditions such as animal, plant and widely used in a lot of therapeutics due to their high water affinity and biocompatibility. For example, natural hydrogels can be used in health care, such as tissue scaffolds, wound dressing, dental materials, and implants, injectable polymeric systems. Furthermore, hydrogels were also studied in biomedical research, such as biosensor, or used as matrices for control of macromolecules release. Especially, they are applied as the potential carrier for drug delivery systems based on their peculiar characteristics and high sensitivity towards temperature and some other stimuli.[90,91]

Interestingly, we could easily find hydrogels in our diet and in popular molecular gastronomy, such as pectin, gelatin, guargum, and starch. Moreover, natural hydrogels can be combined with artificial polymers to achieve better control of material ability; also the molecular system of natural hydrogel can be tailored to intelligently match with environment conditions, which we will not discuss further in this chapter. Table 1 lists the most common natural hydrogels with their corresponding applications.

Table 1. Various applications for appropriate natural polymers.

Tissue engineering, transplantation, microencapsulation
Collagen,[92–94] fibrins,[95] alginate,[96] agarose,[97,98] hyaluronic acid hydrogels[99]
Controlled drug delivery
Polysaccharide,[100] gelatin,[101] cellulose,[102,103] starch,[104,105] chitosan,[106,107] pectin,[108,109] zein[110,111]
Wound dressing
Agar, collagen,[112] cellulose, chitosan,[113,114] gelatin,[115,116] polysaccharide,[117,118] fibrin[119]
Bio-sensor
Dextran,[120,121] gelatin,[122] alginate[123]
Injectable polymers
Chitosan,[124,125] alginate[123] fibrin[126]
Food industry
Pectin, gelatin,[127] starch[128]
Micro patterning, Nano patterning
Collagen,[129] gelatin[130]

4. Future Perspective and Challenge

Nowadays, natural hydrogels have already been employed effectively in tissue engineering and drug delivery due to their unique mechanical and chemical properties. Thanks to advantages of widely existence and great biocompatibility compared to synthetic polymer, natural hydrogels are expected as essential materials for present and future biomedical applications. Furthermore, researchers are attempting to modify natural hydrogel or incorporate with other artificial polymers to form novel materials with highly desired properties. In the case of drug delivery, a fine-tuned hydrogel drug release mechanism is indispensable for controlled release of drugs, because the efficiency of delivering medical active molecules is influenced by various factors, like drug loading, hydrogel breakdown, interactions between drug and hydrogel. Recent days, most of works focus on developing intelligent materials which are not only sensitive but also able to respond to the surrounding environment. Even though it is assumed that natural hydrogels are biocompatible with human body, there are still some impacting factors involved, such as physicochemical and biological properties of polymer, nano assembly drug carriers, controllable drug release rate, bioactivity and maximum cytotoxicity of drug payloads upon polymer degradation, so it is a great challenge for hydrogel drug carrier design to simultaneously optimize all of them. At this moment the synthesis of multidisciplinary technologies, which is urgently needed in intelligent drug-carrier design, seems overly complicated. Moreover, relatively easy degradation without any toxic effect should be taken into consideration during intelligent drug delivery system based on natural hydrogels.

Regarding tissue engineering, the ultimate goal is to fabricate functional tissues and even organs that can successfully replace original organ after implantation, in which extracellular matrix deposition, various remodeling tests, and maybe high shape fidelity maintenance are involved. For example, the fabricated cartilage tissue with natural hydrogels should be able to not only transmit and respond to the mechanical signals, but also be bio-sensitive to the chemical signals. To achieve this goal, desired mechanical, chemical, and biological properties should be engineered into the modified natural hydrogels.[131] It is promising to construct building blocks for a tissue entity and scaffolds with natural or modified hydrogels, however, lacking comprehensive knowledge of bio-inks from physical and rheological perspective to some extent may block the progress of generating larger construct which is expected to replicate tissue organization.[132] Moreover, potential toxicity of bio-inks and possible change of rheological properties of hydrogels should be taken into consideration as well. Another challenge associated with tissue engineering is the trade-off between resolution and speed during biofabrication, especially for the constructs of clinical sizes.[132]

Natural hydrogels can be utilized themselves or modified to fit the requirement for promoting biomedical research and make personalize drug delivery plans. There is already a growing number of routine clinics adopting polymer therapeutics, like the nano-sized medicine.[133] So we can hope that with the development of study on the mechanical properties of natural hydrogels, novel methods of biofabrication and relevant micro-manipulation, natural hydrogels will continue play an important role in biomedical applications.

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