Enzyme-Regulated Healable Polymeric Hydrogels
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ABSTRACT: The enzyme-regulated healable polymeric hydrogels are a kind of emerging soft material capable of repairing the structural defects and recovering the hydrogel properties, wherein their fabrication, self-healing, or degradation is mediated by enzymatic reactions. Despite achievements that have been made in controllable cross-linking and de-cross-linking of hydrogels by utilizing enzyme-catalyzed reactions in the past few years, this substrate-specific strategy for regulating healable polymeric hydrogels remains in its infancy, because both the intelligence and practicality of current man-made enzyme-regulated healable materials are far below the levels of living organisms. A systematic summary of current achievements and a reasonable prospect at this point can play positive roles for the future development in this field. This Outlook focuses on the emerging and rapidly developing research area of bioinspired enzyme-regulated self-healing polymeric hydrogel systems. The enzymatic fabrication and degradation of healable polymeric hydrogels, as well as the enzymatically regulated self-healing of polymeric hydrogels, are reviewed. The functions and applications of the enzyme-regulated healable polymeric hydrogels are discussed.

1. INTRODUCTION
Self-healing is an advanced function existing in many biological systems.1−9 The development of bioinspired self-healing materials possesses a history of more than 40 years since the first self-healing phenomenon was observed in a viscoelastic polymeric system in 1978 (Figure 1).5 The concepts of extrinsic and intrinsic self-healing materials6−10 became clear in early 21st century, after the successful exploitations of microcapsule-based/microvascular self-healing materials,11,12 and Diels−Alder reaction-based thermal-triggered self-healing polymers.13 Nowadays, synthetic self-healing materials can not only repair the structural defects and restore the mechanical properties11,13−19 but also recover the material functions including transparency,20,21 electrical conductivity,22−25 hydrophobicity,26 superhydrophobicity,27−29 and antifouling functions.30,31 Despite these achievements, current synthetic self-healing materials are still far less intelligent and practical than self-healable living organisms.

Self-healing in biological systems is a complex and hierarchically controlled process.1,32 Enzymes play a very important role in the healing of living organisms.33 For example, enzyme kinase is functional for regulating DNA synthesis/mitosis in keratinocytes and re-epithelialization during wound healing.2,3 An epidermal lipoygenase, AmbLOXe, draws significant attention because it has been proven to be key for amphibian epidermal cell proliferation and migration.1,34 Mexican axolotl is a well-known sea creature that employs this enzyme for its intrinsic regeneration.34 Now medical scientists are attempting to use this enzyme to regulate tissue healing and regeneration toward the treatment of human diseases.1 Tough enzyme mediation is a powerful tool for the construction and self-healing of living organisms, it is fragile when introduced into synthetic materials, because the 3-dimensional (3D) structures of enzymes may be altered or destroyed by organic solvents, strongly acidic or basic.
mediums, heating, and drying processes that are frequently employed in artificial material fabrication, leading to loss of the biocatalytic activity. Therefore, the development of a suitable platform to accommodate the rationally selected enzymatic reactions that can effectively work is crucial to mimic the enzyme-regulated self-healing in biological systems.

Self-healing in biological systems is a nutrient intake-promoted, enzyme-assisted, temporally and hierarchically controlled response.

Since the first hydrogel was synthesized by Wichterle and Lim in 1960 (Figure 1), these soft materials have made a significant impact on the developments of electronic sensors,17,18 lyotropic liquid crystals,19,20 smart windows,21 and drug delivery systems,22−27 and scaffolds for tissue engineering.28−31 Polymers hydrogels comprise chemically or physically cross-linked polymer networks and large portions of water, which not only enable the design of reversible interactions in the cross-linked networks for self-healing but also provide a suitable environment to facilitate the occurrence of enzymatic reactions.32−34 The initial use of enzymatic reactions in polymeric hydrogels is for material construction, and then followed by enzymatic fabrication of healable polymeric hydrogels35 and enzymatically regulated healing of polymeric hydrogels.32−37 The latest progress of this field is to use the enzymatic reaction to mediate the transient healing ability of polymeric hydrogels,38,39 which not only enables the fabrication of thermodynamically stable and kinetically inert hydrogels but also endows such hydrogels with an intrinsic healing ability on demand.

In this Outlook, we discuss the perspectives of enzyme-regulated healable polymeric hydrogels. The studies on enzyme-catalyzed cross-linkable hydrogels without self-healing functions and enzyme-triggered sol−gel transitions have been comprehensively reviewed40−42 and, therefore, are not included herein. We summarize representative examples of enzymatic fabrication and degradation of healable polymeric hydrogels and enzymatically regulated self-healing of polymeric hydrogels. The applications of enzyme-regulated healable polymeric hydrogels are discussed. Lastly, we summarize current achievements and give our prospective on the future development trend of the enzyme-regulated healable polymeric hydrogels. For your convenience the main contents of this review are summarized in Figure 2.
2. ENZYMATIC FABRICATION OF HEALABLE POLYMERIC HYDROGELS

The enzymatic strategy for the fabrication of healable polymeric hydrogels aims to use enzymatic reactions to trigger the polymerization or self-assembly of monomers and/or polymers for the construction of healable polymeric hydrogels. The enzymatic cross-linking strategy has been widely used for the fabrication of hydrogels, which acts as an innovative alternative to other cross-linking methods such as the photocross-linking or physical cross-linking. The enzymatic reactions applied in cross-linking of the hydrogels without self-healing functions have been comprehensively reviewed in 2012 by Karperien and colleagues. Emerging methodological studies for enzymatic fabrication of healable polymeric hydrogels have yet to be systematically summarized and discussed. Enzyme-catalyzed reactions are mild but highly efficient to trigger the cross-linking of polymers having functional groups in aqueous mediums, therefore forming hydrogels. The enzyme-regulated healable polymeric hydrogels are synthesized from either monomers or polymers (i.e., building blocks) via noncovalent and/or covalent bonds. In the hydrogels, enzymes usually act as special assisting additives to regulate the synthesis, degradation, and/or self-healing of the hydrogels, but they are usually stably immobilized in the polymer networks based on covalent bonds or supramolecular interactions. For example, glucose oxidase (GOX) was immobilized in the polymer networks based on the metal–ligand interactions between the cobalt ions and the histidine functional groups on GOX in our previous study. However, enzymatic reactions are normally irreversible, which suggests that the enzymatically formed cross-links in the hydrogels also have the tendency to be irreversible, and the hydrogels may lack a self-healing ability. Only rational integration of the cross-linkable functional groups and enzymatic reactions can afford the enzymatic fabrication of healable hydrogels. In this section, we describe some examples of healable polymeric hydrogels fabricated via the enzymatic strategy, and a summary of currently available studies involving the enzyme mediation methods for the construction of healable polymeric hydrogels is presented in Table 1.

Table 1. Overview of the Enzymatically Fabricated Healable Polymeric Hydrogels

| functions for hydrogelation | enzyme | driving force for healing | ref. |
|-----------------------------|--------|---------------------------|------|
| enzymatic polymerization    | acid phosphatase (AP), glucose oxidase (GOX), horseradish peroxidase (HRP) | hydrogen bonding interactions, π–π stacking | 60, 66, and 67 |
| enzyme-mediated reversible covalent bonds | monoamine oxidase B (MAO B), catalase (CAT), plasma acylhydrazide (PAO), urease | inside bond, hydrogen bonding interactions, amine oxide bonds | 68, 69, and 73 |
| enzyme-assisted carbon dioxide fixation | α-glucosidase, GOX | carbon fixation, glucose export, polymerization | 70 |

Polymers hydrogels provide a perfect platform for facilitating the occurrence of enzymatic reactions towards the healing of damages by programming the dynamics of reversible chemical bonds or physical interactions.

2.1. Enzymatic Polymerization for the Fabrication of Healable Polymeric Hydrogels. The early developed approach for enzymatic fabrication of healable polymeric hydrogels is a self-assembly and polymerization method. The hydrogel precursors are typically a composite of substrates, small molecules that have the potential to assemble, monomers, and another small molecules that have the potential to generate radicals. Two different enzymes are usually used in this method to trigger the self-assembly for hydrogel formation and the generation of radicals to initiate the polymerization, respectively. For example, Wang and coworkers in 2014 described the construction of hydrogels with supramolecular-polymeric networks via a dual-enzymatic reaction. Enzyme acid phosphatase (AP) was used to catalyze the hydrolysis of Fmoc-Tyr(PO₃H₂)OH to produce Fmoc-TyrOH, which assembled to produce supramolecular hydrogels. The supramolecular hydrogels were then cross-linked by polymerization of N,N-dimethacrylamide mediated by glucose oxidase (GOX)-catalyzed carbon radical generation. The GOX-catalyzed radical generation system contained N-hydroxyphthalimide (NHPI), GOX, and glucose (0.27 wt %). The hydrogels exhibited a self-healing property and sol–gel transition though they were cross-linked by polymerization. This fact could be attributed to the noncovalent effects of the supramolecular assembly and the π–π stacking between N-hydroxyphthalimide and the nanofibers self-assembled from Fmoc-TyrOH.

Two years after the above-mentioned report, Wei et al. presented the preparation of self-recoverable hybrid hydrogels through a dual-enzymatic polymerization strategy. Different from the previous example, NapFFK-acrylic acid directly self-assembled to form hydrogels (Figure 3Aa,b). Enzyme GOX was selected to catalyze the oxidation of substrate glucose (3.85 mM) to gluconic acid, and the flavin adenine dinucleotide (FAD) cofactor reduced O₂ to H₂O₂. Another enzyme horseradish peroxidase (HRP) was used to catalyze the oxidation of acetyl acetone (AcAc) with H₂O₂, generating AcAc radicals, which could initiate the polymerization of poly(ethylene glycol) methacrylate (PEGMA) with acrylic modified hydrogelators. The cross-linked polymeric hydrogels were formed in this way (Figure 3Ac). The cross-linked hydrogels showed good mechanical properties, which could resist over 80% compression (Figure 3Ad) and could be stretched to about 2.5 times its initial length without collapse (Figure 3Ae). In contrast, the self-assembled hydrogels without cross-linking showed weak mechanical properties. Most importantly, the in situ immobilized enzymes, GOX and HRP, in the hydrogels exhibited superactivity when compared to the free enzymes. This fact was presumably attributed to the synergistic effect of colocalized GOX and HRP minimizing the distances for mass transport between the hydrogels and bulk solutions. In addition, the strong noncovalent interactions between the in situ immobilized enzymes and nanofibers well maintained the stereoconfiguration of the enzymes and made the enzymes have excellent reusability and thermal stability.
This method employing the dual-enzymatic reaction for two-step (self-assembly and polymerization) hydrogel formation looks complicated, but we believe that there are both obstacles and opportunities in the mediation of this issue. Reasonable design not only can improve the enzyme activities as mentioned above but also holds promise for the improvement in other aspects (e.g., the integration of multiple functions in one system).

2.2. Enzyme-Mediated Reversible Covalent Bonds for the Fabrication of Healable Polymeric Hydrogels.

Reversible covalent bonds, such as imide, acylhydrazone, disulﬁde, and borate ester bonds, that can be cleaved under stimuli and subsequently reformed, have been intensively studied to endow synthetic materials with an intrinsic self-healing ability.66,67,68 The earliest study employing reversible covalent bonds to build intrinsic self-healing materials was reported by Wudl and co-workers in 2002, in whose study thermally remendable cross-linked polymer materials were synthesized based on the dynamic Diels–Alder reaction.13 However, enzymatic reactions cannot alter temperature by a large margin, and therefore, the Diels–Alder reaction may not be a good candidate as the reversible covalent bond for enzymatic fabrication of self-healing hydrogels. By comparison, the dynamic covalent bonds capable of being cleaved and reformed at room temperature are promising for building healable hydrogels via the enzyme-induced strategy. The imine bond is one of the most useful reversible covalent bonds that have been applied in intrinsic self-healing materials.58,68,69 The Schiff base reaction is also available for enzymatic fabrication of healable polymeric hydrogels. Enzyme monoamine oxidase B (MAO B) was used by Wei et al. in 2017 to deaminate the benzylamine-difunctionalized poly(ethylene glycol) (PEG-BA) to produce the corresponding aldehyde product (PEG-AL), which became useful for cross-linking the amine-containing macromolecules such as glycol chitosan or gelatin to form dynamic hydrogels.68 Another enzyme catalase (CAT) was also employed to decompose the byproduct H$_2$O$_2$ from the previous MAO B-catalyzed reaction. The reversible imide bonds produced by enzymatic reactions endowed the hydrogels with an excellent healing ability and multiresponsibility.

In addition to the construction of healable hydrogels, enzymatic reactions are useful to reinforce the hydrogels through the formation of reversible covalent bonds. Xu and co-workers in 2017 presented a dual-network ε-poly-L-lysine (EPL)-based hydrogel system.69 The first network of the hydrogel was produced by radical polymerization of 1-vinyl-2-pyrrolidinone (NVP) and N-methylol acrylamide (NMA) in the presence of EPL (Figure 3Ba). As shown in Figure 3Bb, this single network hydrogel system was presented to demonstrate the dual-network self-healing ability of the EPL-g-poly(NVP-co-NMA) hydrogel.
Enzymatically regulated self-healing is a common way for living organisms to repair damage and recover functions.1,3,4 There are two characteristics for enzyme-regulated self-healing in living organisms: (i) The living organisms can only obtain a transient healing ability when needed (e.g., being injured or damaged).5 Most of the time when they are healthy, they will not proliferate unlimitedly nor merge with other living organisms. (ii) Proper nutrition intake is helpful for the self-healing in living organisms.6,7,8 For synthetic materials, enzyme mediation is a biocompatible strategy without energy consuming that can regulate the self-healing of materials at room temperature. The needed mild enzymatic reaction for regulating the healing process can be homogeneously catalyzed with high efficiency and selectivity. In this section, we show some examples of enzymatically regulated self-healing of polymeric hydrogels, and the summary is presented in Table 2.

### 3.1. Improved Self-Healing of Polymeric Hydrogels Mediated by Enzymatic Reaction

To our knowledge, the first study on enzyme-regulated self-healing of synthetic materials was based on a glutaraldehyde-cross-linked bovine serum albumin (BSA) hydrogel system (Figure 4A), which was presented by Liu and co-workers in 2014.9,10 Two enzymes GOX and CAT were prestored in the protein hydrogels. The damaged hydrogels were then brought together with the addition of glucose (0.2 mg) on the breakup (size: 1.0 cm × 0.5 cm). The glucose was oxidized to GL by GOX catalysis, followed by hydrolysis of GL to yield gluconic acid that could decrease the pH of the hydrogel system. The byproduct H₂O₂ generated from the GOX-regulated catalytic reaction could be decomposed into H₂O and O₂ by CAT to avoid the oxidation of imine bonds. The generated O₂ could be reused by GOX-regulated reaction (Figure 4B). With the decrease of pH by gluconic acid, the imine bonds between glutaraldehyde and BSA/GOX/CAT became more active, therefore providing opportunities for healing the damaged protein hydrogels. This enzyme-regulated self-healing can completely repair the structure damage of the hydrogels (Figure 4C) and recover their mechanical property (Figure 4D).

Three years after the above-mentioned study, an enzyme-mediated fast self-healing polymeric hydrogel was produced by Zhang et al. via dynamic aldimine cross-links formed between pillar[5]arene-derivative and benzaldehydes-functionalized PEG (DF-PEG).11 Similar to the previous study,12 two cooperative enzymes, GOX and CAT, were added into the hydrogel scaffold. When the damaged hydrogel was treated with glucose on the breakup, the GOX-regulated catalytic reaction could decompose the GOX-catalyzed hydrogel into H₂O and O₂ by CAT to avoid the oxidation of imine bonds. The generated O₂ could be reused by GOX-regulated reaction (Figure 4B). With the increase of pH by gluconic acid, the imine bonds between glutaraldehyde and BSA/GOX/CAT became more active, therefore providing opportunities for healing the damaged protein hydrogels. This enzyme-regulated self-healing can completely repair the structure damage of the hydrogels (Figure 4C) and recover their mechanical property (Figure 4D).

### 3. ENZYMATICALLY REGULATED SELF-HEALING OF POLYMERIC HYDROGELS

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Table 2. Overview of the Enzymatically Regulated Self-Healing of Hydrogels

| enzyme | hydrogel composition | healing mechanisms |
|--------|----------------------|--------------------|
| GOX | GOX, CAT | (1) Glucose is oxidized to gluconolactone (GL) by GOX catalysis. GL reacts with PMAA and polymerizes to GPMAA, resulting in the healing. | (1) Glucose is oxidized to gluconolactone (GL) by GOX catalysis. GL reacts with PMAA and polymerizes to GPMAA, resulting in the healing. |
| CAT | benzaldehydes-difunctional PEG (DF-PEG); BSA, glutaraldehyde, hydrolysis decreases pH and heals the hydrogel. (3) The byproduct H2O2 is decomposed by CAT. | (3) The byproduct H2O2 is decomposed by CAT. |
| α-glucosidase, chloroplast | chloroplasts export maltose and glucose. (2) Exported glucose and glucose from enzymatic hydrolysis of maltose are converted to GL by GOX. (3) GL reacts with APMA, and polymerizes to GPMAA, resulting in the healing. | (3) GL reacts with APMA, and polymerizes to GPMAA, resulting in the healing. |
| PAM-co-THAM, ADH | GOX | (1) The coordinated Co2+ is rapidly reduced to Co3+ by ascorbic acid, enabling the healing. (2) After healing, coordinated Co3+ is slowly oxidized to Co2+ by H2O2 generated by GOX-regulated catalytic reaction for property recovery. | (2) After healing, coordinated Co3+ is slowly oxidized to Co2+ by H2O2 generated by GOX-regulated catalytic reaction for property recovery. |

Outlook

Self-healing materials with enhanced longevity and reliability as next-generation materials for the realization of a sustainable society have attracted much attention from scientists, and also have found widespread applications in many fields, such as biosensors, drug delivery systems, and electronic devices. Intrinsic self-healing of materials based on the kinetic lability of chemical bonds or physical interactions is one of the research hotspots in healable and renewable materials science because it allows for multiple local healing events to occur. However, the kinetic lability potentially has a negative effect on the stability of materials, which may lead to undesired fusion of intact materials. Please imagine the following situation: two biosensors or smartphones fuse into one whole when they are accidentally stacked together for minutes. It sounds inconceivable but will become true if the intrinsic self-healing materials are used without solving the compatibility problem between intrinsic healing ability and kinetic stability. Therefore, it is critical to reconcile the contradiction between kinetic stability and intrinsic healing ability of synthetic materials.

Living organisms inherently possess both dynamic stability and a self-healing ability. For example, people will not stick together when they shake hands with one another, but they can automatically repair damages when they are hurt. Wound healing in biological systems is a nutrient intake-promoted, enzyme-assisted, hierarchically and temporally controlled process. Specifically, it follows three sequential steps of (i) inflammatory response, (ii) cell proliferation for defect filling, and (iii) matrix remodeling for the recovery of tissue properties. This process endows the living organisms with a transient healing ability, which is adequate to make them recover from injuries. Harnessing this concept could be the ideal platform to create advanced synthetic materials with both high kinetic stability and an intrinsic healing ability. Learning from the above wound healing process, we recently presented two hydrogel systems that were ordinarily stable but became healable when fed with proper chemical nutrients. The healing was essentially realized via the kinetic control of enzyme-regulated competing processes. The temporally modulated healing of kinetically stable polymer hydrogels was also designed to have three steps of (i) actuation by chemical nutrient supply, (ii)
activation of dynamic bonds or interactions for structural healing, and (iii) deactivation of dynamic bonds or interactions for hydrogel property recovery (Figure 5A). It created a transient high healing ability for thermodynamically stable and kinetically inert hydrogels, which was in an analogous fashion to the way living organisms deal with injuries. Three important factors guide the design of polymer hydrogels with an enzyme-regulated transient healing ability: (i) Stability of hydrogels has priority over healing ability. The hydrogels having thermodynamically stable and kinetically inert cross-links are optimal without fusion and self-healing abilities, but they can obtain a transient healing ability on demand. (ii) A transient healing ability is adequate for defect healing and hydrogel property recovery. In comparison, a permanent self-healing ability based on kinetically labile cross-links may reduce the stability of hydrogels. (iii) Enzymes can homogeneously catalyze the reactions that are needed for defect healing with high efficiency and specificity. Furthermore, most enzymatic reactions have

Figure 4. Improved healing of protein hydrogels mediated by enzymatic reactions. (A) Schematic illustration of the protein hydrogels. (B) Enzymatic reactions to promote the healing of hydrogels. (C) Optical images showing the healing behavior of the hydrogels. (D) Healing results for the systems with dual enzymes and without enzymes. Reprinted with permission from ref 32. Copyright 2014 Wiley-VCH.

Figure 5. Transient healing ability of polymeric hydrogels mediated by enzymatic reactions. (A) Hierarchically and temporally controlled healing processes in biological systems and synthetic materials. (B) Schematic representation of the enzyme-regulated transient healing ability of polymeric hydrogels. The transient healing ability of damaged hydrogels is temporally programmed by combining a fast activator with the slow enzymatic generation of a deactivator. (C) Coordination interactions between cobalt ions and histidine-containing polymers. (D) Optical images of GOX-containing, Co2+- and Co3+-cross-linked polymer networks. (E) Healing efficiency of the damaged hydrogels treated with glucose-containing ascorbic acid and ascorbic acid solutions. (F) Optical images showing the healing of hydrogels by treatment with different chemical nutrients. Reprinted with permission from refs 73 and 74. Copyright 2020 American Chemical Society.
fine biological compatibility because a majority of the chemical reactions in living organisms are catalyzed by enzymes. Meanwhile, it does not need to consume fossil fuels during the reactions. Therefore, the enzymatic strategy to trigger the healing of hydrogels is a clean and biocompatible method without energy consumption. However, the long-term stability of enzymes is a concern due to their fragile nature. In addition, the toxic byproducts of some enzymatic reactions make the hydrogel systems have poor biocompatibility. These critical concerns will be emphatic aspects for further studies.

One of our systems was a urease-containing, acylhydrazone-based polymer hydrogel. The hydrogels were rapidly formed by cross-linking of acrylamide-co-diacetone acrylamide polymers with adipic acid dihydrazide at low initial pH. The acylhydrazone bonds had fast formation and exchange rates at low pH, resulting in a hydrogel with a self-healing ability. However, the hydrogels at low pH crept under ambient temperature due to the rapid bond exchange, and undesired fusion of intact hydrogels would occur. The acylhydrazone bonds at pH above 7 were kinetically locked, so that the prepared hydrogels at high pH were kinetically inert and had no self-healing ability. Guo et al. investigated the stability of the hydrogels based on acylhydrazone bonds through the degradation experiments under different pH. When compared to the fast degradation at pH 2.5, the hydrogels had a much slower degradation rate at pH 6. Furthermore, the hydrogels had the slowest degradation rate at pH 7.4, and no significant degradation was found for the hydrogels with high cross-linking densities, implying that the acylhydrazone bonds are kinetically inert under natural conditions. We fabricated kinetically stable hydrogels without creep deformation by increasing the pH of the hydrogels to 7 and above via enzymatic generation of ammonia, wherein the enzyme urease and the substrate urea were prestored in the hydrogels. When the hydrogel was damaged, we smeared urea-containing acidic buffer on the fracture surface. The dynamic acylhydrazone bonds on the fracture surface could be activated by the rapidly decreased pH due to the acidic buffer addition. At the same time, the hydrogels obtained a transient healing ability to repair their structures and mechanical properties. After that, the local

Figure 6. Enzymatic degradation of healable polymeric hydrogels. (A) Schematic illustration of the mass loss owing to the combined erosion and degradation process. Cumulative erosion profiles of (B, C) PAd-HA and (D, E) NAd-HA hydrogels in either (B, D) Type II collagenase or (C, E) MMP-2 at concentrations noted. (F, G) Time course images of near-infrared fluorescence imaging of (F) PAd-HA and (G) NAd-HA hydrogels after injection subcutaneously in the right flank of mice. (H) Quantification of signal intensity in parts F and G. Reprinted with permission from ref 90. Copyright 2015 American Chemical Society.
pH recovered completely by the slow enzymatic generation of ammonia. The enzyme-regulated competing factors composed of a fast acidic activator and a slowly generated basic deactivator were not only efficient for structural healing but also able to completely recover the hydrogel properties, especially the kinetic stability.

Another system was a GOX-containing, Co\textsuperscript{3+}-cross-linked polymer hydrogel (Figure 5C).\textsuperscript{74} The kinetically inert and highly stable hydrogels without self-healing abilities based on metal–ligand interactions were fabricated by oxidation of Co\textsuperscript{2+}-cross-linked polymer networks. The Co\textsuperscript{3+}-cross-linked polymer networks could be switched to yellow viscoelastic liquids (Figure 5D), which had high ligand exchange rates and self-healing abilities, through reducing Co\textsuperscript{3+} to Co\textsuperscript{2+} by ascorbic acid. Based on this phenomenon, the damaged hydrogel could acquire a transient healing ability mediated by kinetic control of competing chemical reactions. To repair the damaged hydrogel, we smeared glucose-containing ascorbic acid solution (53.2 mM glucose and 4 mM ascorbic acid) on the fracture surface. The ascorbic acid reduced the coordinated Co\textsuperscript{3+} to Co\textsuperscript{2+} rapidly to endow the fracture area with high mobility and healing ability. Then, coordinated Co\textsuperscript{2+} was slowly oxidized to Co\textsuperscript{3+} by H\textsubscript{2}O\textsubscript{2} generated from the GOX-regulated catalytic reaction to recover the properties of the Co\textsuperscript{3+}-cross-linked hydrogel. The structure, mechanical property, and kinetic stability could be recovered (Figure 5E,F) by this kind of enzyme-regulated competing chemical reaction. Moreover, multiple local healing events were possible in such enzyme-containing hydrogel systems due to the excess active enzymes immobilized in the hydrogels, implying good stability of the enzyme-containing hydrogels. These two studies showed effectiveness to reconcile the contradiction between the kinetic stability and intrinsic healing ability of synthetic materials, and the enzyme mediation strategy used in these studies was expected to be a broadly applicable approach to make a variety of kinetically stable materials self-healable.

4. ENZYMATIC DEGRADATION OF HEALABLE POLYMERIC HYDROGELS

The enzymatic degradation is important for the implanted or injected materials to be autonomously cleared in a noninvasive manner.\textsuperscript{90,91} The enzymatic degradation of hydrogels is an inverse process of enzymatic fabrication. Rational integration of enzymatic fabrication and degradation processes can adjust the mechanical properties of hydrogels and manipulate the sol–gel transitions, which have already been realized in the peptide-, DNA-, or polymer-based systems.\textsuperscript{92–94} Here, we

Figure 7. Applications of the enzyme-regulated healable polymeric hydrogels. (A) 3D cell printing. (a) Circular grids fabricated by 3D printing. (b) 3D stack of printed cells in the hydrogel stained with FDA and PI. Scale bar is 300 mm. (c, d) Hemostatic effect of the hybrid hydrogel (H Gel). (B) Antibacterial ability. (a) Photographs of the skin defects treated with the EDHs. (b) Images of the wounds treated with PBS, gauze, and EDHs at different days postsurgery, and percentages of wound closure up to 14 days post-transplantation. (c) Images of hematoxylin and eosin (H&E), Masson’s trichrome, and immunohistochemically stained histological sections at 14 days post-transplantation. The black arrows indicate newly formed blood vessels; the dotted black box indicates wavy collagen fibers, and the red arrow indicates the inflammatory factor. (C) Structural coloration. Scanning electron microscopy (SEM) images of (a) template, (b) inverse opal scaffold, and (c) hydrogel surface. Scale bars are 500 nm. (d) Optical images (upper row) and absolute reflection spectra (lower row) of 6 kinds of hydrogel microfibers. Photographs showing the healing of (e) 2 segments of the structural color hydrogel microfiber, and (f) 3 different structural color hydrogel segments. Scale bar is 2 mm. Reprinted with permission.\textsuperscript{66,69,72} Copyright Royal Society of Chemistry, Wiley-VCH, and National Academy of Sciences.
show some recent examples of enzymatic degradation of self-healing polymeric hydrogels.

An injectable and cohesive hydrogel based on reversible bonds between calcium phosphate nanoparticles and bisphosphonate-functionalized hyaluronic acid (HA) was developed by Nejadnik et al. in 2014. The noncovalently cross-linked hydrogels showed a self-healing capacity and strong adhesiveness to mineral surfaces such as enamel and hydroxyapatite. Moreover, the organic–inorganic hybrid hydrogels were surprisingly robust yet biodegradable upon the treatment of enzyme hyaluronidase. These properties of the hydrogels made them potentially useful for bone regenerative applications.

A year after the above-mentioned report, Burdick and co-workers described the fabrication of shear-thinning and self-healing hydrogels through the guest–host interactions between the HAs separately modified by adamantane (Ad) or cyclodextrin (CD). The positively and negatively charged peptide linkers were used in between the HA backbone and the conjugated guest Ads to yield PAD-HA and NAD-HA polymers, respectively. These polymers could form hydrogels by interacting with CD-HA, and the selective proteolytic degradation was studied by employing two enzymes of Type II collagenase and matrix metalloproteinase-2 (MMP-2). The hydrogels containing PAD-HA or NAD-HA had no selectivity for Type II collagenase degradation. The PAD-HA-based hydrogels were designed to allow MMP-2 degradation, while NAD-HA-based hydrogels were designed to limit specific MMP-2 degradation. As shown in Figure 6A, the hydrogel degradation is mediated by the interplay of two distinct mechanisms: On one hand, the guest–host cross-links underwent continuous (i) association and (ii) dissociation processes, which contributed to hydrogel degradation. On the other hand, (iii) the proteolysis of peptide cross-links contributed to hydrogel degradation, which might be hindered by (iv) selective peptide modification. As a result, both PAD and NAD peptides underwent proteolysis and exhibited dose-dependent degradation rates when treated with Type II collagenase (Figure 6B,D). For the MMP-2 degradable sequence, PAD, the enzymatic degradation was observed in response to MMP-2 (Figure 6C). In contrast, no MMP-2-regulated degradation was observed when NAD was used as the cross-linker (Figure 6E). Furthermore, the in vitro degradation behavior is in good agreement with the in vitro proteolysis and degradation results (Figure 6F–H). All of these results demonstrated that the degradation of self-healing polymeric hydrogels could be precisely controlled by rational design of the macromolecular building blocks and the cross-linking interactions, and also by the reasonable selection of enzymes to trigger the hydrogel degradation.

In addition to the protease-mediated degradation of peptides, the nuclease-regulated degradation of DNA can be used for enzymatic degradation of self-healing DNA hydrogels. A supramolecular polypeptide–DNA hydrogel was prepared by Li et al. in 2015. The hydrogels possessed self-healing properties and high mechanical strengths, allowing for 3D bioprinting. Owing to the supramolecularly cross-linked polypeptide–DNA network structures, the hydrogels could be degraded in response to both protease and nuclease. Such biocompatible and biodegradable hydrogels are promising to serve as printable biomaterials for tissue engineering.

5. APPLICATIONS OF ENZYME-REGULATED HEALABLE POLYMERIC HYDROGELS

Currently, most studies of enzyme-regulated healable hydrogels focus on the material fabrication and mechanism investigation. The applications of such materials are mainly limited to the following three aspects: 3D cell printing, antibacterial function, and structural coloration (Figure 7).

In this section, we describe some examples of the enzyme-regulated healable polymeric hydrogels applied in the above-mentioned three aspects.

5.1. Enzyme-Mediated Healable Polymeric Hydrogels for 3D Cell Printing

Hydrogels provide suitable microenvironments for cells, which also enable the diffusion of nutrients, oxygen, and other functional molecules. Physical hydrogels made from proteins, polysaccharides, or self-assembled gelators are suitable materials for 3D printing due to their shear-thinning properties. Hydrogels based on reversible covalent bonds or supramolecular interactions are also promising to serve as printing materials for biomedical applications due to their self-healability at physiological conditions and multiresponsibility.

A bioprintable supramolecular polymeric hydrogel was fabricated via dual-enzyme catalysis by Wang and co-workers in 2016. The prehydrogel solution was prepared by mixing the dual-enzymatic system with a solution of guanidinium-containing hydrogelators (NapFFRK-acryloyl), wherein the dual-enzymatic system included GOX, HRP, glucose, AcAc, and PEGMA. Gluconic acid was first generated by GOX-catalyzed oxidation of glucose. The generated gluconic acid decreased the system pH and triggered the self-assembly of hydrogelators to form supramolecular hydrogels. Meanwhile, the FAD cofactor reduced O2 to H2O2, followed by the oxidation of AcAc via the HRP-catalyzed reaction with the aid of generated H2O2 to produce AcAc radicals, which subsequently initiated the polymerization of PEGMA with hydrogelators to form the supramolecular-polymeric hydrogels. The prehydrogel exhibited self-healing properties due to the π–π stacking and hydrogen bonding interactions. The prehydrogel solution was used to 3D print grids on a printer at the programmed position (Figure 7Aa). For 3D cell printing, cells stained with fluorescein diacetate (FDA) and propidium iodide (PI) were encapsulated in the prehydrogel, followed by printing the cell-containing prehydrogel solution into constructs. Figure 7Ab shows a 3D stack of printed cells in the hydrogel with a cell viability of approximately 98.0%, suggesting good biocompatibility of the hydrogel. The substrate glucose that was critical for hydrogel formation could be entirely provided by the blood glucose, and therefore, blood hybrid hydrogels could be simply fabricated by mixing blood with the prehydrogel solution without glucose. The guanidinium-containing hydrogel could also be used as...
hemostatic materials. As shown in Figure 7Ac,d, a hybrid hydrogel with a dark red color was rapidly formed within 110 s at the bleeding site in a rat model. In contrast, the negative control without any treatment was still bleeding at 110 s. In addition, the groups treated with the prehydrogel without enzymes or the prehydrogel without guanidiniums did not show improved hemostatic ability when compared to the negative control group. These results testified that the guanidinium-containing, enzyme-regulated healable hydrogels are promising printable hemostatic materials.

5.2. Enzyme-Mediated Healable Polymeric Hydrogels for Antibacterial Applications. In addition to the applications of 3D cell printing and hemostasis, enzyme-regulated healable hydrogels can inherit the intrinsic functions (e.g., antibacterial property) of the polymers used for hydrogel preparation. For instance, the previously introduced dual-network EPL-based hydrogels69 (Figure 3B) were reported to have broad-spectrum antimicrobial activity against both Gram-negative (e.g., *Escherichia coli*) and Gram-positive (e.g., *Staphylococcus aureus*) bacteria due to the presence of functional EPL in the hydrogels. Because of the antibacterial ability and excellent biocompatibility, the enzyme-assisted dual-network self-healing hydrogels (EDHs) could be used as dressings for in vivo skin wound healing (Figure 7Ba). The wound closure macroscopic images and quantification analyses in Figure 7Bb indicated that the growth of new epidermis extended to the wound centers, leading to a reduced wound area and depth in the treatment groups, and the wounds treated with the EDHs had significantly (*p < 0.05) faster contraction rates than the control groups. Moreover, more new blood vessels and extensive collagen deposition were observed in the EDH-treated group when compared to the control groups (Figure 7Bc). All of these results testified that the EPL-containing enzyme-regulated healable hydrogels had the ability to accelerate wound healing, which was because of the suitable environment for skin regeneration, as well as the excellent antimicrobial function and biocompatibility of the hydrogels. The dual-network EPL-containing enzyme-regulated hydrogels with high self-healing efficiency and robust mechanical strength are promising to be useful in the fields of artificial muscles and tissue engineering.

In addition to the enzyme-regulated antibacterial hydrogels, the hydrogels with enzyme-like activity can also be used for antimicrobial applications. For instance, the hydrogel with peroxidase-like activity developed by Qu and colleagues showed broad-spectrum antimicrobial activity against both Gram-positive bacteria and Gram-negative bacteria due to the reactive oxygen species (ROS) formed via the transformation of H2O2, which was mediated by the hydrogel-based artificial enzyme.106 Meanwhile, copper ions in the hydrogels could accelerate the wound healing process. The novel artificial enzyme-based antibacterial hydrogels are promising to be used for tissue engineering.

5.3. Enzyme-Mediated Healable Structural Color Hydrogels. Structural coloration originates from intrinsic periodic nanostructures, which interact with light to produce brilliant colors.72,107 This can be seen everywhere in nature; for example, the brilliant colors of hummingbirds, butterflies, and beetles are typical structural colors.108,109 The structural color is important for creatures to realize their biological functions.
such as communication, and shielding. Learning from nature, synthetic materials with structural colorations, including structural color hydrogels, have been developed for the potential applications such as switches, \textsuperscript{110,111} sensors, \textsuperscript{112,113} optical devices, \textsuperscript{14,115} and wearable electronics. \textsuperscript{116} However, such materials may accumulate damages during usage. Another bioinspired function, enzyme-regulated self-healing, can be useful to repair the structural defects and to recover the materials' structural colors.

Zhao and colleagues in 2017 developed self-healing structural color hydrogels by adding a GOX- and CAT-containing glutaraldehyde-cross-linked BSA hydrogel into methacrylated gelatin (GelMA) inverse opal scaffolds.\textsuperscript{72} The GelMA hydrogel inverse opal scaffolds were prepared by replicating silica colloidal crystal templates, which were fabricated by the self-assembly of silica nanoparticles in silica capillaries or on glass slides to yield the closely packed and ordered structures after dehydration (Figure 7Ca). The GelMA prehydrogel solutions were filled into the voids of the templates, followed by UV-induced polymerization to produce a hydrogel. The inverse GelMA hydrogel scaffolds were then obtained by etching the silica nanoparticles (Figure 7Cb). The glutaraldehyde-cross-linked BSA hydrogels with GOX and CAT were filled into the inverse opal scaffold to endow the structural color hydrogels with a self-healing ability, and finally, the hybrid hydrogels with both structural colorations and enzyme-regulated healing ability were fabricated (Figure 7Cc). Furthermore, the structural colors and different diffraction peaks could be precisely adjusted by using different sizes of silica nanoparticles for the colloidal crystal templates (Figure 7Cd). Notably, the enzymatic strategy could enable the healing of microfiber segments with identical (Figure 7Ce) or different (Figure 7Cf) structural colors, which warranted the construction of multiplex structural color hydrogels.

5.4. Future Applications. In addition to the above-mentioned three applications, the enzyme-regulated healable polymer hydrogels are also potentially useful in many other fields when combining with functional guest materials (Figure 8). On one hand, nanomaterials such as quantum dots,\textsuperscript{117} metal nanoparticles,\textsuperscript{1,18,119} carbon nanotubes,\textsuperscript{120} and graphene\textsuperscript{122} show excellent optical, electronic, magnetic, thermal, mechanical, and other properties. These nanomaterials also have good compatibility with polymers and can be introduced into polymer hydrogels to improve their fluorescence performance, mechanical toughness, and electrical conductivity. The nanomaterial-doped, enzyme-regulated healable polymeric hydrogels have potential applications in the fields of remote sensing, electronics, and energy. In addition to nanomaterials, conductive polymers, a kind of intelligent materials, which have responsiveness to external stimuli can also be used to construct enzyme-regulated healable polymeric hydrogels. Doping organic conductors into the enzyme-regulated healable polymeric hydrogels can endow the hydrogels with more new functions. Moreover, the reactions between enzymes and the target substrates could create detectable electrochemical signals. In this case, the enzyme-regulated healable polymeric hydrogels containing conductive polymers which have the advantages of both conductors and enzyme-regulated healable polymeric hydrogels have a wide range of applications in advanced biosensors which have high intrinsic selectivity.\textsuperscript{121} In addition, the organic conductor-doped, enzyme-regulated healable polymeric hydrogels have potential applications in the fields of energy storage/conversion,\textsuperscript{122,123} and solar water purification.\textsuperscript{124,125} However, for the hydrogels used in these fields, the stability/durability of the enzymes in the hydrogels is a major concern. On the other hand, enzymes are biomacromolecules which have good biocompatibility, and many chemical reactions in living organisms are catalyzed by enzymes. The high specificity of enzymes makes them respond to some specific components in living organisms. Based on this, the enzyme-regulated healable polymeric hydrogels can incorporate drugs and cells for the applications of therapeutic delivery and tissue engineering. For example, GOX-containing, healable hydrogels can respond to glucose in the blood to change the local pH and release H$_2$O$_2$, so they are promising for controlling the release of insulin. Meanwhile, the self-healing ability of hydrogels can increase their service life and reliability. In this case, the biocompatibility of the polymers for hydrogel construction and the products of enzymatic reactions become a major issue. For example, the urease-regulated reaction that generates toxic ammonia$^5$ should be insulated from the biomedical applications.

6. CONCLUSIONS AND OUTLOOK

In this Outlook, we have summarized the recent advances in the field of enzyme-regulated healable polymeric hydrogels. We have especially discussed the enzymatic fabrication of healable hydrogels and enzymatically regulated healing of hydrogels. In the studies on enzymatic fabrication of healable polymeric hydrogels, enzymatic reactions mainly play the roles of triggering the self-assembly,\textsuperscript{68,69} polymerization,\textsuperscript{50,66,67,70} and formation of reversible covalent bond,\textsuperscript{68,69} in hydrogels. The dual-enzymatic self-assembly and polymerization is a valid strategy developed by Wang and co-workers for the preparation of self-healing polymeric hydrogels, and the resulting hydrogels have found applications in 3D cell printing and hemostasis.\textsuperscript{60,66,67} The dynamic imide bond is one of the most frequently used reversible covalent bonds for the enzymatic formation of healable hydrogels, wherein amine oxidases are employed to partially oxidize the amine-containing macromolecules to the corresponding aldehyde products, therefore allowing the occurrence of the Schiff base reactions.\textsuperscript{68,69} Single-network and dual-network healable polymeric hydrogels have been fabricated in this way for the applications of 3D printing and skin wound healing.\textsuperscript{68,69} In the studies on enzymatically regulated self-healing of hydrogels, GOX is the most frequently used enzyme because the GOX-regulated catalytic reaction can generate both glucose acid and H$_2$O$_2$.\textsuperscript{32,71,72,74} On one hand, the glucose acid can decrease the system pH and facilitate the healing of hydrogels by activating the dynamic imide or aldime bonds.\textsuperscript{32,71,72} On the other hand, the oxidizing agent H$_2$O$_2$ can trigger the oxidation state change of metal ions, therefore facilitating the self-healing or property recovery in metal cross-linked polymer networks.\textsuperscript{74} Most importantly, the enzymatic strategy can impart a transient healing ability to hydrogels, which makes it feasible to heal the defects of thermodynamically stable and kinetically inert materials.\textsuperscript{73,74}

Despite these achievements, three problems currently exist in the field of enzyme-regulated healable polymeric hydrogels: (i) The reversible interactions and enzymatic reactions that have been used for hydrogel fabrication and self-healing are very limited. Enzymatic reactions and reversible interactions are inherently a pair of closely connected actors for developing self-healing materials and regulating healing processes because
enzyme-mediated environmental change can act as an effective tool to control the reversible interactions. Rational integration of different types of reversible interactions and enzymatic reactions can result in various advanced healable materials. However, it is worth noting that the unreasonable combination of functional groups and enzymes may lead to the loss of enzyme activity. For example, the catechol groups significantly inhibit urease activity by a radical-based autocatalytic multistep mechanism.126,127 (ii) Most current enzyme-regulated healable polymeric hydrogels are constructed based on kinetically labile bonds or interactions, and therefore, the poor kinetic stability/inertness is a major concern in these systems, which may lead to noticeable shape change, creep, and unwanted fusion of intact materials. Our study on the self-regulated transient healing ability of materials 25,74 may illuminate an entirely new avenue for the development of advanced materials possessing both high kinetic stability/inertness and an excellent intrinsic healing ability. (iii) The fragile nature of bioactive enzymes may limit the further practical application of the enzyme-containing polymeric hydrogels. The enzym mimetics119,128,129 or other environmentally friendly man-made catalytic agents can be the adequate substitutes of bioactive enzymes, not only because of their high catalytic activities but also due to their high tolerance to harsh conditions such as organic solvents, high salt concentrations, drying, and high or low temperature. In addition to the catalytic reactions, synthetic reaction cycles 130−132 are also promising to contribute to the regulation of healable polymeric hydrogels. Future work in this field may focus on solving the above-mentioned three problems. In addition, learning from the extrinsic self-healing materials,11 the chemical nutrients including enzymes and/or substrates can be prestored in microcapsules or hollow fibers, which can be further embedded into the polymer materials containing reversible interactions. When the materials are damaged, the chemical nutrients can be released from the cracked microcapsules and trigger the defect healing. In this way, a spontaneous self-healing can be realized.

Overall, enzyme catalysis provides a powerful tool to control the surrounding physicochemical environments, which enables the efficient fabrication and degradation of polymeric hydrogels, as well as the healing of structural defects and recovery of hydrogel functions. The enzyme-regulated healable polymeric hydrogels are expected to be a new generation of biomaterials useful in many fields.

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Notes

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