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Weak Bond-Based Injectable and Stimuli Responsive Hydrogels for Biomedical Applications

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Abstract

Here we define hydrogels crosslinked by weak bonds as physical hydrogels. They possess unique features including reversible bonding, shear thinning and stimuli-responsiveness. Unlike covalently crosslinked hydrogels, physical hydrogels do not require triggers to initiate chemical reactions for *in situ* gelation. The drug can be fully loaded in a pre-formed hydrogel for delivery with minimal cargo leakage during injection. These benefits make physical hydrogels useful as delivery vehicles for applications in biomedical engineering. This review focuses on recent advances of physical hydrogels crosslinked by weak bonds: hydrogen bonds, ionic interactions, host-guest chemistry, hydrophobic interactions, coordination bonds and π - π stacking interactions. Understanding the principles and the state of the art of gels with these dynamic bonds may give rise to breakthroughs in many biomedical research areas including drug delivery and tissue engineering.

Keywords

hydrogel; shear thinning; stimuli responsive; drug delivery; reversible crosslink

Introduction

Polymeric materials, ceramics and metals are three major categories of materials that are used to design medical devices.^{1–4} Recent developments seek more sophisticated designs with multiple functionalities to control their performance. For example, the materials are engineered to possess stimuli responsiveness to surrounding environments, such as pH,

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ionic, enzyme, thermal, electric, light and magnetic, to manipulate performance and enhance therapeutic efficacy.^{5–7} These functionalities can be designed by either selecting appropriate functional monomers for synthesis or modifying existing substances, such as natural biopolymers or medical devices, to impart stimuli responsiveness.^{6, 8–11} Regardless of the challenges, these advanced materials are enabling the development of new solutions for treating various human diseases, such as diabetes, cardiac repairs, wound healing and cancer theranostics.^{12–16} Although we attempted to cover as much as possible researches published in the last six years that are related to the weak bond-based injectable hydrogels for biomedical applications, it is impossible to cover every single paper in this field. We attempt to cover the most extensively studied weak bond-based hydrogels in this review.

Polymeric biomaterials have many advantages and play critical roles in bioengineering fields. The physical, chemical and biological properties of polymeric materials can be manipulated at the molecular level to achieve the desired properties. For example, the viscoelasticity of a material can be controlled by molecular weight, molecular architecture, covalent or non-covalent crosslinking types and crosslinking density.^{17–20} The degradation rate of a polymer can be adjusted by introducing enzymatically cleavable bonds or dissociable non-covalent bonds.^{19, 21, 22} The stiffness and toughness of a scaffold can be adjusted by selecting appropriate polymer blends, designing block copolymers, or incorporating nanoparticles into the polymer matrix to form nanocomposite materials.^{23, 24}

Among a variety of polymeric biomaterials, hydrogel represents one major class. It is used for cell and drug delivery, soft fillers and tissue regeneration scaffolds.^{25–30} A hydrogel is a three-dimensional (3D) hydrophilic polymeric network swollen in aqueous solution that typically carries a large amount of water. The hydrogels possess excellent porosity that resembles the properties of extracellular matrix to provide biological and mechanical cues to promote cell activities for tissue regeneration.^{31–33} Generally, two categories of injectable hydrogels have been extensively investigated over the past decades. One type is a chemically or covalently crosslinked hydrogel (chemical hydrogel) and the other is a physically or non-covalently crosslinked one (physical hydrogel).^{33, 34} Chemical hydrogels are not covered in this review. Readers are referred to several excellent recent reviews.^{25, 35–38}

A physical hydrogel is generated based on physical bonding to form a network structure.^{33, 39} Physical hydrogels possess features of dynamic crosslinks, reversible sol-gel transition, shear thinning properties and stimuli responsiveness.³³ Thus, it is a relatively new minimally invasive delivery platform for biomedical applications. The advantages of physical hydrogels over chemical hydrogels are: (1) the gelation does not require active agents or triggers to initiate the *in situ* reaction, (2) the drugs can be fully loaded in a pre-formed hydrogel, (3) the gel-sol transition occurs at the interface between the gel and needle wall during injection to minimize the pre-mature leakage of the payloads, (4) quick recovery of gel properties after injection, and (5) can be designed to be responsive to external stimuli (e.g., pH, ionic strength, and temperature) to control the performance for specific applications.^{33, 35} This review provides an overview of the most recent advances in shear thinning and stimuli-responsive hydrogels with the widely used non-covalent bonds including hydrogen bonds, ionic interaction, host-guest chemistry, hydrophobic interaction, π - π stacking interaction and coordination bonds. Detailed examples are given to illustrate

the features of the shear thinning properties and stimuli-responsiveness for their applications.

Biopolymer/nanomaterial composite hydrogel is another important category formed by binding polymers with nanomaterials. The nanomaterials can be either modified or unmodified organic and inorganic nanoparticles.^{40–55} The physical interactions between biopolymer matrices and nanomaterials are also mainly classified into the above listed six categories of non-covalent bonds and sometimes together with strong physical absorption dominating the composite gelation process. Nanocomposite hydrogels are not included in the current work. Readers are referred to several excellent reviews.^{56–59}

1. Hydrogen bond-based hydrogels

A hydrogen bond (H-bond) is one of the most frequently used dynamic bonds to build up a supramolecular network with stimuli responsive properties. The functional moieties, such as 2-ureido-4-[1H]-pyrimidinone (UPy), 6-aminocaproic acid (6-ACA) and diaminotriazine (DAT), have been employed to design biomaterials with shear thinning properties and specific stimuli responsiveness (Fig. 1).^{60–62}

UPy-UPy dimer can form strong quadruple H-bonds. The monomer can either be incorporated into a polymer backbone or be immobilized to chain ends to generate UPy-functionalized materials. For example, a diisocyanate functionalized UPy was reacted with PEG to generate chain extended UPy-PEG, or a UPy-hexyl isocyanate was attached to PEG ends to yield end-capped UPy-PEG (Fig. 1A).^{63, 64} Both biomaterials could form injectable hydrogels with different toughness and degradation rates because of the different crosslinking density formed by UPy-UPy interactions along the polymer chains. It was demonstrated in recent reports that both UPy-functionalized PEGs were able to form shear thinning hydrogels to deliver growth factors (e.g., bone morphogenetic protein-7, hepatocyte growth factor and insulin-like growth factor-1) for renal repair and infarcted myocardium regeneration.^{60, 64, 65} The chain extended UPy-PEG formed a tougher hydrogel because of multiple UPy-UPy interactions that were established along the polymer chains. The hydrogel was stable for more than 15 days that was dominated by degradation of the polymer backbone (Fig. 1A(a)). However, the end-capped UPy-PEG yielded a soft hydrogel with degradation dominated by UPy-UPy dissociation within a week (Fig. 1A(b)).⁶⁰ In addition to tunable toughness and degradation rate, it was also demonstrated that the UPy-based supramolecular hydrogel showed pH responsiveness. The end-capped UPy-PEG hydrogel switched into a liquid at pH > 8.5 that was able to pass through a one-meter long catheter lumen for delivery into a porcine myocardial infarction zone. The injected solution was then instantaneously transformed into a gel state at the tissue pH level. Such sensitive pH responsiveness enables this material to be an efficient injectable drug delivery system either through a syringe or a state-of-the-art of catheter system.^{60, 65}

Another H-bond-based stimuli-responsive hydrogel is engineered using 6-aminocaproic acid (6-ACA) as a functional moiety, showing reversible self-healing ability upon damages stimulated by pH conditions (Fig. 1B).⁶¹ The flexible pendant 6-ACA is key to mediate H-bonding formation for this performance. The 6-ACA segment possesses an optimal balance

between hydrophobic (methylene groups) and hydrophilic moieties (amide and carboxylic acid groups) so that the pendant groups are sufficiently long and flexible across the interfaces at a damaged site. However, the 6-ACA is short enough to minimize steric hindrance between the interacting functional groups and prevent hydrophobic collapse of the side segments. The designed poly(acryloyl-6-aminocaproic acid) (PA-6-ACA) demonstrated that the H-bond association and dissociation were reversible responding to pH conditions. When two pieces of hydrogels approach each other, the H-bonds are established for healing between amide and carboxylic acid groups at pH 3. The healed hydrogel could be separated into two pieces again when it was exposed to basic solution (pH 9) as a result of the deprotonation of the terminal carboxylic acid groups, leading to electrostatic repulsion. Such a healing and separation cycle could be repeated for more than 12 times without sacrificing the healing efficiency.⁶¹ Because of the excellent self-healing capability under acidic conditions and good adhesion to fresh gastric mucosa (rabbit), this material was investigated for biological applications as a tissue adhesive for stomach perforations and a delivery vehicle for sustained release in a low pH environment.

A diaminotriazine-diaminotriazine (DAT-DAT) H-bond shows a unique responsiveness to nucleosides that have been used to design another type of stimuli-responsive hydrogel for controlled drug release. For example, a PEG-crosslinked poly(2-vinyl-4,6-diamino-1,3,5-triazine) (PVDAT) hydrogel was reported to show nucleoside (thymidine) responsiveness for the controlled release of protein.⁶² The general principle is illustrated in Fig. 1C. The constructed PVDAT hydrogel possesses PEG covalent crosslinks and DAT-DAT dynamic H-bonding crosslinks. When the PEG crosslinks remain constant, the entire crosslinking density is modulated by the DAT-DAT H-bonds. The DAT-DAT interactions can thus control the subsequent swelling ratio and the protein release behavior from the hydrogel in the presence of nucleosides. It was shown that when PVDAT hydrogel was exposed to thymidine solutions with concentrations at 2 and 4 wt%, the hydrogel showed a larger swelling ratio responding to the higher thymidine concentration.^{62, 66} The more thymidine molecules that diffuse into the hydrogel network, the more that initial DAT-DAT dual H-bonds switched to stronger DAT-thymidine triple H-bonds (Fig. 1C). This process led to the reduction of physical crosslinks and also to network swelling to a larger pore size for protein permeation.⁶² Though this DAT-DAT H-bond-based hydrogel was designed to combine with covalent crosslinks to form a responsive hydrogel, it is expected that such nucleoside-responsive H-bonds can be expanded to combine with other physical dynamic bonds to construct a hydrogel possessing shear thinning properties along with nucleoside responsiveness for relevant applications.

2. Ionic interaction-based hydrogels

Inspired by natural species that produce biological complexes in aqueous environments, the electrostatic interaction is a straightforward and efficient strategy to generate ionic complexes, mediate biomolecular attachment, and stabilize molecular configuration to prolong biomolecular activities.^{67–70} This strategy is receiving more attention for the construction of supramolecular hydrogels for controlled drug or cell delivery in tissue regeneration.^{71–79} The hydrogels formed by electrostatic interactions from polyelectrolytes possess several advantages: (1) cationic and anionic polyelectrolytes are typically water

soluble, so they alleviate the use of organic solvent during gel construction; (2) ionic interaction is highly specific and efficient to accomplish quick gelation; (3) payloads, such as bioactive proteins, can better retain their molecular configuration and preserve their bioactivities in aqueous environments; and (4) electrostatic interactions are sensitive to polyelectrolyte concentration and medium conditions, including pH, ionic strength and temperature, to tune the performance for specific applications.^{78, 80} In this section, we will discuss ionic interaction-based hydrogels using engineered ionic complementary peptides, synthetic polyelectrolytes and natural polyelectrolytes.

2.1 Ionic complementary peptide-based hydrogels

Ionic complementary peptides contain alternating hydrophobic and hydrophilic amino acids in peptide sequences. This type of peptide can spontaneously self-assemble into stable β -sheet nanofibers and eventually yield a nanofibrous hydrogel induced by entanglement and ionic interactions between the nanofibers. The peptide can be designed to possess both positive and negative charges of amino acid residues in one sequence to form a self-complementary ionic peptide for self-assembly.^{81–83} Alternatively, two sequences can be designed for complementary ionic interactions, one bearing positive charges, and the other bearing negative charges.⁸⁴ In this case, the individual peptide is self-repulsive, but the pair is mutually attractive. When the two sequences are combined, they can self-assemble into peptide nanofibers.^{84, 85} Typically, lysine (K) and arginine (R) are used to introduce positive charges, and glutamic acid (E) and aspartic acid (D) are used to generate negative charges.

The ionic complementary peptides possess two distinct surfaces, one hydrophilic and the other hydrophobic, allowing precise assembly into a predetermined architecture. In an aqueous environment, the hydrophobic residues of two peptide sequences bury themselves to form dimers with the hydrophilic surface toward the aqueous phase; the ionic interactions drive self-assembly to form β -sheet nanofibers. The supramolecular architecture is eventually formed based on the electrostatic interactions and entanglement between the fibers as long as it is above the critical gelation concentration.^{81, 85} Fig. 2 illustrates the general process of an anti-parallel self-assembly to form β -sheet nanofibers from a self-complementary ionic peptide, e.g., FEFEFKFK.⁸³

In past decades, many ionic complementary peptides have been designed using the solid phase peptide synthesis technique that typically produces peptides with amino acids between 5–50 units.^{81, 85} A classic example of ionic complementary peptides is RAD16-II (ARARADADARARADAD), inspired by EAK16-II (EAEAKAKAEAEAKAKA) that is found in Zuotin protein, showing robust self-assembly into a stable β -sheet fibrous hydrogel and versatile applications in drug or cell delivery for tissue regeneration.^{85, 86} Recently, more ionic peptides have been designed for a variety of applications; stimuli-responsive properties are also introduced to tune the performance of the materials.

It is generally agreed that longer peptide sequences, such as the RAD16-II or EAK16-II organized by 16 amino acids, will more efficiently yield stable β -sheet nanofiber hydrogels, but this is not always the case. As long as appropriate hydrophobic and hydrophilic amino acids are selected in a sequence, a shorter ionic complementary peptide can also effectively and quickly self-assemble into stable β -sheet nanofiber hydrogels. This is well illustrated by

a recently reported designer peptide, FEFEFKFK (half-length compared to EAK16-II) that efficiently yields nanofiber gels in aqueous media.⁸³ In this case, F (phenylalanine), a more hydrophobic amino acid than A (alanine), was selected to organize with E and K to form an octapeptide. This designer peptide can spontaneously self-assemble into anti-parallel β -sheet nanofibers with an average diameter of 3 nm, eventually forming a stable fibrous network at a concentration above 5 mg/mL (Fig. 2). Interestingly, the viscoelasticity of the fibrous hydrogel can be tuned by the concentration of the peptide, pH value and ionic concentration in the media. For example, the storage modulus (G') is 10–20 Pa when the peptide hydrogel is prepared in deionized water at pH 3 with a concentration between 20–40 mg/mL. At the same concentration range, if the peptide solution is adjusted to pH 7, the G' increases to 1–11 kPa, and further increases to 6–35 kPa if prepared in tissue culture medium at the same pH.⁸³ Such easily tunable mechanical properties make this material useful for many potential applications. One example is 3D cell culture to promote chondrocyte attachment, proliferation and extracellular matrix deposition, showing potential for cell delivery and tissue scaffolding for cartilage regeneration.⁸³

In addition to responding to pH conditions, ionic strength and peptide concentrations, thermal responsiveness can also be introduced into the ionic complementary peptide hydrogel. This can be achieved by conjugating a thermally responsive polymer to the peptide end and co-assembling with the free polymers to form a hybrid gel with thermal responsiveness. For example, a simple one pot synthesis method was proposed to prepare a PNIPAAm-FEFEFKFK conjugate and PNIPAAm hybrid system (Fig. 3A).⁸⁷ In this case, a thiol-functionalized peptide (HS-FEFEFKFK) was used as a chain transfer agent that was mixed with N-isopropylacrylamide (NIPAAm) monomer and azoisobutyronitrile (AIBN) as an initiator for free radical polymerization to directly obtain a mixture of PNIPAAm-peptide and PNIPAAm. After reaction, the chemical residues, unreacted HS-FEFEFKFK, and short PNIPAAm chains were removed together by dialysis in one step. The ratio of the PNIPAAm-peptide conjugate to free PNIPAAm could be readily adjusted by the ratio of monomer to HS-FEFEFKFK. In this way, multiple processes including peptide and polymer synthesis, purification, and a coupling reaction between peptide and polymer can be avoided. The products can be directly used to fabricate the hybrid gel with thermo-responsiveness for applications.

Usually the peptides are synthesized using the solid phase peptide synthesis technique. Recently, an enzymatically triggered reverse hydrolysis method was proposed to synthesize self-complementary ionic peptides and *in situ* formed peptide hydrogel.^{88, 89} The general process is illustrated in Fig. 3B.⁸⁹ An initially non-gelling short peptide (FEFK) solution is mixed with a protease, thermolysin, at an appropriate concentration to trigger the gelation. The thermolysin is known to catalyze the hydrolysis of peptide bonds,⁹⁰ but here the enzyme is used to perform reverse hydrolysis to generate octapeptides from the initial tetrapeptides. This process was confirmed in recent reports that the tetrapeptides first underwent hydrolysis catalyzed by the enzyme to produce dipeptides along with some initial tetrapeptides. Interestingly, the short peptide mixture was then favorably converted into octapeptides, mainly a FEFKFEFK and FEFEFKFK mixture because these octapeptides preferably self-assemble into thermodynamically stable β -sheet nanofibers. Once forming the stable β -sheet structures, they would not be hydrolyzed back to short peptides again.

This process shifted the chemical reaction to production of octapeptides and eventually formed a β -sheet nanofiber hydrogel when the concentration was above the critical gelation concentration (Fig. 3B). The gelation rate and viscoelasticity of the gel are dependent on the initial tetrapeptide concentration at a fixed enzyme concentration. For example, when the initial concentration of FEFK was 200 mg/mL, it took approximately 1 h to form a gel, but gelation could be achieved within 10 min when the initial concentration was 300 mg/mL. The oscillatory rheometry test showed that the storage modulus could go up to 25 kPa at 300 mg/mL.⁸⁸ The enzyme concentration would not change the composition of the final products, but affected the reaction kinetics and the shear moduli because of a quicker reaction and denser network formed around the enzymes if using a higher enzyme concentration.⁸⁹ This method of forming longer ionic peptides and a fibrous hydrogel from initially short, readily prepared and non-gellable peptides shows promise for peptide synthesis and potential for 3D cell culture and cell delivery for tissue engineering.

As a delivery platform, the ionic complementary peptides are typically used to encapsulate hydrophilic biomolecules or cells for delivery.^{83, 91} However, a recent study reported an ionic complementary peptide for the controlled release of a hydrophobic anticancer agent (ellipticine) to overcome its low solubility in aqueous solution and enhance anticancer therapy.⁸² This self-complementary peptide was designed to be Ac-NH-LDLKLELKLDLKLELK-CONH₂. When mixing the designer peptide with ellipticine at an appropriate ratio (e.g., 12.5:1 by weight), peptide-stabilized ellipticine complexes were formed and uniformly distributed in the peptide hydrogel matrix. The quantity of the ellipticine in aqueous solution was increased up to ca. 19-folds in the presence of the peptide compared to that of free ellipticine. *In vitro* anticancer efficiency was evaluated using Hepatic Carcinoma cell (SMMC7721) and Esophageal Carcinoma cell (EC9706). The results indicated that the anticancer efficacy of the drug was significantly enhanced possibly as a result of the release of protonated ellipticine from peptide-drug complexes, rather than the less effective forms of neutral or crystalline ellipticine released from free drug control.

Not only limited to the above-mentioned versatility of the designer ionic peptides, more controllable features, such as cell adhesion sequence RGD and enzymatically cleavable motif LRG, can also be introduced to a designer peptide. These properties can be used to enhance cell-matrix interactions and control the degradation of peptide hydrogels for cell infiltration, migration and proliferation to achieve appropriate conditions for biomedical applications.^{92, 93} Here, we focused on recent advances in ionic complementary peptides for bioengineering applications; other peptide designs and applications are found in several recent reviews.^{81, 85, 86, 94}

2.2 Synthetic polyelectrolyte-based hydrogels

It is known that directly mixing two oppositely charged homopolymers usually leads to complex coacervate and eventually precipitate out of the aqueous phase.^{73, 74} Although the complex coacervate is also an important drug delivery system for biomedical applications, it will not be included in this review and readers can refer to several recent publications for their interesting applications.^{75, 95–100} To use a synthetic polyelectrolyte to form a hydrogel, an ABA triblock copolymer architecture is used.^{78, 101} Fig. 4A illustrates the general design

of triblock copolyelectrolytes and hydrogel formation based on a complex coacervate as physical crosslinks.⁷⁸ The ABA triblock copolyelectrolytes are designed to have a hydrophilic middle block with two anionic or cationic end blocks that are derived from a common triblock copolymer precursor. Once the two oppositely charged copolyelectrolytes are mixed at equimolar ratio, the cationic and anionic blocks are complexed to form coacervates as crosslinks that are connected by the hydrophilic middle block to yield a network structure. The merit of using a common copolymer precursor is to produce cationic and anionic copolyelectrolytes with the same molecular weight distribution. This is essential to control the stoichiometric balance for coacervation crosslinking via electrostatic interactions when mixing two copolyelectrolyte equimolar solutions.

To develop an ABA triblock copolymer precursor for easy functionalization with either cationic or anionic groups on the end blocks, a precursor by ring-opening polymerization was made using allyl glycidyl ether (AGE) as the monomer. This triblock copolymer was synthesized using a difunctional potassium alkoxide PEG as the macroinitiator to obtain poly(allyl glycidyl ether-b-ethylene glycol-b-allyl glycidyl ether) (P(AGE-b-EG-b-AGE)) (Fig. 4B).⁷⁸ This precursor bears pendent alkene units on the end blocks that readily react with functional moieties via thiol-ene chemistry to introduce either anionic or cationic functional groups. In this way, the P(AGE-b-EG-b-AGE) end blocks were functionalized with sulfonate, carboxylate, ammonium and guanidinium, respectively, to yield four types of triblock copolyelectrolytes (Fig. 4B). These four functional moieties possess different pK_a and consequently generate different ionic strengths for coacervate crosslinking. Therefore, the viscoelastic properties of the formed gels are tunable by selecting a different copolyelectrolyte pair. For example, when mixing the pair of copolyelectrolytes bearing strong ionic groups of sulfonate and guanidinium (a and d), the most stable and mechanically resilient gel was generated within 30 s at a concentration as low as 3–5 wt%. However, mixing carboxylate-functionalized copolyelectrolyte with the guanidinium-functionalized one yielded a mechanically robust hydrogel but required 10 wt% of each polymer solution. As expected, mixing weak ionic group functionalized copolyelectrolytes (e.g., carboxylate and ammonium, b and c) only formed a viscous fluid.⁷⁸ As a result of being sensitive to pH conditions and ionic concentration, the coacervate strength and crosslinking density of the designed hydrogels could be tuned by adjusting the medium pH level or ionic concentrations.^{78, 101} These features show interesting potentials for applications in 3D cell culture in which the hydrogel matrix can be dissociated and removed by adjusting cell medium conditions and leaving the developed 3D tissues for studies.

It is noted that stoichiometry between the cationic and anionic blocks is crucial to achieve efficient gelation if using the two oppositely charged triblock copolyelectrolytes. To explore more convenient strategies to fabricate polyelectrolyte-based hydrogels, an anionic thermosensitive hydrogel was developed from poly(organophosphazene) to load charged protein for sustained release with a suppressed initial burst (Fig. 5).⁷⁹ In this case, the negatively charged protein (e.g., human growth hormone, hGH) is complexed with a polycation at an appropriate ratio to form polyelectrolyte-protein complexes (PECs) with net positive charges. The PECs are then incorporated into the anionic poly(organophosphazene) solution. Upon injection into a tissue location, the anionic thermosensitive polymer solution undergoes the sol-gel transition at body temperature. The positively charged PECs and

negatively charged hydrogel forms additional electrostatic interactions to control the protein release with an effectively suppressed initial burst.⁷⁹

Either synthetic or natural polycations, such as poly-L-arginine, poly-L-lysine, or protamine sulfate can be used to complex with a negatively charged protein (e.g., hGH) at a suitable ratio to yield the protein complexes before incorporating into the anionic hydrogel.^{102, 103} For example, poly-L-arginine possesses stronger ionization than poly-L-lysine or protamine sulfate that can easily complex with hGH to form poly-L-arginine/hGH complexes. After the complexes were incorporated into the poly(organophosphazene) hydrogel, the initial burst release was reduced in proportion to the amount of poly-L-arginine. Both *in vitro* release and *in vivo* pharmacokinetic studies in SD rats showed that the hGH was sustainably released over 14 days with a significantly suppressed initial burst compared to that of the free hGH solution. However, the cumulative release of hGH was decreased more than 2-folds compared to the free hGH control because of the difficult dissociation between poly-L-arginine and hGH.¹⁰³ Thus, in a further study, the less positively charged protamine sulfate was selected to complex with hGH to form protamine sulfate/hGH complexes for loading into the anionic gel. The protamine sulfate is a natural cationic peptide that is rich in arginine and has lower molecular weight than poly-L-arginine. The positive and negative charge distribution in protamine sulfate and its lower molecular weight made protamine sulfate/hGH complexes more easily dissociated than poly-L-arginine/hGH. The hGH could be nearly fully released over 14 days but with a higher initial release rate compared to poly-L-arginine/hGH complexes.⁷⁹ It is worth noting that the half-life ($t_{1/2}$) of hGH and duration in SD rat serum were increased 13- and 10.7-folds respectively compared to the free hGH control.⁷⁹ Therefore, this delivery system demonstrated an easy way to control the release rate by either selecting different polycations to complex with the proteins or adjusting the weight ratio between the polycation and the proteins. The increased half-life *in vivo* indicated that the released hGH showed effective protection from proteolytic degradation. The features of injectability, temperature-triggered gelation, easy control over release rate and enhanced bioactivity of the released proteins demonstrated the versatility of this delivery platform for practical applications.

2.3 Natural polyelectrolyte-based hydrogels

Natural polyelectrolytes, such as chitosan, hyaluronic acid, alginate and heparin, typically show less controllability over the molecular architecture and molecular weight distribution compared to synthetic polyelectrolytes. To date, the most well-established hydrogel directly constructed from a natural polyelectrolyte uses alginate to complex divalent cations (e.g., Ca^{2+}) for gelation based on ionic crosslinks. Divalent cations are considered to bind solely to guluronate blocks of the alginate chains because the guluronate blocks allow a high degree of coordination with the divalent cations. The guluronate blocks on one chain then form junctions with the guluronate blocks on adjacent chains with divalent ions in an egg-box model for crosslinking. This system has been widely used for biomedical applications.¹⁰⁴ More often, natural polyelectrolytes are combined with synthetic polyelectrolytes to generate hydrogels. The latter have better control over properties and molecular architecture to achieve gelation.^{79, 105, 106}

Other injectable hydrogels derived from natural polyelectrolytes have been more focused on modification to form covalently crosslinked gels. These modifications for gelation typically include thiol-ene chemistry, EDC/NHS coupling reaction, photo-initiated polymerization and enzymatic oxidation.^{107–110} Several recent reviews overviewed such modifications on natural polyelectrolytes for biomedical applications.^{23, 39}

3. Host-guest chemistry-based hydrogels

The host-guest interaction or self-assembly between the inclusion complexes are employed as physical crosslinks to generate shear thinning hydrogels. A widely used host is cyclodextrin (CD) that is able to accommodate one or two guest molecules to form an inclusion complex.¹¹¹ CDs are natural water-soluble cyclic oligosaccharides with a torus-like molecular shape and are named α -, β - or γ -CD according to the number of the repeat unit such as 6, 7, or 8 D-glucoses, respectively (Fig. 6A).¹¹² The molecular structure of the CDs consists of a hydrophobic inner cavity and hydrophilic outer surface. The hydroxyl groups are located at the outer surface with the primary hydroxyls at the narrow end and the secondary hydroxyls at the wider end. The inner diameter of the hydrophobic cavity is 0.45, 0.70 and 0.85 nm with a depth of 0.67, 0.70 and 0.70 nm for α -, β - or γ -CD, respectively.³³ This section is grouped into two subsections to discuss how to design biopolymers that are able to complex with α -CD and β -CD to form physical hydrogels.

3.1 PEG-functionalized biomaterials complex with α -CD to form hydrogels

Linear polymers such as poly(ethylene glycol) (PEG) and poly(ethylene oxide) (PEO) have a cross-sectional area of the polymer chain that matches the inner diameter of α -CD cavity and can penetrate the cavity to form necklace-like inclusion complexes.¹¹² The threading process is driven by van der Waals and hydrophobic interactions as well as hydrogen bonding between the neighboring threaded CDs.¹¹³ The self-assembly between the PEG/ α -CD complex strings forms microcrystals that serve as physical crosslinks to generate hydrogels. However, the gelation depending on the PEG/ α -CD complex alone typically takes hours or longer with a slow recovery of gel properties after a shear thinning process.¹¹⁴ Researchers are seeking to combine the PEG/ α -CD assembly with other physical interactions that could facilitate the gelation process and enhance hydrogel performance for real-world applications. Fig. 6B, C and D show several examples of the PEG/ α -CD inclusion complex together with hydrogen bonding, ionic or hydrophobic interactions to form supramolecular hydrogels with dual physical crosslinks.^{105, 115, 116} Compared with PEG/ α -CD alone, the dual physical crosslinks not only significantly reduce gelation time, but also control the release kinetics for either a single drug or multiple drugs. For example, the PEG/ α -CD complex with either hydrogen-bonding or electrostatic interactions as additional crosslinks shows gelation within 30–40 min with a storage modulus (G') approaching 1000 Pa (Fig. 6B and C), while PEG (5k or 10 kDa)/ α -CD control requires more than 1.5 h and yields a weak gel.^{105, 115}

The drug release kinetics depends on the encapsulation fashion in these dually crosslinked hydrogels. A burst release is usually shown if a hydrophilic drug is directly incorporated into the gel as a result of easy diffusion through the microscopic porous structure, such as the

doxorubicin releasing from a (thymine/adenine)-terminated PEGs (T-PEG-T/A-PEG-A) and α -CD gel (Fig. 6B).¹¹⁵ However, a steady release without an initial burst can be achieved if the drug is anchored into the network structure and serves as additional physical crosslinks. Two strategies have been proposed for a steady release of either bioactive proteins or hydrophobic molecules (Fig. 6C and D). For the sustained release, proteins are complexed with heparin at an appropriate ratio to form heparin-protein complexes that are then added to a polycation, mPEG-grafted poly(ethylene argininyaspartate diglyceride) (mPEG-g-PEAD), and α -CD mixture to yield protein-loaded hydrogel. The gel is formed based on self-assembly between mPEG-graft/ α -CD complexes and the electrostatic interactions between the heparin-protein complexes and pendent cationic groups on the polymer backbone. The protein is thus released steadily without an initial burst (Fig. 6C).¹⁰⁵ This delivery system effectively employed the merits of the bio-mimetic heparin-binding protein that is known to protect protein configuration and prolong bioactivity with regard to proteolysis in an extracellular matrix.¹⁰⁶

Another strategy can simultaneously load hydrophobic and hydrophilic anticancer drugs, camptothecin (CPT) and 5-fluorouracil (5-FU), in a single hydrogel for sequential release with enhanced anticancer therapeutic efficacy.¹¹⁶ The combined chemotherapy of CPT and 5-FU is known to have higher anticancer efficacy compared to individual use. This is realized by coupling mPEG-COOH with CPT to yield an amphiphilic mPEG-CPT prodrug that could self-assemble into prodrug micelles. When α -CD and hydrophilic 5-FU are added, the mPEG blocks on the prodrug micelles are complexed with α -CDs to form the hydrogel containing both CPT and 5-FU. In this case, the hydrophobic interaction between CPT moieties and self-assembly between mPEG-block/ α -CD complexes served as dual physical crosslinks. The 5-FU is released in phase I by diffusion through microscopic pores along with dissociation of PEG-blocks/ α -CD complexes and minor release of mPEG-CPT prodrug. CPT is steadily released mainly in phase II after dissociation of the mPEG-CPT prodrug micelles and hydrolysis of mPEG-CPT (Fig. 6D).¹¹⁶ *In vitro* cytotoxicity assays using A549 lung and Hep G2 liver cell lines indicated that the 5-FU loaded mPEG-CPT/ α -CD hydrogel showed a higher cytotoxicity to both cells than either free CPT or free 5-FU. However, an appropriate ratio of 5-FU to CPT in the hydrogel is desired for an optimal synergistic effect because a high dose of 5-FU would suppress the cytotoxicity activity to the cell lines.

3.2 Adamantane or azobenzene functionalized biomaterials complex with β -CD to form hydrogels

β -CD can also accommodate polymer chains such as poly(propylene oxide) to form an inclusion complex like PEG/ α -CD.¹¹⁷ However, the host-guest interactions between β -CD and adamantane (Ad) or azobenzene (Az) are more often used as crosslinks to build up supramolecular hydrogels (Fig. 7A and B).^{118, 119} Either natural or synthetic biopolymers can be functionalized with Ad or Az that are then complexed with another batch of β -CD-functionalized polymer or β -CD dimer to form gels. For example, hyaluronic acid (HA) was separately functionalized with Ad and β -CD to generate Ad_x-HA (x=20–50 mol%) and β -CD_x-HA (x=20 mol%) (Fig. 7A(a)).¹¹⁸ When mixing the two macromers, quick gelation occurred between them to yield a shear thinning hydrogel, e.g., Ad₂₀-HA mixed with β -

CD₂₀-HA at a total concentration of 5wt%. Such rapid gelation and shear thinning properties are attributed to the reversible host-guest complexation between Ad and β -CD. The crosslinking density, dissociation rate of the hydrogel and release rate of the payloads are adjustable by controlling the polymer concentration and molar ratio of Ad to β -CD moieties on the polymers (Fig. 7A(b, c)). Generally, a higher concentration yields more densely crosslinked gel with slower hydrogel dissociation and release rate of the payloads (Fig. 7A(c)). In addition, the stoichiometric balance between the Ad and β -CD moieties on the two macromers also plays a key role in the stability of the hydrogel. For example, Ad₂₀-HA and β -CD₂₀-HA at 7.5 wt% generated a more stable structure with the lowest hydrogel dissociation and protein release compared to hydrogels formed from other Ad-HA and β -CD₂₀-HA at the same concentration.¹¹⁸

The reversible isomerization of Az molecule (conversion between trans and cis isomers) triggered by different wavelengths gives Az/ β -CD complex light-responsiveness absent in the Ad/ β -CD complexation because β -CD only complexes with the trans Az isomer. This light responsiveness together with other stimuli-responsiveness can be engineered into a single hydrogel to enable multiple controls over gel properties. Fig. 7B shows an example of such a hydrogel exhibiting a triple stimuli-responsiveness in one system.¹¹⁹ A thermal-responsive polymer, PNIPAM, was functionalized with Az moieties (PNIPAM-Az) that was complexed with a disulfide bond bridged β -CD dimer. The hydrogel formed from a PNIPAM-Az/ β -CD dimer showed thermal responsiveness with a lower critical solution temperature (LCST) at 25 °C. Additionally, the hydrogel also exhibited a gel-sol transition when exposed to UV (λ =365 nm) for a while and recovered to gel again after exposure to Vis (λ =450 nm) for approximately 2 h (Fig. 7B(c)). The disulfide bonds in the β -CD dimers were cleavable using a reductant, dithiothreitol (DTT), leading to a gel-to-sol transition as well. However, such a gel-sol transition by disulfide reduction was not reversible because re-establishing the disulfide bonds was inefficient due to the inhibited mobility and reactivity of the thiol groups on the cleaved β -CD.¹¹⁹ Compared to the straightforward complexation between PEG and α -CD, β -CD usually requires a modification with hexanediamine to form mono-amine-functionalized CD that is then used to synthesize a β -CD dimer or couple to a polymer.^{118, 119} Although it needs additional modifications, the advantages of the stimuli-responsiveness from the disulfide-bridged β -CD dimer and light-induced association/dissociation of the Az/ β -CD complex offers great versatility.

4. Hydrophobic interaction-based hydrogels

Hydrophobic interactions play an important role in biomolecular complexation or folding. Hydrophobic interactions can also be used as physical crosslinks to design supramolecular hydrogels.^{120–123} The hydrophobic segment or block is balanced with a hydrophilic polymer to form an amphiphilic block copolymer and enable the whole polymer chain to be water-soluble. Such amphiphilic block copolymers are designed to be thermo-responsive to trigger gelation or mix with liposomes to interact with a hydrophobic bilayer to establish crosslinks for gelation (Fig. 8A and B).^{120, 121} Two good examples of thermo-triggered gelation that have been reported recently are illustrated by biocompatible and biodegradable polymers, poly(ethylene glycol)-b-poly(serinol hexamethylene urethane) (ESHU) and its derivative poly(ethylene glycol)-b-poly(propanol serinate hexamethylene urethane) (EPSHU).^{120, 123}

Here, the hydrophobic block, poly(serinol hexamethylene urethane) or poly(propanol serinate hexamethylene urethane), is balanced with a hydrophilic PEG block on their chain ends. Both materials undergo a reversible phase transition induced by changes of solubility upon heating and cooling. A rapid sol-gel transition occurs as the polymeric solution temperature is elevated to body temperature (Fig. 8A). Such rapid temperature-triggered gelation demonstrated potential for applications in injectable cell or drug delivery.

To evaluate the biocompatibility of ESHU, *in vivo* subcutaneous implantation studies were performed to examine the host response to the material. H&E staining showed a native histological structure with regular muscle alignment and cell morphology. ED-1 staining revealed only mild inflammatory reactions to the subcutaneous implants of ESHU gel. These results demonstrate good biocompatibility and make it attractive as minimally invasive drug delivery platform. For example, bevacizumab (Avastin), a VEGF antibody used to treat age-related macular degeneration, was loaded in ESHU gel and showed a sustained release *in vitro* over 17 weeks at 37 °C.¹²⁴ Such a long-term release is very attractive for intraocular drug delivery application. To investigate the suitability of ESHU gel for this application, an *in vitro* cytocompatibility test was performed on two different cell lines, bovine corneal endothelial cells and human ARPE-19 cells. These two cell types were chosen because they were likely to be exposed to ESHU gel and its degradation components after intravitreal injection. The *in vitro* results showed that neither the ESHU gel nor its degradation components caused negative impacts on the cell morphologies, viability or growth.

Furthermore, intraocular injection of bevacizumab loaded ESHU gel in rabbits demonstrated that bevacizumab concentration in the treated eyes was maintained approximately 5-folds higher over 9 weeks than that of bolus administered control. The histological study revealed that such long-term retention of ESHU gel in rabbit eyes did not cause significant inflammatory response or affect retinal structures. These *in vivo* results demonstrated effective protection of bevacizumab from degradation. The sustained release profile and good biocompatibility suggested that ESHU is a promising vehicle for intraocular drug delivery.¹²⁵

Here, the slow degradation and long-term sustainable release of the payloads from ESHU gel are suitable for intraocular drug delivery applications, but some applications might require faster biodegradation of the designed materials. Thus, the EPSHU was designed to biodegrade more quickly than ESHU by introducing more hydrolysable ester bonds along the polyurethane backbone; the thermo-triggered gelation kinetics and other gel properties remained similar to ESHU.¹²³

Hydrophobic interactions can also be established between the hydrophobic segments on polymer ends and liposome particles to form a liposome gel (Fig. 8B).¹²¹ Liposome has been widely used as a versatile drug delivery vehicle in applications ranging from chemotherapy to gene therapy.¹²⁶ However, administration of the dispersed particles to a localized site in the body remains a challenge. To expand the applications of the liposome delivery platform, a liposome gel was designed by mixing the liposome suspension with a hydrophobic segment end-capped polymer, e.g., cholesterol-end capped PEG (Chol-PEG-Chol).¹²¹ When Chol-PEG-Chol is mixed with a liposome suspension, the hydrophobic ends

of cholesterol are separately embedded into the liposome bilayers via hydrophobic interaction to bridge the adjacent liposome particles to yield a network structure. The interactions between the polymer ends and liposome particles have three categories, including the above-mentioned “bridge” between adjacent particles, “loop” when both cholesterol ends are inserted into the same bilayer and “dangling” when only one end is embedded into the liposome bilayer (Fig. 8B). The “loop and dangling” chains do not form effective crosslinks. The crosslink density and viscoelastic properties are dependent on the ratio of Chol-PEG-Chol to liposome. For example, when a 20 mM liposome suspension was mixed with Chol-PEG-Chol solution, the storage modulus was elevated from tens to thousands when the polymer concentration increased from 1 to 6 wt% because of the formation of more effective crosslinks at a higher polymer concentration. Such a liposome gel could be transformed into the sol state readily under mild mechanical stress (ca. 59 Pa) and be able to rapidly recover gel properties after removal of the stress, showing a reversible interaction between liposome and Chol-PEG-Chol.

Although only dimethyldioctadecylammonium bromide (DODAB) was used to prepare the liposome suspension for study in this example, this principle can be expanded to other liposome particles as long as the hydrophobic polymer ends can insert into the liposome bilayers to form crosslinks. The hydrophobic segments used to functionalize the hydrophilic polymer ends are not only limited to cholesterol. Other hydrophobic moieties such as $C_{12}H_{15}$, $C_{18}H_{37}$ and $C_{24}H_{49}$ can also be used to end-cap hydrophilic polymers for use, but cholesterol is favored because it is naturally occurred and is metabolized in the body. All these features make the liposome gel a promising platform for controlled drug delivery and will definitely expand the versatility of the liposome delivery system.

5. Coordination bond-based hydrogels

Marine mussel secretes prepolymerized collagens and mussel foot proteins to form mussel byssus, byssal cuticle and plaques, in which the byssal plaques achieve attachment to many substrate surfaces in seawater.¹²⁷ The mussel byssus and foot proteins are rich in amino acids of 3,4-dihydroxyphenylalanine (DOPA) and histidine that mediate complexation with metal ions such as divalent and trivalent cations of Ca^{2+} , Zn^{2+} , Cu^{2+} , Al^{3+} and Fe^{3+} .^{127–129} The DOPA- and histidine-chelation with metal ions via coordination bonds play critical roles in maintaining the mechanical properties of the byssal threads and mediate plaque adhesion at the pH conditions in seawater.¹²⁷ For example, the histidine in byssal threads complexing with Zn^{2+} or Cu^{2+} enables byssal threads to dissipate stress and self-repair damages caused by ocean waves. The DOPA and Fe^{3+} complexation in mussel foot proteins mediates the oxidation state of DOPA and its adhesion to substrates.^{127, 128, 130} Inspired by the marine mussel anchoring mechanism, scientists design biomaterials functionalized with DOPA, its analogues such as dopamine, nitrodopamine and 3-hydroxy-4-pyridinone (HOPO), or histidine for wet-resistant adhesives, coatings and particularly the ligand-metal coordination bond-based supramolecular hydrogels.^{127–133}

Generally, the catechol or its analogues can be immobilized or end-capped on either natural or synthetic biopolymers with linear or branched architectures. These polymers usually bear functional groups of carboxylic acid or amine groups to react with the counterparts in

catechol and the analogues. DOPA or histidine can also be incorporated into a protein or peptide by solid peptide synthesis techniques.^{127–132} Fig. 9 summarizes the typical polymer architectures functionalized with catechol or its analogues to yield marine mussel-mimetic biopolymers chelating with metal ions to generate supramolecular hydrogels.^{127–129}

To form ligand-metal complexation crosslinks, the molar ratio of the catechol or HOPO to Fe^{3+} is typically favored at 3:1, while histidine is preferred to chelate with divalent metal ions (e.g., Zn^{2+} or Cu^{2+} , Co^{2+} or Ni^{2+}) at 2:1 by mole (Fig. 9B).^{128–130} In addition, the pH condition has similar importance in mediating the formation of ligand-metal complexation. The histidine can effectively chelate with M^{2+} to form bis complexation for crosslinking at pH 7.¹²⁸ However, the catechol chelates with Fe^{3+} preferably at pH above 9 to effectively form tris catechol- Fe^{3+} complexation.¹³⁰ However, the catechol is prone to oxidation at such an alkaline pH level, leading to conversion of the catechol into quinone and susceptibility to covalent crosslinks, thus losing the dynamic nature.^{129, 130}

To address these challenges, two analogues including nitrocatechol (e.g., 6-nitrodopamine) and HOPO are used as chelating functionalities with high coordination affinity and lower susceptibility to oxidation.¹²⁹ The two analogues possess electron-withdrawing functional groups that withdraw electron density from phenolate moieties but do not sacrifice the bidentate phenolate nature of the catechol ligand. The electron-withdrawing effect leads to reduced phenolic pK_a values (pK_{a1} and pK_{a2} , 9.1 and 14 for catechol, 6.7 and 10.3 for nitrocatechol, and 3.6 and 9.9 for HOPO) and increased oxidation potentials.¹³⁴ It was shown that the measured stability constants for tris complexation of nitrocatechol- Fe^{3+} ($\log \beta_3 = 39.2$) and HOPO- Fe^{3+} ($\log \beta_3 = 37.6$) were considerably high, although they were slightly lower than that of catechol- Fe^{3+} complexation ($\log \beta_3 = 43$).¹²⁹ These properties make nitrocatechol and HOPO functionalized polymers more feasible to form robust hydrogels at a physiological pH level for biomedical applications. For example, comparison among the gelation of catechol, nitrocatechol and HOPO end-capped 4-arm PEG complexing with Fe^{3+} revealed that HOPO-based gel could predominantly form tris-coordination crosslinks at pH 7.4. The nitrocatechol-based gelation was dominated by tris coordination at pH 9, but the catechol-based one had a large amount of bis coordination at pH 9.¹²⁹ A bis catechol- Fe^{3+} has a stability constant of $\log \beta_2 = 33$, much lower than that of the tris complex ($\log \beta_3 = 43$) and also lower than either the nitrocatechol or HOPO tris complexes.¹²⁹ In addition, a HOPO-functionalized polymer solution with a chelator: Fe^{3+} ratio at 3:1 was able to immediately form a gel upon injection into a pH 7.4 buffer solution. The HOPO- Fe^{3+} -based hydrogel retained almost a similar dynamic nature of the coordination bonds after incubation for one month under ambient conditions even with a pH at 9. This indicated the high stability of HOPO resistant to oxidation and thus little covalent crosslinking occurred.¹²⁹ Such rapid gelation kinetics and anti-oxidation properties have not been observed in either catechol or nitrocatechol chelators. Compared to catechol- and nitrocatechol-based hydrogels, a HOPO-based supramolecular network is more promising under physiological conditions. Overall, these different properties of catechol and its analogues for chelation and surface adhesion apparently enriched the potentials of marine mussel-inspired biomaterials for different biomedical applications.

6. π - π stacking interaction-based hydrogels

π - π stacking interactions form between aromatic rings via a plane-to-plane or an edge-to-plane orientation that commonly exist in aromatic-motif-containing proteins to offer a stabilizing force for protein organization.¹³⁵ The aromatic rings have relatively compact volumes that reduce the steric hindrances, and thus the π - π stacking is typically stronger than the van der Waals interaction formed between alkyl chains. These features make π - π stacking interactions more predictable and efficient self-assembly forces to form a stable supramolecular structure.¹³⁵ Aromatic ring containing amino acids such as phenylalanine (F), tyrosine (Y) and tryptophan (W), and the compounds including fluorenylmethyloxycarbonyl (Fmoc), 1-pyrenebutyric acid, 2-naphthalene acetic acid and nitrophenyl methacrylate are usually used to design peptides or biopolymers bearing aromatic moieties to form π - π stacking interaction for gelation.^{136–140}

Different from ionic complementary peptides that typically require a minimal sequence of eight amino acids for efficient self-assembly to yield a thermodynamically stable structure (see section 2.1), a π - π stacking interaction assembly can be realized using a peptide sequence as short as only two amino acid residues. The self-assembly is versatile and can construct many micro or nanoscale architectures, including fibrous hydrogel, nanotube, nanowire and vesicle, that have been demonstrated by diphenylalanine (FF) or FF-based building blocks.^{140–142} Recently, an aromatic moiety containing short peptides and N-terminal Fmoc-amino acids have been reported for robust self-assembly into supramolecular architectures for biomedical applications.^{136–138, 143, 144} Table 1 summarizes the categories of these gelators and their gelation conditions.

It is interesting to note that even the individual amino acids such as F, Y, W, M, G, I and DOPA coupled with Fmoc moiety on N-termini can efficiently drive the small molecules to self-assemble into hydrogels (Table 1). In these cases, aromatic-aromatic stacking along the Fmoc moieties plays a key role in driving the self-assembly into nanofibrils. In addition to the π - π stacking interaction on N-terminal Fmoc groups, the final fibrous gel formation is also dependent on other intermolecular interactions such as the hydrogen bond, ionic interaction and additional π - π stacking between the side chains on the amino acids.^{137, 138, 143, 144} For example, F, Y and W molecules bear an aromatic ring on their side chains. This makes Fmoc-F-OH, Fmoc-Y-OH and Fmoc-W-OH self-assemble into hydrogels at a lower critical concentration than Fmoc-G-OH and Fmoc-I-OH without an aromatic group on the side chains (Table 1).¹⁴⁴ This phenomenon was also observed in the pentapeptide derivatives. The aromatic rings on N-termini of VYGGG and YGFGG together with the aromatic ring on Y and F side chains enabled the two pentapeptide derivatives to more easily assemble into a hydrogel compared to other pentapeptide derivatives (Table 1).¹³⁶

Although the π - π stacking is important for directing the assembly of these designer peptides or Fmoc amino acids, pH is similarly important to trigger gelation by forming hydrogen bonds between the molecules for most of the species listed in Table 1.^{136–138, 143, 144} This is because they all have free carboxylic acid groups on the C-termini and some species, such as VTTEEI derivatives, have carboxylic acid on the E side chain as well. The hydrophilicity and

hydrophobicity of these molecules are balanced by the degree of deprotonation of the carboxylic acid group, their hydrophobic side groups and the aromatic moieties on N-termini. Thus, pH is very important to mediate the deprotonation and protonation of the carboxylic acid group for dissolution and gelation processes.^{136, 137, 144} The general trend is that the increase of hydrophobicity on amino acid side chain leads to gelation at a relatively higher pH condition.¹³⁶ For example, 3,4-dihydroxy-phenyl on the DOPA side chain is more hydrophilic than the phenyl group on F. This leads to Fmoc-DOPA gelling at pH 2 but Fmoc-F-OH at pH 7.4 (Table 1).^{138, 143} A similar trend was also observed in the gelation of pentapeptidic derivatives.¹³⁶

These Fmoc amino acids or aromatic moiety-coupled short peptides are either commercially available or are easily synthesized by the solid phase peptide synthesis technique. Their versatile gelation ability demonstrated the attractiveness for biomedical applications, but some Fmoc amino acids and short peptides require harsh conditions to dissolve the molecules in aqueous solution for gelation; this most likely limited their applications (Table 1). The examples cited here illustrate the use of π - π stacking to trigger gelation of hydrogels. This is undoubtedly another highly useful and relatively simple way to design hydrogels crosslinked by weak bonds.

Conclusion and Perspectives

We have presented recent advances of weak bond-based hydrogels according to six categories of dynamic bonds. Each type of these weak bonds has its own features to establish interactions and thus govern gelation kinetics. Recent studies show a trend that multiple weak bonds are combined in a single system to more efficiently control hydrogel performance. These multiple dynamic interactions synergistically serve as reversible crosslinks to facilitate the gelation process, manipulate viscoelastic properties, and introduce stimuli-responsiveness to control the sol-gel phase transition. Several examples have been given in section 2.1 (PNIPAAM-FEFEFKFK gel), section 2.2 (anionic and thermo-responsive poly(organophosphazene) and positively charged polycation/protein complex), section 3.1 (PEG/ α -CD complexation in combination with electrostatic interactions, hydrogen bonding, or hydrophobic interactions), and section 6 (π - π stacking interactions in combination with hydrogen bonding interactions). Several These examples have been are provided to illustrate the benefits from such synergistic effects, such as ionic interaction combined with thermo-triggered gelation in either ionic peptide self-assembly or polyelectrolyte gels, host-guest interaction combined with ionic interaction, hydrogen bond, hydrophobic interaction, and π - π stacking interaction combined with hydrogen bond. We hope these recent developments will spark even more activities in this exciting field and inspire new designs of gels using weak bonds.

The injectable biomaterials are designed for use as soft scaffolds alone, or for cell and drug delivery. We would like to note that this review is focused on the design of these biomaterials at a proof-of-concept stage for biomedical applications. Most of the materials were evaluated in small animal models. A few more traditional physical gels such as collagen, gelatin, fibrin-alginate composite, self-assembling peptide fibers and UPy based hydrogels have progressed to evaluations in large animal models. Several excellent reviews

on these progresses in preclinical testing were published recently.^{145–147} On the other hand, it is exciting to note that some of the more recent designs demonstrated great potential and further studies are underway to evaluate the therapeutic efficacy in large animals and clinical trials. For example, Ad-HA and β -CD-HA complex gel was tested in sheep for heart failure treatment and is moving forward to clinical trials.¹⁴⁸ In addition, alginate based hydrogel and its delivery method showed promising results in treating porcine myocardial infarction models and thus it is transitioned into phase I and now phase II clinical trials.^{149, 150} The progress of these new generation of physical gels in preclinical and clinical testing will undoubtedly intensify the interests in these materials and uncover new design criteria for further development of these exciting gels.

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Fig. 1.

Examples of H-bond-based shear thinning and stimuli responsive hydrogels for biomedical applications. (A) (a) Hydrogel formed from chain extended UPy-PEG polymers containing multiple UPy moieties in polymer backbone, yielding a tougher hydrogel for long-term applications. (b) Hydrogel formed from end-capped UPy-PEG chains, yielding a soft hydrogel for short-term applications. X represents an alkyl (CH_2) spacer between PEG and UPy units forming a hydrophobic pocket shielding the UPy-UPy interaction from aqueous attack. Adopted from ref. 60. Copyright © 2012, Elsevier Ltd. (B) PA-6-ACA hydrogel shows reversible H-bonding for self-healing via pH stimulation. At pH 3, two separate pieces can self-heal by forming H-bonds between carboxylic acid and amide groups at the interface. At pH 9, the H-bonds at the interface dissociate because of the deprotonation of termini carboxylic acid groups leading to repulse each other. Adopted from ref. 61.

Copyright © 2012, National Academy of Sciences. (C) PVDAT hydrogel responds to nucleoside (Thymidine) to switch H-bonds from the initial DAT-DAT dual H-bonds to stronger DAT-Thymidine triple H-bonds. Adopted from ref. 62. Copyright © 2015, Elsevier Ltd.

Fig. 2. General process of anti-parallel self-assembly from a self-complementary ionic peptide, FEFEFKFK, to generate a β -sheet nanofiber hydrogel. Adopted from ref. 83. Copyright © 2013, Elsevier Ltd.

Fig. 3.

(A) One pot synthesis of PNIPAAm-PEFEFKFK conjugate and PNIPAAm for self-assembling into a polymer/conjugate hybrid gel with thermo-responsiveness. The HS-peptide as a chain transfer agent is mixed with a monomer (NIPAAm) and initiator (AIBN) for free radical polymerization to simultaneously yield a PNIPAAm and PNIPAAm-peptide conjugate for self-assembly. Adopted from ref. 87. Copyright © 2011, Royal Society of Chemistry. (B) Illustration of an enzymatically triggered gelation using a short tetrapeptide solution as a starting material and self-assembling into a thermodynamically stable octapeptide β -sheet architecture. The initially non-gelling tetrapeptide, FEFEFK solution, is mixed with thermolysin at an appropriate concentration. The ionic self-complementary octapeptide is favorably generated for the molecular assembly via reverse hydrolysis in the presence of the enzyme. A thermodynamically stable network is eventually formed when the concentration is above the critical gelation concentration (CGC). Adopted from ref. 89. Copyright © 2013, American Chemical Society.

(A) General principle for triblock copolyelectrolyte design and hydrogel formation through coacervate crosslinking. An ABA triblock copolymer is used as a precursor to synthesize two copolyelectrolytes with oppositely charged end blocks. Equimolar aqueous solutions of the two copolyelectrolytes are mixed to generate a network structure driven by coacervate crosslinking. (B) Synthesis of ABA triblock copolyelectrolytes from a common copolymer precursor. The A blocks are conveniently functionalized with (a) sulfonate, (b) carboxylate, (c) ammonium and (d) guanidinium to bear negative or positive charges with different ionic strength in aqueous conditions for complex coacervate crosslinking. Adopted from ref. 78. Copyright © 2011, John Wiley & Sons, Inc.

Fig. 5.

(A) Schematic illustration of incorporating polycation/hGH complexes (PECs) into an anionic thermosensitive poly(organophosphazene) hydrogel for sustained release of hGH. The polycation can be either synthetic or natural biopolymers, such as poly-L-arginine, poly-L-lysine or protamine sulfate, to complex with hGH to form PECs. When above the lower critical solution temperature (LCST), the dehydration of the polymer chains occurs and the hydrophobic moieties interact with each other to form a network structure. The positively charged PECs are incorporated into the hydrogel network through electrostatic interactions with the negatively charged moieties along the polymer backbone. (B) Chemical composition of the anionic poly(organophosphazene). The polymer is derived from poly(dichlorophosphazene) functionalized with hydrophobic L-isoleucine ethyl ester (a, IleOEt), hydrophilic α-amino-ω-methoxy-poly(ethylene glycol) (c, AMPEG) and anionic moiety of glycyl-glycine (b, GGOH). The LCST is adjusted by the ratio of a to c along the polymer backbone. Adopted from ref. 79. Copyright © 2013, Elsevier Ltd.

Fig. 6.

(A) Chemical structure of α -, β - and γ -CD comprising 6, 7 and 8 repeat units of D-glucose.

A representative 3D structure of CD is shown in right. (B) A supramolecular hydrogel is made from (thymine/adenine)-terminated PEGs (T-PEG-T/A-PEG-A) and α -CD. The thymine/adenine-terminated PEG and α -CD forms inclusion complexes for self-assembly into microcrystals as crosslinks. The hydrogen-bonding between A and T moieties acts as additional crosslinks to facilitate gelation and enhance the elastic modulus. Adopted from ref. 115. Copyright © 2014, Royal Society of Chemistry. (C) A supramolecular hydrogel is formed based on host-guest chemistry and electrostatic interaction. The hydrogel is made by mixing mPEG-grafted poly(ethylene argininy laspartate diglyceride) (mPEG-g-PEAD) with α -CD and heparin. The self-assembly between mPEG-graft/ α -CD inclusion complexes serves as first crosslinks. Additionally, the electrostatic interaction between heparin and the

pendent cationic groups are secondary crosslinks. Such dual physical crosslinks significantly reduced gelation time with enhanced viscoelasticity compared with mPEG-g-PEAD/ α -CD alone or mPEG/ α -CD control. A growth factor (GF) is complexed with heparin to form heparin-binding proteins that are then loaded into the gel and steadily released over 16 days without an initial burst. Adopted from ref. 105. Copyright © 2016, Royal Society of Chemistry. (D) A supramolecular hydrogel is designed for dual-phase drug release based on host-guest chemistry and hydrophobic interaction. The hydrophobic camptothecin (CPT) is coupled with mPEG-COOH to yield mPEG-CPT prodrug that can self-assemble into prodrug micelle. When α -CD and hydrophilic 5-fluorouracil (5-FU) are added, the prodrug micelles are complexed with α -CD to form a hydrogel that contains both 5-FU and CPT anticancer drugs. The 5-FU is released in phase I through diffusion along with the dissociation of mPEG-segments/ α -CD complexes; while CPT is released in phase II after dissociation of the prodrug micelles and hydrolysis of mPEG-CPT. Adopted from ref. 116. Copyright © 2013, Royal Society of Chemistry.

Fig. 7.

(A) Adamantane-functionalized hyaluronic acid (Ad-HA) and β -CD-functionalized hyaluronic acid (β -CD-HA) form a shear thinning hydrogel. (a) Chemical structures of Ad-HA and β -CD-HA. The host-guest interactions between Ad and β -CD serve as physical crosslinks. Either the concentrations of Ad-HA and β -CD-HA macromers or the molar ratio between Ad and β -CD moieties are used to control the crosslinking density to obtain (b) low crosslinking and (c) high crosslinking gels. Adopted from ref. 118. Copyright © 2013, American Chemical Society. (B) Azobenzene-functionalized PNIPAM (PNIPAM-Az) and β -CD dimer form a hydrogel with multiple stimuli-responsiveness, including temperature, light and disulfide reductant. (a) Chemical structures of PNIPAM-Az and a disulfide bond-connected β -CD dimer. (b) The Az-functional groups are complexed with β -CD dimer acting as physical crosslinks. The hydrogel shows LCST at 25 °C because of the thermal-responsiveness of the PNIPAM backbone. (c) A reversible gel-sol transition is triggered by subsequent exposure to UV ($\lambda=365$ nm) and Vis ($\lambda=450$ nm). Under UV exposure, Az trans isomer converts into cis conformation and the Az/ β -CD complexes are dissociated, leading to gel-to-sol transition. On the contrary, a gel is formed again under Vis exposure. (d) The disulfide bonds in the β -CD dimers are cleaved by dithiothreitol (DTT) as the reductant, also leading to gel-to-sol transition. Adopted from ref. 119. Copyright © 2014, Royal Society of Chemistry.

Fig. 8.

Two types of hydrophobic interaction-based hydrogels. (A) Temperature-triggered gelation of a thermo-responsive amphiphilic block copolymer, ESHU. As the temperature is elevated to 37 °C, the polymer chains are dehydrated and the hydrophobic blocks interact and entangle each other to form a hydrogel. (B) Formation of a liposome gel by mixing cholesterol-end capped PEG (Chol-PEG-Chol) and dimethyldioctadecylammonium bromide (DODAB) liposome suspension. The hydrophobic cholesterol ends are embedded into the bilayers of the adjacent liposome particles to form the effective physical crosslinks. Some of the Chol-PEG-Chol chains forming either a “Loop” or “Dangling” structures are not effective crosslinks for gelation. Adopted from ref. 121. Copyright © 2011, Elsevier Ltd.

Fig. 9.

(A) Catechol or its analogue functionalized polymers with different architectures. (a) End-capped linear polymer, (b) pendant on linear polymer, (c) end-capped 4-arm-polymer and (d) end-capped 8-arm-polymer. Adopted from ref. 127. Copyright © 2011, Annual Reviews. (B) Schematic illustration of the catechol or its analogues functionalities chelating with divalent or trivalent metal ions to form coordination bond-based crosslinks. (a) DOPA, dopamine, or nitro-dopamine chelating with trivalent metal ion, e.g., Fe³⁺; (b) 3-hydroxy-4-pyridinone (HOPO) chelating with Fe³⁺. Adopted from ref. 129. Copyright © 2013, Royal Society of Chemistry. (c) Histidine chelating with divalent metal ions, e.g., Zn²⁺ or Cu²⁺, Co²⁺ or Ni²⁺. Adopted from ref. 128. Copyright © 2013, American Chemical Society.

Table 1

π - π stacking interaction-based hydrogels from Fmoc- amino acids or aromatic moiety containing peptides.

Gelator	Gelation condition	Reference
Fmoc-F-OH	6 mM in PBS 7.4 buffer; ultrasonicate and heat at 80 °C to dissolve; cool to r.t. for gelling.	138
Fmoc-L-DOPA	2.5 mg/mL in aqueous solution at pH 2; heat to dissolve; incubate at 25 °C overnight for gelation.	143
Fmoc-F-OH Fmoc-Y-OH Fmoc-W-OH Fmoc-M-OH Fmoc-G-OH Fmoc-I-OH	General procedure: Fmoc-amino acid (e.g., 10 mg/mL) disperse in basic deionized water, heat at 40 °C for seconds to dissolve; remove extra residue; adjust pH to acidic level by adding glucono- δ -lactone (1–5 mg/mL) for gelation.	144
Fmoc-2-Nal-OH Fmoc-FG-OH Fmoc-FF-OH Fmoc-FRGD-OH Fmoc-RGDF-OH	General procedure: Fmoc-amino acid or peptide dissolve in DMSO (100 mg/mL) as precursor solution; dilute with ultrapure water to yield peptide solutions (1–5 mg/mL) for gelation.	137
Fmoc-GAGAS-OH Pyr-GAGAS-OH Fmoc-GVGVP-OH Pyr-GVGVP-OH Fmoc-VTEEI-OH Pyr-VTEEI-OH Nap-VTEEI-OH Fmoc-VYGGG-OH Nap-VYGGG-OH Fmoc-YGFGG-OH Pyr-YGFGG-OH Nap-YGFGG-OH	General procedure: At r.t., slowly add 1N NaOH to suspension of the pentapeptide derivative in deionized water to dissolve; adjust pH to a certain acidic level for each peptide solution using 1N HCl for gelation; typical gel concentration is between 0.5–2.8 wt%.	136

Abbreviation denotation: A=alanine, D=Aspartic acid, E=Glutamic acid, F=Phenylalanine, G= Glycine, I=Isoleucine, M=methionine, P=Proline, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine, DOPA= 3,4-dihydroxy-L-phenylalanine, Fmoc=Fluorenylmethyloxycarbonyl, 2-Nal= β -(2-naphthyl)-L-alanine, Pyr=Pyrenyl, Nap=Naphthalenyl.