Recent Studies on Hydrogels Based on H$_2$O$_2$-Responsive Moieties: Mechanism, Preparation and Application

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Abstract: H$_2$O$_2$ is essential for cellular processes and plays a vital role in the regulation of cell signaling pathways, which can be viewed as a warning signal for many kinds of disease including cancer, cardiovascular disease, reproductive abnormalities, diabetes, and renal failure. A H$_2$O$_2$-responsive hydrogel (H$_2$O$_2$-Gel) is a promising candidate for biomedical applications because of its good biocompatibility, similarity to soft biological tissues, ease of preparation, and its ability to respond to H$_2$O$_2$. In this study, the H$_2$O$_2$-responsive moieties used to fabricate H$_2$O$_2$-Gels were reviewed, including thioethers, disulfide bonds, selenides, diselenium bonds, diketones, boronic, and others. Next, the preparation method of H$_2$O$_2$-Gel was divided into two major categories according to their reaction mechanisms: either self-crosslinking or mechanisms entailing the addition of difunctional crosslinkers. Last, the applications of H$_2$O$_2$-Gels were emphasized, which have been viewed as desirable candidates in the fields of drug delivery, the detection of H$_2$O$_2$, glucose-responsive systems, ROS scavengers, tissue engineering, and cell-encapsulation.

Keywords: stimuli-responsive; drug delivery; halloysite nanotube; biomedical; self-crosslinking; difunctional crosslinkers

1. Introduction

In recent years, relevant researchers have developed a variety of stimulation mechanisms by investigating the changes in the microenvironment of the human body [1–3]. Following this research, quite a number of stimuli-responsive materials have been developed with sensitivity to acidity [4], alkali [5], temperature [6], mechanical force [7], magnetic fields [8], ultrasound [9], reactive oxygen species (ROS) [10], etc. ROS are emerging as critical signaling molecules which are abundant in the human microenvironment [11]. The term ROS encompasses a wide range of molecules, such as hydrogen peroxide (H$_2$O$_2$), singlet oxygen (¹O$_2$), hydroxyl radical, superoxide, etc. [12]. The stable control of reactive oxygen species level is of great significance to maintain the normal physiological activities of human bodies [13]. The overexpression of reactive oxygen species can easily destroy the structure of many biological macromolecules, such as proteins and nucleic acids, and thus result in a variety of diseases [14]. Therefore, it is of great theoretical and practical significance to design and develop smart materials with ROS responsive behaviors.

H$_2$O$_2$ is a representative case of ROS that is particularly important in the physiological regulation in organisms [15]. It plays an important role in cell signal transduction, differentiation, and proliferation, and also plays an irreplaceable role in the diagnosis and treatment of many diseases [16]. Especially in diseases related to oxidative stress, a change in the H$_2$O$_2$ level is a good indication of the location of the lesion site [17]. When oxidative stress and injury occur in the body, the concentration of H$_2$O$_2$ is higher than the normal level [18]. Therefore, the over expression of H$_2$O$_2$ in vivo is directly related to inflammation, bleeding, Alzheimer’s disease, diabetes, renal function decline or tumors, and other
diseases (Scheme 1) [19]. For example, the H$_2$O$_2$ level of tumor tissue is significantly higher than that of normal tissue. The H$_2$O$_2$ concentration in normal tissue usually maintains about $10^{-8}$ M, while the H$_2$O$_2$ concentration in inflammatory tissue or tumors is about dozens or even thousands of times higher than that in normal tissue [16]. Therefore, H$_2$O$_2$ is more suitable to serve as a pathological marker than pH, temperature, ionic charge, etc. The concentration of H$_2$O$_2$ is also overexpressed during the proliferation and metastasis of tumor cells. Therefore, the overexpression of H$_2$O$_2$ can be used to monitor changes in the microenvironment. The development of H$_2$O$_2$-responsive materials with high specificity, sensitivity, and accuracy is a meaningful and interesting topic, which may exhibit much more promising applications for early diagnosis than other stimuli responsive materials such as those that respond to pH, temperature, ionic charge, etc.

![Scheme 1](image-url)

**Scheme 1.** Relation between the over expression of H$_2$O$_2$ and related diseases.

Until now, different types of H$_2$O$_2$-responsive materials have been explored, including MRI contrast agents, fluorescent sensors, transistors, drug delivery systems, mitochondrial-directed tools, etc. [20,21]. Gu et al. published a review paper on the topic of H$_2$O$_2$-responsive materials, in which the biomedical applications were emphasized [17]. It is worth noting that hydrogels are widely used in the fields of drug delivery, tissue engineering, surgical dressing, etc. [22–25]. As a result, hydrogel based on H$_2$O$_2$-responsive moieties is a major kind of H$_2$O$_2$-responsive material [26,27] which can be defined as H$_2$O$_2$-responsive hydrogel (H$_2$O$_2$-Gel). There is a great deal of significance in performing a review of the development of H$_2$O$_2$-Gel and in the explication of the guidelines for designing suitable formations, which is also our interest in this study. Hydrogels are three-dimensional (3D) cross-linked polymer networks, which can absorb and retain large amounts of water [28]. Due to their flexible structure, stimuli-responsive hydrogels can perform work by converting an external stimulation into mechanical motions or chemical conversions, which can be an effective framework for soft actuators. In this review, we focus on overviewing the responsive mechanism of H$_2$O$_2$-responsive moieties and the applications of H$_2$O$_2$-Gel, especially in biomedicine. The preparation methods are also summarized. We expect to provide guidance and designate the direction of further studies on H$_2$O$_2$-responsive materials.
2. H$_2$O$_2$-Responsive Moieties

2.1. Thioether (–S–)

Thioether can be regarded as a compound analogous to ether in which the oxygen is replaced by sulfur. Thioether is commonly synthesized from potassium or sodium sulfide and halohydrocarbon and is widely distributed in proteins [29]. Benefiting from the higher atomic number of sulfur compared to oxygen, thioether usually shows higher chemical activity than ether and can be transferred into sulfoxide and sulfone with the treatment of H$_2$O$_2$ (Table 1) [30].

**Table 1. Summary of responsive mechanism to H$_2$O$_2$.**

| No. | Mechanism | Literature |
|-----|-----------|------------|
| 1   | $R'\cdot S'\cdot R' \xrightarrow{H_2O_2} R'\cdot S\cdot R' \xrightarrow{H_2O_2} R'\cdot S\cdot R'$ | [31,32] |
| 2   | $R'\cdot S\cdot S\cdot R' \xrightarrow{H_2O_2} R'\cdot S\cdot SH + R'\cdot S\cdot SH$ | [33,34] |
| 3   | $R'\cdot Se\cdot R' \xrightarrow{H_2O_2} R'\cdot Se\cdot R'$ | [35] |
| 4   | $R'\cdot Se\cdot Se\cdot R' \xrightarrow{H_2O_2} R'\cdot Se\cdot OH + R'\cdot Se\cdot OH$ | [36] |
|     | $Ar\cdot B\cdot OH \xrightarrow{H_2O_2} Ar\cdot OH + HO\cdot B\cdot OH$ | [37] |
| 6   | $\text{[Diagram]} \xrightarrow{H_2O_2} \text{[Diagram]} + R\cdot OH$ | [38] |
| 7   | $\text{[Diagram]} \xrightarrow{H_2O_2} \text{[Diagram]} + \text{[Diagram]}$ | [39,40] |

Napoli et al. investigated the mechanism for the oxidative destabilization of poly(propylene sulfide) [31]. Even at concentrations as low as 0.03 vol.% H$_2$O$_2$, the transformation from thioether to sulfoxide or sulfone can be achieved. The NMR shifts of the methyl group in the $\alpha$ and $\beta$ position to sulfur were examined in detail to reveal the changes. After treating the material with H$_2$O$_2$, a doublet at ~1.53 ppm apparated corresponding to the methyl in $\beta$ position to $-SO_{\alpha}$ moieties. Moreover, a high-frequency shift of the peaks corresponding to the protons in the $\alpha$ position to the $-S-$ ($2.65$ and $2.93$ ppm) was also observed. These phenomena indicate that both the sulfoxide and sulphone moieties are included in the backbone of H$_2$O$_2$-treated poly(propylene sulfide). Kramer and Deming
further demonstrated the H$_2$O$_2$-sensitive mechanism of thioether, in which the thioether moiety in a pyranose derivative can be oxidized into sulphone-containing product with a high yield [32].

2.2. Disulfide Bond (-S-S-)

Disulfide bonds are commonly included in a variety of proteins, which can be generated from the oxidation of thiol groups. An S anion from one sulfhydryl group can act as a nucleophile and attack another sulfhydryl moiety to create a disulfide bond. Particularly, H$_2$O$_2$ can be used to break the S-S bond and yield corresponding thiolated compounds (Table 1). Due to the high activity of sulfhydryl groups, the degraded thiolated compounds have the capacity of forming the original disulfide-bearing substance. Therefore, the disulfide-based H$_2$O$_2$-responsive behavior can be regarded as a reversible process [33].

2.3. Selenide (-Se-)

Selenium (Se) is a nonmetallic element in the oxygen family that mainly exists in several allotropic forms. It is widely spread in volcanic areas and sulfide ores, which can be used in photocells, solar cells, and in xerography. Selenium can be bound into proteins and serves as an indispensable microelement in humans. Its chemical properties are similar to sulfur, but with a higher reactivity. As a result, the H$_2$O$_2$-treated selenide derivatives are usually transferred into the selenone form (Table 1).

Zhang’s group illustrated the structural change with the oxidation of a selenide block copolymer with H$_2$O$_2$ at 0.1%, and the oxidized residue was analyzed by XPS. The binding energy of Se 3d’s shift from 55.9 eV to 59.5 eV was clearly observed, suggesting the higher valency of selenium which greatly matches the formation of selenone groups. The presence of O=Se=O groups can also be demonstrated based on the characteristic absorption bands at 904 and 880 cm$^{-1}$. $^1$H-NMR and $^{77}$Se-NMR further confirmed the transformation from -Se- to O=Se=O groups, which shows a H$_2$O$_2$-responsive behavior [35].

2.4. Diselenium Bond (-Se-Se-)

A diselenium bond can be regarded as a linkage containing two atoms of selenium combined with an element or radical. Due to the similar chemical properties of selenium and sulfur, a diselenium bond can also be broken with treatment from H$_2$O$_2$. The diselenide bond has an energy of around 176 kJ/mol, indicating its easy oxidization in the presence of H$_2$O$_2$ while undergoing a phase transition from hydrophobic diselenide to hydrophilic selenic acid (Table 1) [41]. An attractive advantage of diselenide and disulfide bonds is their hydrolytic stability in physiological conditions [36]. Moreover, the diselenid-based H$_2$O$_2$-responsive behavior can also be regarded as a reversible process. The degraded hydrophilic selenic acid can rebuild the diselenium bond under ambient atmosphere.

Diselenium derivatives can be prepared by the oxidation of aryl selenols [42]. However, as aryl selenols are most conveniently prepared from the diselenides themselves, this method is not likely to have preparative value. Thus, diselenium derivatives are usually synthesized via other routes. For example, electron-deficient aryl halides can be used as substrates [43], and their reaction with the diselenide dianion gives rise to the desired molecules. Arylamines can also serve as potential precursors via the reaction of diazonium salts with sodium hydroselenide. Another typical route to diaryl diselenides is based on the reaction of arylaldehydes with sodium hydroselenide, which, after treatment with sodium borohydride and piperidine, leads to the target compounds.

2.5. Diketone

Benzil is a yellow crystalline containing a diketone moiety made by oxidizing benzoin [44]. Sawaki et al. have reported that benzil can transform into benzoic anhydride via a Baeyer-Villiger type reaction with H$_2$O$_2$ in alkaline organic solvents [39]. Then the products are usually presented in benzoic form due to hydrolysis (Table 1). However, the reported conditions were far from biological. Nagano’s group developed a simple strategy to adjust
the reactivity between benzil and H$_2$O$_2$ based on the modification of the benzene ring [40]. Following this method, 5-benzoylcarbonylfluorescein derivatives were synthesized for the detection of hydrogen peroxide at the cellular level together with the d-PeT mechanism to control fluorescence. Moreover, other peroxalate ester-containing derivatives have been reported to show similar H$_2$O$_2$-responsive properties [45–47].

2.6. Boronic

The reaction between H$_2$O$_2$ and benzeneboronic acid in aqueous solutions can generate phenol and boric acid in high yields, which can be viewed as a homolytic oreelectrophilic substitution in the benzene ring (Table 1) [48]. Generally, the rate of the reaction follows a dose-dependent manner. Moreover, in addition to benzeneboronic acid, quite a number of aryl-substituted boronic acids were developed, which can be exploited as H$_2$O$_2$-responsive tools with high accuracy, specificity, and sensitivity. Chang’s group proposed a strategy for the optical detection of H$_2$O$_2$ relying on the selective H$_2$O$_2$-triggered transformation of arylboronates to phenols [37]. With the H$_2$O$_2$-triggered hydrolytic deprotection of the boronates, our groups have developed a series of open, colored, and fluorescent products [49–52].

Interestingly, when the arylboronic acid ester is connected to a suitable linker, e.g., a typical –Ar–CH$_2$–O– linkage, after the formation of a phenol derivative triggered by H$_2$O$_2$, a sequential self-immolative process can take place via a 1,4-/1,6 elimination reaction. This character of arylborates has enabled the development of H$_2$O$_2$-triggered degradable materials [38].

2.7. Others

To meet the increasing demand for H$_2$O$_2$-responsive materials and ROS scavengers, some other types of H$_2$O$_2$-sensitive linkages have been employed. Tellurium (Te)-containing organic compounds have gained attention because of their lower electronegativity, which is thought to be more easily oxidized by H$_2$O$_2$ than selenium/sulfur–containing ones [53]. Moreover, tellurium-containing compounds were evidenced to exhibit less toxicity than selenium analogues. It has been reported that the tellurium atoms in Te-containing organic compounds can be completely oxidized with 100 µM H$_2$O$_2$.

Peptides and enzymes have also been used to fabricate H$_2$O$_2$-responsive materials. Sung et al. reported a peptide oligomer as a H$_2$O$_2$-responsive crosslinker for long-term tissue engineering applications. The degradation can take place by reacting with 5 mM H$_2$O$_2$ [54,55].

Otherwise, some metallic oxides and metal salts can be used as H$_2$O$_2$-responsive moieties owing to their catalytic action or redox activity. For example, the catalytic reaction between the H$_2$O$_2$ and MnO$_2$ contributes to the formation of H$_2$O and O$_2$ [56]. Fe(II) can react with H$_2$O$_2$ to generate hydroxyl radicals [57].

3. Preparation

Generally, hydrogels fall into two categories [58–60]:

(i) Chemical gel: Hydrogels that are covalently cross-linked networks in which the equilibrium swelling state depends on the crosslink density and interaction parameters [61,62];

(ii) Physical gel: The networks are constructed by noncovalent linkages, e.g., hydrogen bonding, ionic bonding, hydrophobic interactions, molecular entanglements, etc. These interactions are reversible.

As for H$_2$O$_2$-responsive hydrogels, the responsiveness is mainly based on the linkages summarized in the abovementioned section. Such responsiveness is quite different from a response to temperature, pH, or electric fields, and the latter of these is mainly presented in physical gels. As a result, H$_2$O$_2$-responsive hydrogels are mainly presented as chemical gels in the literature, in which a chemical cross-linking approach is usually involved. The cross-linking approach can be classified into two generic groups.
3.1. Self-Crosslinking

For this case, the H$_2$O$_2$-Gels were usually prepared based on the self-crosslinking behavior of the polymeric chains without the addition of any other crosslinkers. The H$_2$O$_2$-sensitive moieties, e.g., sulfydryl, selenol, phenol, etc., are mainly incorporated in the polymeric chains as pendent or terminal groups which can be further involved in the condensation reactions to achieve the three-dimensional structures.

Wan’s group synthesized a sulfydryl-bearing oligomer (o-DHLA) by treating dihydrolipoic acid with equimolar 2,2-dimethoxypropane under the catalysis of p-toluenesulfonic acid [63]. The obtained o-DHLA was achieved with sulfydryl units as terminal groups, the latter of which can react with each other to yield –S–S– containing microgels with the addition of polyvinyl alcohol. The formation of an –S–S– linkage makes the obtained microgels desirable H$_2$O$_2$–responsive drug delivery systems. Yang’s group employed cysteine-containing keratin as the base material, in which the sulfydryl units can afford –S–S– linkages under the effect of oxygen [64].

Ma et al. [65] prepared a boronate-based copolymer comprising N-isopropylacrylamide, hydroxyethyl methacrylate, and (4-(hydroxymethyl)-phenylboronic acid. The presence of N-isopropylacrylamide endowed the obtained copolymer’s temperature-responsive characters. As a result, the sol-gel transformation occurs when the temperature is higher than a certain value ranging from 18 to 37 °C. The lower critical solution temperature (LCST) and upper critical solution temperature (UCST) can be controlled by the proportion of N-isopropylacrylamide. The presence of boronate moieties makes the obtained hydrogel exhibit H$_2$O$_2$–responsive characters. A similar strategy can also be found in another patent [66].

3.2. Addition of Difunctional Crosslinkers

Zhao et al. [67] prepared H$_2$O$_2$–responsive hydrogels using cystamine dihydrochloride as the difunctional crosslinkers. In their strategy, catecholamine-modified chitosan was prepared and used to fabricate the hydrogels. The amino groups in cystamine can react with catecholamine moieties via the formation of benzoquinone. As a result, the disulfide bonds were introduced into the matrix of the obtained hydrogels, which endowed the hydrogels with H$_2$O$_2$–responsive degradation abilities.

Our group proposed an alternative approach to synthesizing H$_2$O$_2$–responsive hydrogels, in which the difunctional crosslinkers and polyhydric matrix are used. For example, the aryloboronic acid unit on 1,4-phenylenediboronic acid can react with the vicinal diol groups of a polysaccharide to form a chemical hydrogel with high efficiency. The B–C bond in the obtained hydrogel can be broken with the addition of H$_2$O$_2$ in a dose-dependent manner, which can give rise to the degradation behavior and endow the H$_2$O$_2$–responsive behavior to the hydrogel (Figure 1) [50]. The boronate-based difunctional crosslinkers have been also reported in other studies [68].
Figure 1. The H$_2$O$_2$-responsive release mechanism of the 1,4-phenylenediboronic acid-crosslinked hydrogels reported by our group (Reprinted/adapted with permission from Ref. [50]. Copyright year: 2022, copyright owner’s name: Hailei Zhang).

4. Application

4.1. Drug Delivery

Drug-loaded hydrogels have generated increasing interest in recent years because of their extraordinary advantages in versatile drug administration, ease of preparation, high biocompatibility, and improved patient compliance [69]. Moreover, the stimuli-responsiveness can be achieved by including the responsive linkages into the hydrogel networks, e.g., temperature-responsive, pH-responsive, photo-responsive, etc. H$_2$O$_2$ is usually involved in a variety of pathological effects in organisms, in which the aberrant expression of H$_2$O$_2$ is directly linked with cancer, inflammation, phyma, etc. The exploration of H$_2$O$_2$-responsive drug delivery systems with high selectivity and specificity has great significance for improving antitumor efficacy and preventing inflammation.

In Wan’s study, a series of thioketal-containing microgels have been developed that show a dual responsive behavior to H$_2$O$_2$ and glutathione. Paclitaxel was loaded into the dual responsive thioketal microgels to achieve an antitumor preparation which is sensitive enough to the tumor-site H$_2$O$_2$ and glutathione levels and able to release paclitaxel accordingly. Moreover, the obtained dual responsive thioketal microgels can be degradable into biocompatible and low toxic byproducts after performing their delivering tasks. Following this method, the antitumor efficacy of chemotherapy drugs can be significantly improved by loading them into the dual responsive platform. Meanwhile, side effects can also be effectively reduced [63].

Duvall et al. [70] explored poly(propylene sulfide) nanoparticles and hydrogels as H$_2$O$_2$-responsive drug carriers. Generally, the conversion between sulfoxides/sulfones and hydrophobic sulfide can cause solubility changes. The hydrophobicity of poly(propylene sulfide) makes it a good candidate for loading hydrophobic drugs, similar to curcumin. After delivery in vivo, the exposure of poly(propylene sulfide) to H$_2$O$_2$ enables a transition to its hydrophilic form, which triggers particle swelling and H$_2$O$_2$-responsive drug release.

Our group developed H$_2$O$_2$-Gels by using difunctional crosslinkers. A high level of H$_2$O$_2$ can destroy the B-C bond in the obtained hydrogels and thereby break the three-dimensional networks in H$_2$O$_2$-Gels. When the drug was loaded into the hydrogel, a
typical \( \text{H}_2\text{O}_2 \)-responsive behavior was easily achieved. It should be noted that hydrogels, which are diffusion-controlled drug delivery systems, usually exhibit an initial burst release. Since drug-release during the initial stage depends mostly on diffusional escape, a sudden release in a short period usually takes place. It can be regarded as an “initial burst release” [71,72]. A serious initial burst release may be toxic and give rise to suppressed bioavailability. In our study, the model drug was poured into halloysite nanotubes, a natural aluminosilicate clay mineral with a hollow tubular structure, before loading it into the matrix of \( \text{H}_2\text{O}_2 \)-Gels (Figure 2, DLHCH-1: the therapeutic agent was pre-loaded into the halloysite nanotubes; DLHCH-2: the therapeutic agent was directly loaded into the matrix of hydrogels). Following this method, the “initial burst effect” was effectively suppressed due to the halloysite nanotubes. Our strategy provides an alternative way to design \( \text{H}_2\text{O}_2 \)-Gels which may meet the requirements for the application of topical preparations to prevent inflammation (Figure 2) [50].

![Figure 2](image)

**Figure 2.** The \( \text{H}_2\text{O}_2 \)-responsive behaviour of 1,4-phenylenebisboronic acid-crosslinked hydrogels reported by our group (A): \( \text{H}_2\text{O}_2 \)-Gel in a transparent vial; (B): \( \text{H}_2\text{O}_2 \)-Gel immersing in \( \text{H}_2\text{O}_2 \) aqueous solution for 100 min; (C): \( \text{H}_2\text{O}_2 \)-Gel immersing in aqueous solution for 100 min; (D): the drug release profiles of DLHCH-1 and DLHCH-2) (Reprinted/adapted with permission from Ref. [50]. Copyright year: 2022, copyright owner’s name: Hailei Zhang).

Zhen et al. focused on exploiting the boronate-based polymeric network as microneedles, which can be used as skin patches for acne vulgaris treatment. Due to the presence of boronate moieties, the antibiotic loaded composition possesses a \( \text{H}_2\text{O}_2 \)-responsive release behavior [68].

There numerous reports focused on the development of \( \text{H}_2\text{O}_2 \)-Gel as drug delivery systems [73,74]. However, there is still a lack of commercially available products. Further studies should pay attention to this point. Some other studies are still required. The potential side effects of the degraded products should also be investigated. Additionally, the specificity in non-enzyme systems should also be improved since the body fluid is a very complicated system.

4.2. Detection

\( \text{H}_2\text{O}_2 \) has been demonstrated to be a metabolite of many biochemical reactions. Qualitative \( \text{H}_2\text{O}_2 \) detection is an important tool which can be used to discover early lesions such as those present in cancer. As typical soft materials, hydrogels show a biophysical similarity to soft biological tissues and thereby possess a desirably good affinity with them. Moreover, the chemosensor moieties can be covalently linked or physically incorporated into the matrix of hydrogels to endow the product with various diagnostic applications. In past decades, studies on utilizing \( \text{H}_2\text{O}_2 \)-Gels as detection tools were reported [75,76].
4.2.1. Electrical

Tian’s group developed a hydrogel-based biosensor system by incorporating Cytochrome c into the matrix of hydrogel consisting of Fmoc-L-lysine, Fmoc-L-phenylalanine, and sodium carbonate, in which the inherent bioactive activity of Cytochrome c was retained. After treatment with H₂O₂, the biochemical reaction between Cytochrome c and H₂O₂ can result in a fast and sensitive change in the redox formal potential. Moreover, the cellular level H₂O₂ can be quantitatively determined. Such H₂O₂-responsive behavior was demonstrated to exhibit excellent specificity over commonly used ions, ascorbic acid, and other ROS. The good stability and reproducibility, as well as the high specificity and sensitivity, may pave a new path to further understanding the role of H₂O₂ in pathological changes [18].

Li et al. prepared luminophore-incorporated polyaniline-polyacrylamide hydrogel that exhibited favorable biocompatibility and good conductivity. The obtained hydrogel showed a high detection sensitivity upon H₂O₂ based on an electrochemiluminescence method. The limit of detection of H₂O₂ was as low as 2.9 × 10⁻⁹ M. The detection concentration ranged from 5 × 10⁻⁵ to 1 × 10⁻⁸ M. The results indicated that the prepared H₂O₂-Gel can be utilized to detect cellular H₂O₂ [77].

The electrochemical detection of H₂O₂ can also be achieved by constructing Hemin-G4/Au-containing composite hydrogels. In Hou’s study [78], the excellent catalytic activity of Hemin-G4 and the high conductivity of gold nanoparticles were employed to endow the obtained hydrogels with a good electrochemical response towards H₂O₂. Moreover, A549 cells can adhere to the surface of the obtained hydrogels, which can be used to detect extracellular H₂O₂.

Electrical detection methods usually show low limit of detection (LOD) levels. The enzyme-incorporated ones exhibit good specificity, making them desirable detection tools in bioanalysis. Nevertheless, there is still a need for other detention methods that are low-cost and free of energy.

4.2.2. Fluorescent

Hydrogels with a fluorescent response to H₂O₂ are fundamentally important in biology and pathophysiology which have practical value in early clinical diagnosis. In Yang’s study [79], an intensive chemiluminescence hydrogel was proposed by incorporating luminol and hemin into the matrix of guanosine-derived hydrogel. The obtained composite hydrogel demonstrated enzyme-like activity towards the H₂O₂-mediated oxidation of luminol, in which the blue light can be visibly observed.

Our group synthesized a novel type of fluorescein-derived fluorescence probe by installing boronic acid groups at the 3′ and 6′-positions of the xanthenone moiety in fluorescein. The obtained fluorescein derivative displayed almost no emission behavior (Φ₁ = ca.0.01). After treatment with H₂O₂, the fluorescein derivative can be degraded into fluorescein with strong fluorescence (Φ₁ = 0.94) [80]. The “turn-on” response in fluorescence resulted in a H₂O₂-detecting ability. Moreover, the two arylboronic acid groups in fluorescein derivative can react to diol units, which makes it a desirable crosslinker to fabricate the H₂O₂-responsive fluorescein change in the obtained hydrogels. Following this method, hydrogels with a “turn-on” fluorescence behavior upon H₂O₂ administration were developed (Figure 3A,B). The obtained hydrogel shows no emission behavior under the physiological H₂O₂ concentration, while a fast non-fluorescent to fluorescent change can be achieved under pathological H₂O₂ concentration [51].

Concerning the lack of an indicator to monitor the release behavior in real time for H₂O₂-Gels, the obtained hydrogels were also explored as drug carriers. As shown in Figure 3C, a very small amount of agent was released from DHNTs@PVA@PA under a low level of H₂O₂ (0.02 µM), while the 4-fold increased initial-burst amount can be tracked for PTX@PVA@PA (DHNTs@PVA@PA: the therapeutic agent was pre-loaded into the halloysite nanotubes; PTX@PVA@PA: the therapeutic agent was directly loaded into the matrix of hydrogels). The significant increase of the release rates can be observed in 200 µM H₂O₂.
and compared to those in 0.02 μM H₂O₂, suggesting a typical H₂O₂-responsive release behavior. Figure 3D shows a remarkable increase of the fluorescence intensity over time from 5 to 40 min. The relationship between the release rate and fluorescence intensity was investigated and displayed in Figure 3E. The good relationship is quite beneficial to invisibly monitoring the drug release behavior.

Figure 3. H₂O₂-responsive behaviors of the fluorescein-crosslinked hydrogels reported by our group: (A) The transformation mechanism of fluorescein-crosslinked hydrogels from arylboronates to phenols to afford fluorescein with high fluorescence in the presence of H₂O₂; (B) the changes of the hydrogel from non-fluorescent to fluorescent; (C) the drug release profiles in different concentration of H₂O₂; (D) the fluorescence spectra of the release medium (H₂O₂ = 200 μM) after addition of DHNTs@PVA@PA; (E) plots of fluorescence intensity vs. the drug release rate (Reprinted/adapted with permission from Ref. [51]. Copyright year: 2022, copyright owner’s name: Hailei Zhang).

4.2.3. Colorimetric

As a typical pH indicator, phenolphthalein solution can present a red color under an alkalescence environment based on the transformation between the phenolic hydroxyl groups and the benzoquinone unit. In our study, this colorimetric behavior was employed for the visual detection of H₂O₂. Boronic acid pinacol ester groups were used to substitute the hydrogel groups in phenolphthalein. As a result, the transformation between the phenolic hydroxyl groups and benzoquinone unit was blocked. Then, the phenolphthalein derivative was used to crosslink polyvinyl alcohol chains in a weak basic solution to yield a H₂O₂-Gel based on the dynamic covalent bonds. After treatment with H₂O₂ under a
pathological concentration, the B–C linkage in the network can be rapidly degraded into Ar-OH groups and B-OH groups result in the formation of phenolphthalein. Since the hydrogel was prepared under an alkalescence environment, the obtained hydrogel showed a distinct colorimetric response to H$_2$O$_2$ (Figure 4) [52].

Yang et al. [81] reported a 2D photonic crystal-based horseradish peroxidase/bovine serum albumin (HRP/BSA) composite hydrogel. BSA and HRP was crosslinked by glutaraldehyde, in which BSA and HRP acts as a scaffold and a recognition unit, respectively. The obtained hydrogel can monitor H$_2$O$_2$ since HRP can selectively decompose H$_2$O$_2$ along with heme inactivation. The conformational change of HRP can decrease the crosslinking density and enlarge the particle spacing. As a result, the Debye ring of the 2-D photonic crystals also decreased, which provided a colorimetric response to H$_2$O$_2$ for the obtained hydrogel.

Compared to electrical and fluorescent methods, the colorimetric method shows a superior low cost, an easy operation, and operates free of energy. Moreover, the latter method can be used without any sophisticated or expensive analytical instruments, which makes it a desirable detection tool for actual production and daily life.

4.3. Glucose-Responsive

Diabetes is regarded as a serious metabolic disorder, which affects more than one-tenth of the population in the world [82,83]. Long-term high glucose levels can result in serious cardiovascular disease, renal failure, abnormal bone metabolism, and many other complications. The glucose-controlled insulin release system is usually required in the treatment of diabetes, especially for suppressing diabetes ulcers [84]. Among these systems,
glucose oxidase is an important intermediary for the transformation of glucose signals into the generation of H$_2$O$_2$ [85]:

$$\text{Glucose} + \text{O}_2 \xrightarrow{\text{Glucose oxidase}} \text{H}_2\text{O}_2 + \text{Gluconic acid} \quad (1)$$

Therefore, with the addition of glucose oxidase, H$_2$O$_2$-Gel can also be used as glucose-responsive systems. Kiritoshi et al. prepared H$_2$O$_2$-Gel via a typical radical copolymerization of 2-methacryloyloxyethyl phosphorylcholine and triethylene glycol dimethacrylate. The obtained polymer can be easily degraded in the presence of H$_2$O$_2$ (974 mmol/L). In Kiritoshi’s strategy, the glucose oxidase can be loaded into the matrix of the obtained hydrogel without a loss in catalytic activity. Following this method, the obtained H$_2$O$_2$-Gel is also available in glucose-responsive insulin delivery systems in a concentration-dependent manner [86].

In past decades, an increasing number of studies have focused on transforming H$_2$O$_2$-Gels into glucose-responsive systems with the addition of glucose oxidase [64,87]. For example, Yang et al. developed a hydrogel that formed in situ by loading glucose oxidase into keratin hydrogels, which showed promising applications for the treatment of diabetic wounds [64].

### 4.4. ROS Scavengers

An excessive ROS level can raise the oxidative damage to cellular macromolecules and thereby deteriorate many inflammation-related diseases. Moreover, the ROS accumulated in a wound can induce strong inflammation and serve as a strong barrier that inhibits tissue regeneration, which is a puzzling problem for diabetic ulcers [88]. Some H$_2$O$_2$-Gels are promising ROS scavengers in clinical applications [35,89], especially those that have the capacity to consume H$_2$O$_2$ in a concentration-dependent manner [90].

Liu’s group developed a type of ROS-scavenger by using a difunctional arylboronic derivative as crosslinker [91]. The obtained H$_2$O$_2$-Gels can act as an effective ROS-scavenger agent to promote wound-healing by reducing the ROS level around the wound, which is quite beneficial to inhibiting aseptic inflammation. Moreover, the H$_2$O$_2$-Gel was also explored as a drug carrier to allow the release of therapeutic agents, such as antibacterial agents and growth factors. As a result, the H$_2$O$_2$-Gel supported a systemic therapy strategy to promote wound-healing in complicated infections.

Gao et al. presented a novel type of thioether-containing injectable hydrogel with H$_2$O$_2$-responsive behaviors, which can be used as ROS-scavengers for myocardial infarction treatment [92]. The resulting animal model showed that the obtained H$_2$O$_2$-Gels can significantly consume excessive ROS, accelerate angiogenesis, and improve cardiac functions.

### 4.5. Tissue Engineering

Hydrogels have been demonstrated to be a desirable candidate for cartilage regenerative substrates owing to their attractive advantages including mild reaction conditions and tunable structures. The controllable gelatinizing process, especially for the gelation time, is the key issue when hydrogel is used for articular cartilage engineering. Rahbarghazi et al. developed a library of alginate-based hydrogels crosslinked via horseradish peroxidase. Generally, horseradish peroxidase can serve as an efficient enzyme catalysis in catalyzing the conversion from H$_2$O$_2$ to H$_2$O and O$_2$ [93]:

$$\text{H}_2\text{O}_2 \xrightarrow{\text{Horseradish peroxidase}} 2\text{H}_2\text{O} + \text{O}_2 \quad (2)$$

As a result, the gelation time can be well-controlled based on the concentration of H$_2$O$_2$, which is a result of the deactivation effect of horseradish peroxidase by H$_2$O$_2$ [94].

It is worth noting that the tissue engineering materials are usually composed of different parts to meet the demand in terms of therapeutics. Liu’s group developed an injectable, post-trauma microenvironment-responsive, H$_2$O$_2$ depleting, and drug-loaded...
hydrogel [95], which can be used to lower the H$_2$O$_2$ levels in damaged brain tissue. The poly (propylene sulfide)-containing matrix endowed the obtained hydrogel with H$_2$O$_2$-responsive abilities. The released curcumin can further eliminate the ROS, promoting the regeneration and recovery of neurons. Following this method, a systematic and multi-channel promoting effect can be achieved, which is quite useful in tissue engineering materials.

4.6. Cell-Encapsulation

Hydrogel particles have been studied in the latest decade for encapsulating and delivering cells in humans, which meets many of the demands for treating diseases and producing antibodies for vaccine use. The main advantage of hydrogel-based cell-encapsulation technology is a mild reaction condition which does not threaten the lives of the enclosed cells.

Sakai et al. reported a facile approach for preparing mammalian cell-encapsulating hydrogel particles based on the horseradish peroxidase-crosslinked hydrogelation [96]. Gelatin bearing phenolic hydroxyl moieties were employed as the polymeric substrate and dissolved in a cell-suspending solution containing horseradish peroxidase. The mixed solution was then dropped into 1 mM H$_2$O$_2$ to form hydrogels with a spherical shape. The α-H in phenolic hydroxyl moieties usually exhibits high activity with the presence of horseradish peroxidase and H$_2$O$_2$ [19]. These phenomena suggested that the H$_2$O$_2$-responsive behavior can be used to establish a cell-encapsulation method in hydrogel particles.

In Sakai’s proposed strategy [19], the individual cell can be encapsulated into a thin hydrogel sheath. This approach can be controlled by the addition of HRP and exhibits no significant adverse effects to the encapsulated cells. Apparently, the proposed approach based on the H$_2$O$_2$-responsive behavior is expected to be used to extend the applications of cell encapsulation technology.

5. Conclusions and Perspective

Recent studies of H$_2$O$_2$-Gels have been reviewed in this paper. The summarized H$_2$O$_2$-responsive linkages and preparation methods may serve as guides for researchers who are interested in H$_2$O$_2$-Gels. We expect to provide guidance and designate the direction of further studies on H$_2$O$_2$-Gels. In combination with drug delivery, the detection of H$_2$O$_2$, glucose-responsive systems, ROS scavengers, tissue engineering, and cell-encapsulation, H$_2$O$_2$-Gels could be advanced to have much more promising applications in biomedicine. Nevertheless, commercially available H$_2$O$_2$-Gels, as well as official standards or criteria, are also urgently needed. There are still some limitations for this issue. The level of H$_2$O$_2$ usually differs among individuals, and the complexity of bodily fluids should also be considered. Therefore, it is far more difficult to establish in vitro and in vivo correlation (IVIVC), which is rarely studied but indeed remains a key issue that should be focused on. On the other hand, costly proteinases seriously limit large-scale production. Non-proteinase ones usually suffer from other problems such as stability, complexity in the synthesis approach, the potential toxicity of the degraded by-products, etc. Thus, longer-duration studies are encouraged to conduct deeper investigations to make H$_2$O$_2$-Gels “over the counter”.

Author Contributions: Conceptualization, H.Z.; data collection: W.S., J.Y., Y.Z., Q.Y., J.J.; Original draft preparation: W.S. and H.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Project of Science & Technology Bureau of Baoding City (no.1941ZF081).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

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

1. Lavrador, P.; Esteves, M.R.; Gaspar, V.M.; Mano, J.F. Stimuli-responsive nanocomposite hydrogels for biomedical applications. *Adv. Funct. Mater.* 2021, 31, 2009941. [CrossRef]

2. Panja, S.; Adams, D.J. Stimuli responsive dynamic transformations in supramolecular gels. *Chem. Soc. Rev.* 2021, 50, 5165–5200. [CrossRef] [PubMed]

3. Yang, P.; Zhu, F.; Zhang, Z.; Cheng, Y.; Wang, Z.; Li, Y. Stimuli-responsive polydopamine-based smart materials. *Chem. Soc. Rev.* 2021, 50, 8319–8343. [CrossRef] [PubMed]

4. Meng, J.; Jin, Z.; Zhao, P.; Zhao, B.; Fan, M.; He, Q. A multistage assembly/disassembly strategy for tumor-targeted co delivery. *Sci. Adv.* 2020, 6, eaba1362. [CrossRef] [PubMed]

5. Li, Q.; Wang, B.; Fang, X.; Zong, B.; Liu, Y.; Li, Z.; Mao, S.; Ostríkov, K.K. Rapid synthesis of multifunctional beta-cyclodextrin nanospheres as alkali-responsive nanocarriers and selective antibiotic adsorbents. *Chem. Commun.* 2021, 57, 1161–1164. [CrossRef] [PubMed]

6. Guo, Y.; Bae, J.; Fang, Z.; Li, P.; Zhao, F.; Yu, G. Hydrogels and hydrogel-derived materials for energy and water sustainability. *Chem. Rev.* 2020, 120, 7642–7707. [CrossRef]

7. Gao, G.; Yang, F.; Zhou, F.; He, J.; Lu, W.; Xiao, P.; Yan, H.; Pan, C.; Chen, T.; Wang, Z.L. Bioinspired self-healing human-machine interactive touch pad with pressure-sensitive adhesiveness on targeted substrates. *Adv. Mater.* 2020, 32, 2004290. [CrossRef]

8. Liu, Z.; Li, J.; Cui, X.; Wang, X.; Zhang, L.; Tang, P. Recent advances on magnetic sensitive hydrogels in tissue engineering. *Front. Chem.* 2020, 8, 124. [CrossRef]

9. Lea-Banks, H.; Meng, Y.; Wu, S.-K.; Belhadjhamida, R.; Hamani, C.; Hynynen, K. Ultrasound-sensitive nanodroplets achieve targeted neuromodulation. *J. Control. Release* 2021, 332, 30–39. [CrossRef]

10. Lin, X.; Liu, S.; Zhang, X.; Zhu, R.; Chen, S.; Chen, X.; Song, J.; Yang, H. An ultrasound activated vesicle of janus au-mn nanoparticles for promoted tumor penetration and sono-chemodynamic therapy of orthotopic liver cancer. *Angew. Chem. Int. Ed.* 2020, 59, 1682–1688. [CrossRef]

11. Hoestis, N.; Rosen, E.D.; Lander, E.S. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 2006, 440, 944–948. [CrossRef]

12. Fubini, B.; Hubbard, A. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis. *Free Radical. Bio. Med.* 2003, 34, 1507–1516. [CrossRef]

13. Wang, S.; Wang, Z.; Yu, G.; Zhou, Z.; Jacobson, O.; Liu, Y.; Ma, Y.; Zhang, F.; Chen, Z.-Y.; Chen, X. Tumor-specific drug release and reactive oxygen species generation for cancer chemo/chemodynamic combination therapy. *Adv. Sci.* 2019, 6, 1801986. [CrossRef]

14. Wang, Y.Q.; Li, L.L.; Zhao, W.B.; Dou, Y.; An, H.J.; Tao, H.; Xu, X.Q.; Jia, Y.; Lu, S.; Zhang, J.X.; et al. Targeted therapy of atherosclerosis by a broad-spectrum reactive oxygen species scavenging nanoparticle with intrinsic anti-inflammatory activity. *ACS Nano* 2018, 12, 8943–8960. [CrossRef] [PubMed]

15. Rhee, S. H$_2$O$_2$, a necessary evil for cell signaling. *Science* 2006, 312, 1882–1883. [CrossRef]

16. Sies, H. Role of metabolic h$_2$o$_2$ generation: Redox signaling and oxidative stress. *J. Biol. Chem.* 2014, 289, 8735–8741. [CrossRef]

17. Wang, J.; Zhang, Y.; Archibong, E.; Ligler, F.S.; Zhen, G. Leveraging H$_2$O$_2$ levels for biomedical applications. *Adv. Biosyst.* 2017, 1, 1700884. [CrossRef]

18. Zhou, J.; Liao, C.; Zhang, L.; Wang, Q.; Tian, Y. Molecular hydrogel-stabilized enzyme with facilitated electron transfer for determination of h$_2$o$_2$ released from live cells. *Anal. Chem.* 2014, 86, 4395–4401. [CrossRef]

19. Cheng, Y.; Dai, J.; Sun, C.L.; Liu, R.; Zhai, T.Y.; Lou, X.D.; Xia, F. An intracellular H$_2$O$_2$-responsive aiegen for the peroxidase-mediated selective imaging and inhibition of inflammatory cells. *Angew. Chem. Int. Edit.* 2018, 57, 3123–3127. [CrossRef]

20. Saxon, E.; Peng, X. Recent advances in hydrogen peroxide responsive organoborons for biological and biomedical applications. *ChemBioChem* 2022, 23, e202103566. [CrossRef]

21. Yang, N.; Xiao, W.; Song, X.; Wang, W.; Dong, X. Recent advances in tumor microenvironment hydrogen peroxide-responsive materials for cancer photodynamic therapy. *Nano-Micro Lett.* 2020, 12, 15. [CrossRef] [PubMed]

22. Bastianich, C.; Danhier, P.; Preat, V.; Danhier, F. Anticancer drug-loaded hydrogels as drug delivery systems for the local treatment of glioblastoma. *J. Control. Release* 2016, 243, 29–42. [CrossRef] [PubMed]

23. Daly, A.C.; Riley, L.; Segura, T.; Burdick, J.A. Hydrogel microparticles for biomedical applications. *Nat. Rev. Mater.* 2020, 5, 20–43. [CrossRef]

24. Liu, X.; Liu, J.; Lin, S.; Zhao, X. Hydrogel machines. *Mater. Today* 2020, 36, 102–124. [CrossRef]

25. Yang, J.; Bai, R.; Chen, B.; Suo, Z. Hydrogel adhesion: A supramolecular synergy of chemistry, topology, and mechanics. *Adv. Funct. Mater.* 2020, 30, 1901693. [CrossRef]

26. Yoshii, T.; Onogi, S.; Shigemitsu, H.; Hamachi, I. Chemically reactive supramolecular hydrogel coupled with a signal amplification system for enhanced analyte sensitivity. *J. Am. Chem. Soc.* 2015, 137, 3360–3365. [CrossRef]

27. Xu, Q.; He, C.; Ren, K.; Xiao, C.; Chen, X. Thermosensitive polypeptide hydrogels as a platform for ROS-triggered cargo release with innate cytotoxic ability under oxidative stress. *Adv. Healthc. Mater.* 2016, 5, 1979–1990. [CrossRef]

28. Mateen, R.; Ali, M.M.; Hoare, T. A printable hydrogel microarray for drug screening avoids false positives associated with promiscuous aggregating inhibitors. *Nat. Commun.* 2018, 9, 602. [CrossRef]

29. Tabor, A.B. The challenge of the lantibiotics: Synthetic approaches to thioether-bridged peptides. *Org. Biomol. Chem.* 2011, 9, 7606–7628. [CrossRef]
30. Kholdeeva, O.A.; Maksimov, G.M.; Maksimovskaya, R.I.; Kovaleva, L.A.; Hill, C.L. A dimeric titanium-containing polyoxometalate. Synthesis, characterization, and catalysis of H₂O₂-based thioether oxidation. *Inorg. Chem.* 2000, 39, 3828–3837. [CrossRef]

31. Napoli, A.; Valentini, M.; Tirelli, N.; Müller, M.; Hubbell, J.A. Oxidation-responsive polymeric vesicles. *Nat. Mater.* 2004, 3, 183–189. [CrossRef] [PubMed]

32. Kramer, J.R.; Deming, T.J. Glycopolyptides with a redox-triggered helix-to-coil transition. *J. Am. Chem. Soc.* 2012, 134, 4112–4115. [CrossRef]

33. Miller, E.W.; Bian, S.X.; Chang, C.J. A fluorescent sensor for imaging reversible redox cycles in living cells. *J. Am. Chem. Soc.* 2007, 129, 3458–3459. [CrossRef] [PubMed]

34. Xu, Q.; Venet, M.; Wang, W.; Creagh-Flynn, J.; Wang, X.; Li, X.; Gao, Y.; Zhou, D.; Zeng, M.; Lara-Saez, I.; et al. Versatile hyperbranched poly(beta-hydrazide ester) macromers as injectable antioxidant hydrogels. *ACS Appl. Mater. Interfaces* 2018, 10, 39494–39504. [CrossRef] [PubMed]

35. Ma, N.; Li, Y.; Ren, H.; Xu, H.; Li, Z.; Zhang, X. Selenium-containing block copolymers and their oxidation-responsive aggregates. *Polym. Chem.* 2010, 1, 1609–1614. [CrossRef]

36. Deepagan, V.G.; Kwon, S.; You, D.G.; Nguyen, V.Q.; Park, J.H. In situ diselenide-crosslinked polymeric micelles for ROS-mediated anticancer drug delivery. *Biomaterials* 2016, 103, 56–66. [CrossRef]

37. Chang, M.; Pralle, A.; Isacoff, E.Y.; Chang, C.J. A selective, cell-permeable optical probe for hydrogen peroxide in living cells. *J. Am. Chem. Soc.* 2004, 126, 15392–15393. [CrossRef]

38. Broaders, K.E.; Grandhe, S.; Fréchet, J.M. A biocompatible oxidation-triggered carrier polymer with potential in therapeutics. *J. Am. Chem. Soc.* 2011, 133, 756–758. [CrossRef]

39. Sawaki, Y.; Foote, C.S. Acyclic mechanism in the cleavage of benzils with alkaline hydrogen peroxide. *J. Am. Chem. Soc.* 1959, 81, 6292–6296. [CrossRef]

40. Abo, M.; Urano, Y.; Hanaoka, K.; Terai, T.; Komatsu, T.; Nagano, T. Development of a highly sensitive fluorescence probe for hydrogen peroxide. *J. Am. Chem. Soc.* 2011, 133, 10629. [CrossRef]

41. Chivers, T. Main-group elements, including noble gases. In *Comprehensive Inorganic Chemistry II*; Elsevier: Amsterdam, The Netherlands, 2013.

42. Etal, A. Synthesis: Carbon with one heteroatom attached by a single bond. In *Comprehensive Organic Functional Group Transformations III*; Taylor: Oxfordshire, UK, 1995.

43. Wang, Y.; Lv, M.-Z.; Song, N.; Liu, Z.-J.; Wang, C.; Yang, Y.-W. Dual-stimuli-responsive fluorescent supramolecular polymer based on a diselenide-bridged pillar 5 arene dimer and an AIE-active tetraphenylethylene guest. *Macromolecules* 2017, 50, 5759–5766. [CrossRef]

44. Zhang, K.-M.; Dou, W.; Li, P.-X.; Shen, R.; Ru, J.-X.; Liu, W.; Cui, Y.-M.; Chen, C.-Y.; Liu, W.-S.; Bai, D.-C. A coumarin-based two-photon probe for hydrogen peroxide. *Biosens. Bioelectron.* 2015, 64, 542–546. [CrossRef] [PubMed]

45. Lee, Y.-D.; Lim, C.-K.; Singh, A.; Koh, J.; Kim, J.; Kwon, I.C.; Kim, S. Dye/peroxalate aggregated nanoparticles with enhanced and tunable chemiluminescence for biomedical imaging of hydrogen peroxide. *ACS Nano* 2012, 6, 6759–6766. [CrossRef] [PubMed]

46. Seo, Y.H.; Singh, A.; Cho, H.-J.; Kim, Y.; Heo, J.; Lim, C.-K.; Park, S.Y.; Jang, W.-D.; Kim, S. Rational design for enhancing inflammation-responsive in vivo chemiluminescence via nanophotonic energy relay to near-infrared aie-active conjugated polymer. *Biomaterials* 2016, 84, 111–118. [CrossRef] [PubMed]

47. Liu, C.; Zhu, X.; Duan, J.; Liang, X.; Li, X.; Yang, J. Hydrogen peroxide-responsive peroxalate ester-linked PCL-PEG micelles as drug carrier. *J. Control. Release* 2017, 259, e17. [CrossRef]

48. Cui, Y.; Zhang, M.; Du, F.S.; Li, Z.C. Facile synthesis of H₂O₂-cleavable poly(ester-amine) by passerini multicomponent polymerization. *ACS Macro Lett.* 2016, 6, 11–15. [CrossRef]

49. Zhang, H.; Ren, T.; Ji, Y.; Han, L.; Wu, Y.; Song, H.; Bai, L.; Ba, X. Selective modification of halloysite nanotubes with 1-pyrenylboronic acid: A novel fluorescence probe with highly selective and sensitive response to hyperoxide. *ACS Appl. Mater. Interfaces* 2015, 7, 23808–23811. [CrossRef]

50. Liu, F.; Bai, L.; Zhang, H.; Song, H.; Hu, L.; Wu, Y.; Ba, X. Smart H₂O₂-responsive drug delivery system made by halloysite nanotubes and carbohydrate polymers. *ACS Appl. Mater. Interfaces* 2017, 9, 31626–31633. [CrossRef]

51. Cheng, C.; Gao, Y.; Song, W.; Zhao, Q.; Zhang, H.; Zhang, H. Halloysite nanotube-based H₂O₂-responsive drug delivery system with a turn on effect on fluorescence for real-time monitoring. *Chem. Eng. J.* 2020, 380, 122474. [CrossRef]

52. Tang, B.; Zhang, H.; Cheng, C.; Jiang, H.; Bai, L.; Ba, X.; Wu, Y. Development of halloysite nanotube-based hydrogel with colorimetric H₂O₂-responsive character. *Appl. Clay Sci.* 2021, 212, 106230. [CrossRef]

53. Li, F.; Li, T.; Cao, W.; Wang, L.; Xu, H. Near-infrared light stimuli-responsive synergistic therapy nanoplatforms based on the coordination of tellurium-containing block polymer and cisplatin for cancer treatment. *Biomaterials* 2017, 133, 208–218. [CrossRef] [PubMed]

54. Yu, S.S.; Koblin, R.L.; Zachman, A.L.; Perrien, D.S.; Hofmeister, L.H.; Giorgio, T.D.; Sung, H.-J. Physiologically relevant oxidative degradation of oligo(proline) cross-linked polymeric scaffolds. *Biomacromolecules* 2011, 12, 4357–4366. [CrossRef] [PubMed]

55. Lee, H.S.; Boire, T.C.; Lee, J.B.; Gupta, M.K.; Zachman, A.L.; Rath, R.; Sung, H.-J. ROS-cleavable proline oligomer crosslinking of polyca pro lactone for pro-angiogenic host response. *J. Mater. Chem. B* 2014, 2, 7109–7113. [CrossRef] [PubMed]
56. Ye, Y.; Sun, X.; Zhang, Y.; Han, X.; Sun, X. A novel cell-based electrochemical biosensor based on mno2 catalysis for antioxidant activity evaluation of anthocyanins. Bioins. Bioelectrochem. 2022, 202, 113990. [CrossRef]
57. Xie, M.; Liu, X.; Wang, S. Degradation of methylene blue through fenton-like reaction catalyzed by mos2-doped sodium alginate/se hydrogel. Colloids Surf. B 2022, 214, 112443. [CrossRef]
58. Sallach, R.E.; Cui, W.; Wen, J.; Martinez, A.; Conticello, V.P.; Chaikoff, E.L. Elastin-mimetic protein polymers capable of physical and chemical crosslinking. Biomaterials 2009, 30, 409–422. [CrossRef]
59. Leonardi, A.B.; Fasce, L.A.; Zucchi, I.A.; Hoppe, C.E.; Soule, E.R.; Perez, C.J.; Williams, R.J.J. Shape memory epoxies based on networks with chemical and physical crosslinks. Eur. Polym. J. 2011, 47, 362–369. [CrossRef]
60. Xu, J.; Liu, X.; Ren, X.; Gao, G. The role of chemical and physical crosslinking in different deformation stages of hybrid hydrogels. Eur. Polym. J. 2018, 100, 86–95. [CrossRef]
61. Nezhad-Mokhtari, F.; Ghobarni, M.; Roshangar, L.; Rad, J.S. Chemical gelling of hydrogels-based biological macromolecules for tissue engineering: Photo- and enzymatic-crosslinking methods. Int. J. Biol. Macromol. 2019, 139, 760–772. [CrossRef]
62. Annaka, M.; Ogata, Y.; Nakahira, T. Swelling behavior of covalently cross-linked gellan gels. J. Phys. Chem. B 2000, 104, 6755–6760. [CrossRef]
63. Chen, D.; Zhang, G.; Li, R.; Guan, M.; Wang, X.; Zou, T.; Zhang, Y.; Wang, C.; Shu, C.; Hong, H.; et al. Biodegradable, hydrogen peroxide, and glutathione dual responsive nanoparticles for potential programmable paclitaxel release. J. Am. Chem. Soc. 2018, 140, 7373–7376. [CrossRef] [PubMed]
64. Chen, Y.; Li, Y.; Yang, X.; Cao, Z.; Nie, H.; Bian, Y.; Yang, G. Glucose-triggered in situ forming keratin hydrogel for the treatment of diabetic wounds. Acta Biomater. 2021, 125, 208–218. [CrossRef] [PubMed]
65. Ma, J.; Guan, J.; Li, H.; Niu, H.; Guan, Y.; Tan, T. Cosmeceutical Composition.WO Patent 2021150383A1, 29 July 2021.
66. Duvall, G.L.; Gupta, M.K.; Martin, J.R.; Dollinger, B.R. ROS-Degradeable Hydrogels. U.S. Patent 201603003241A1, 20 October 2016.
67. Zhao, A.; Liu, L.; Lu, B.; Yang, P. Hydrogel with Ros Response and Preparation Method and Application. CN Patent 113101264A, 8 April 2021.
68. Gu, Z.; Zhang, Y. ROS-Responsive Microneedle Patch for Acne Vulgaris Treatment. U.S. Patent 20210145984A, 20 May 2021.
69. Lin, Z.Q.; Gao, W.; Hu, H.X.; Ma, K.; He, B.; Dai, W.B.; Wang, X.Q.; Wang, J.C.; Zhang, X.; Zhang, Q. Novel thermo-sensitive hy- drogel system with paclitaxel nanocrystals: High drug-loading, sustained drug release and extended local retention guaranteeing better efficacy and lower toxicity. J. Control. Release 2014, 174, 161–170. [CrossRef]
70. Duvall, C.L.; Martin, J.R.; O’Grady, K.P.; Nelson, C.E. Reactive Oxygen Species (ROS)-Responsive Compositions and Method. U.S. Patent 10695288B2, 23 June 2016.
71. Yamaguchi, Y.; Takenaga, M.; Kitagawa, A.; Ogawa, Y.; Mizushima, Y.; Igarashi, R. Insulin-loaded biodegradable plga microparticles: Initial burst release controlled by hydrophilic additives. J. Control. Release 2002, 81, 235–249. [CrossRef]
72. Allison, S.D. Analysis of initial burst in PLGA microparticles. Expert Opin. Drug Del. 2008, 5, 615–628. [CrossRef] [PubMed]
73. Williams, G.T.; Sedgwick, A.C.; Sen, S.; Gwynne, L.; Gardiner, J.E.; Brewster, J.T.; II; Hiscock, J.R.; James, T.D.; Jenkins, A.T.A.; Sessler, J.L. Boronate ester cross-linked pva hydrogels for the capture and release of active fluorophores. Chem. Commun. 2020, 56, 5516–5519. [CrossRef]
74. Wang, W.; Liu, X.; Ding, L.; Jin, H.J.; Li, X. RNA hydrogel combined with MnO2 nanoparticles as a nano-vaccine to treat triple negative breast cancer. Front. Chem. 2021, 9, 797094. [CrossRef]
75. Wang, Z.; Shen, Y.-H.; Sun, X.; Li, Z.-H.; Wang, X.-Y.; Zhao, Z. High biocompatible auncs-silk fibroin hydrogel system for visual detection of H2O2. Microchem. J. 2020, 157, 105036. [CrossRef]
76. Manickam, P.; Vashist, A.; Madhu, S.; Sadasivam, M.; Sakhivela, A.; Kaushik, A.; Nair, M. Gold nanocubes embedded biocompati- ble hybrid hydrogels for electrochemical detection of H2O2. Bioelectrochemistry 2020, 131, 107373. [CrossRef]
77. Xiaojin, G.; Li, Y.; Yingchun, L.; Ye, Z.; Zhang, J.; Zhu, T.; Li, F. An l012@pani-paam hydrogel composite-based electrochemiluminescence biosensor for in situ detection of H2O2 released from cardiomyocytes. Electrochim. Acta 2020, 354, 136763.
78. Zhou, S.Y.; Wang, X.F.; Jiang, L.Y.; Sun, H.M.; Huo, D.Q.; Hou, C.J. A three-dimensional hydrogel-modified indium tin oxide electrode with enhanced performance for in situ electrochemical detection of extracellular H2O2. Analyst 2021, 146, 5403–5412. [CrossRef] [PubMed]
79. Ye, J.; Zhu, L.P.; Yan, M.X.; Xiao, T.; Fan, L.B.; Xue, Y.; Huang, J.S.; Yang, X.R. An intensive and glow-type chemiluminescence of luminol-embedded, guanosine-derived hydrogel. Talanta 2021, 230, 122351. [CrossRef] [PubMed]
80. Dong, J.; Zhao, Z.; Liu, R.; Zhang, H.; Wu, Y.; Ba, X. Investigation of a halloysite-based fluorescence probe with a highly selective and sensitive “turn-on” response upon hydrogen peroxide. RSC Adv. 2017, 7, 55067–55073. [CrossRef]
81. Liu, R.X.; Cai, Z.Y.; Zhang, Q.S.; Yuan, H.; Zhang, G.L.; Yang, D. Colorimetric two-dimensional photonic crystal biosensors for label-free detection of hydrogen peroxide. Sens. Actuators B Chem. 2022, 354, 131236. [CrossRef]
82. Diabetes Control and Complications Trial Research Group; Nathan, D.M.; Genuith, S.; Lachin, J.; Cleary, P.; Crofford, O.; Davis, M.; Rand, L.; Siebert, C. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N. Engl. J. Med. 1993, 329, 977–986. [CrossRef]
83. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global prevalence of diabetes - estimates for the year 2000 and projections for 2030. Diabetes Care 2004, 27, 1047–1053. [CrossRef]
84. The ADVANCE Collaborative Group; Patel, A.; MacMahon, S.; Chalmers, J.; Neal, B.; Billot, L.; Woodward, M.; Marre, M.; Cooper, M.; Glassiou, P.; et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N. Engl. J. Med.* **2008**, *358*, 2560–2572. [CrossRef]

85. Zhu, H.G.; Srivastava, R.; Brown, J.Q.; McShane, M.J. Combined physical and chemical immobilization of glucose oxidase in alginate microspheres improves stability of encapsulation and activity. *Bioconjug. Chem.* **2005**, *16*, 1451–1458. [CrossRef]

86. Uchiyama, T.; Kiritschi, Y.; Watanabe, J.; Ishihara, K. Degradation of phospholipid polymer hydrogel by hydrogen peroxide aiming at insulin release device. *Biomaterials* **2003**, *24*, 5183–5190. [CrossRef]

87. Jeong, D.I.; Kim, S.; Lee, S.Y.; Kim, H.-J.; Lee, J.; Lee, K.; Cho, H.-J. Iron sulfate-reinforced hydrogel reactors with glucose deprivation, serial reactive oxygen species generation, ferroptosis induction, and photothermal ablation for cancer therapy. *Chem. Eng. J.* **2022**, *438*, 135584. [CrossRef]

88. Kim, Y.E.; Kim, J. Ros-scavenging therapeutic hydrogels for modulation of the inflammatory response. *ACS Appl. Mater. Interfaces* **2021**, *14*, 23002–23021. [CrossRef] [PubMed]

89. Phuong Le, T.; Lee, Y.; Dieu Linh, T.; Thai Thanh Hoang, T.; Kang, J.I.; Park, K.M.; Park, K.D. In situ forming and reactive oxygen species-scavenging gelatin hydrogels for enhancing wound healing efficacy. *Acta Biomater.* **2020**, *103*, 142–152.

90. Wu, T.; Liu, W. Functional hydrogels for the treatment of myocardial infarction. *NPG Asia Mater.* **2022**, *14*, 9. [CrossRef]

91. He, Z.A.; Jie, H.A.; Yan, L.A.; Xi, A.; Hz, A.; Hv, A.; Yx, A.; Chao, W.B.; Jian, W.A.; Zhuang, L.B. ROS-scavenging hydrogel to promote healing of bacteria infected diabetic wounds. *Biomaterials* **2020**, *258*, 120286. [CrossRef]

92. Ding, J.; Yao, Y.; Li, J.; Duan, Y.; Nakkala, J.R.; Feng, X.; Cao, W.; Wang, Y.; Hong, L.; Shen, L.; et al. A reactive oxygen species scavenging and $O_2$ generating injectable hydrogel for myocardial infarction treatment in vivo. *Small* **2020**, *16*, 2005038. [CrossRef]

93. Chen, Z.; Xu, L.; Liang, Y.; Zhao, M. Ph-sensitive water-soluble nanospheric imprinted hydrogels prepared as horseradish peroxidase mimetic enzymes. *Adv. Mater.* **2010**, *22*, 1488–1492. [CrossRef]

94. Saghati, S.; Khoshfetrat, A.B.; Tayefi Nasrabadi, H.; Roshangar, L.; Rahbarghazi, R. Fabrication of alginate-based hydrogel cross-linked via horseradish peroxidase for articular cartilage engineering. *BMC Res. Notes* **2021**, *14*, 384. [CrossRef]

95. Qian, F.; Han, Y.; Han, Z.; Zhang, D.; Liu, H. In situ implantable, post-trauma microenvironment-responsive, ROS depletion hydrogels for the treatment of traumatic brain injury. *Biomaterials* **2021**, *270*, 120675. [CrossRef]

96. Sakai, S.; Ashida, T.; Ogino, S.; Taya, M. Horseradish peroxidase-mediated encapsulation of mammalian cells in hydrogel particles by dropping. *J. Microencapsul.* **2014**, *31*, 100–104. [CrossRef]