Hydrogel-based composites: Unlimited platforms for biosensors and diagnostics

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Abstract

The need for biosensing systems, which are capable of reliable and accurate detection of physiological signals and disease biomarkers along with biocompatible surface chemistry and textures for device–human interface, has driven the continuous search for advanced sensing materials, sensing strategies, and device structures. Hydrogels are hydrophilic polymers with high water content and thus akin to human tissues. They are not only able to act as polymeric matrices to load functional materials for bio-signal transducing, but also stimuli-responsive to cooperate with the filler materials for further enhanced sensing performance. A vast combination of hydrogels and functional fillers such as biomacromolecules, metal nanostructures, carbon nanomaterials, and two-dimensional materials beyond graphene has been demonstrated over the last decade for fabrication of biosensors for disease diagnosis and health monitoring. This review article aims to provide an overview of the various hydrogel-based composites, introduce their preparation protocols, and describe the working principles of their corresponding sensors. Recent development of these sensors for potential diagnosis of diseases such as diabetes, cancers, and cardiovascular diseases is then summarized, followed by stating the current challenges and prospects in this field.

KEYWORDS
biosensors, disease diagnosis, hydrogels, hydrogel composites

1 | INTRODUCTION

Sensing devices that are capable of monitoring human physiological signals and quantifying disease biomarkers are important for the early prevention/interference of diseases, provision of treatments, and accurate assessment of the treatment outcomes.1–6 Over the past years, the development of these sensing systems have experienced tremendous advances in terms of transducing strategies, transducing materials (typically electronic and
optoelectronic micro- and nano-structured materials), and device designs and architectures. While the demand for miniaturization, cost-reduction and high-sensing efficiency continuously drives the development of high-performance biosensing systems, the increasing need for wearable and implantable devices has imposed additional challenges in the design and preparation of functional materials that can interface with soft tissues without losing signal-transducing capabilities.

Hydrogels are hydrophilic polymer networks with a large number of water molecules retained in them. These networks can be prepared through the chemical (e.g., via coupling reactions and additive reactions) and/or physical (e.g., via hydrophobic interactions, hydrogen bonding, and ionic interactions) cross-linking between one or more kinds of hydrophilic polymers, such as polyaniline (PANI), poly(N-isopropylacrylamide) (PNIPAM), polyvinyl alcohol (PVA), and polyacrylamide (PAM), which are synthesized before or in situ polymerized during the cross-linking. Because of their inherent softness, flexibility with controllable mechanical strength, porous structure with high surface area and easy functionalization, stimuli responsibility and excellent biocompatibility, hydrogels are attractive building blocks for fabrication of biosensors. However, most hydrogels are nonconductive and nonspecific, which have limited their performance in signal transducing and target recognition. To counter these problems, hydrogels have been composited with other functional materials in addition to biological recognition elements such as enzymes, antibodies, and nucleic acids. For example, noble metal nanostructures of Au, Ag, Pd, and Pt have been introduced into hydrogel structures, offering added optical and catalytic functionalities. Carbon nanomaterials, such as graphene and its derivatives, carbon nanotubes (CNTs), and carbon dots (CDs), have also been composited with hydrogels because of their large surface area, high electrical and thermal conductivity, and good chemical and thermal stability, as well as the rich carbon chemistry for functionalization. Moreover, two-dimensional (2D) materials beyond graphene (e.g., transition metal dichalcogenides [TMDs], MXenes and black phosphorus [BP]) with the unique electronic, optoelectronic and photothermal properties have recently been composited with hydrogels, enabling versatile biosensing strategies and broadened applications.

Up to now, various hydrogel composite-based biosensors, working via various electrical and optical transducing pathways, have been successfully used in the sensitive and selective detection of various biomarkers or physiological signals, such as diabetes-related glucose and lactate, cancer-related microRNAs and protein, cardiovascular disease-related triglyceride (TG) and heart-beat (pulse), and arthritis-related collagenase and uric acid, demonstrating their great potential in future portable and wearable health monitoring and diagnostic toolsets.

There are recent review papers specifically on hydrogel-based immunoassays/immunosensors and metal nanoparticle–hydrogel composites for diagnosis. This review focuses more on the various kinds of hydrogel-based composites and their applications in different electrical and optical biosensors for disease diagnosis and health monitoring (Figures 1 and 2). First, we describe and compare the synthetic protocols for the various hydrogel-based composites. Depending on the properties of the filler materials, we describe the sensing mechanisms of the corresponding sensors. Significantly, we then provide a survey on the recent applications of the hydrogel composite sensors in health monitoring and diagnosis. Finally, potential research directions in the development of the hydrogel composite-based sensing platforms will be suggested.

2 | CLASSIFICATION AND PREPARATION OF HYDROGEL-BASED COMPOSITES

Up to now, hydrogels have been composited with a wide range of functional materials, and depending on the type of filler materials, they are classified as hydrogel–biomacromolecule composites, hydrogel–metal nanostructure composites, hydrogel–carbon...
nanomaterial composites,[28,31,34,36] and hydrogel–2D material beyond graphene composites (Figure 2).[83–89]

2.1 Hydrogel–biomacromolecule composites

Like in many conventional biosensors, the incorporation of biomacromolecules into hydrogels, such as enzymes, antibodies and nucleic acids, is necessary for the enhanced sensitivity and selectivity.[69–73] Moreover, the structural conformation and bioactivity of these biomacromolecules can be well protected and retained in the hydrogel matrices.[90–92] In this section, we introduce the common methods used to prepare hydrogel–biomacromolecule composites.

2.1.1 Hydrogel–enzyme composites

Enzymes, as proteins with catalytic activities toward biological reactions, are able to interact with hydrogels via weak intermolecular interactions, such as the van der Waals force, hydrogen bonding, π–π interaction, electrostatic force, and hydrophobic interaction.[93] For example, Kim et al. synthesized a PVA/β-cyclodextrin (PVA/β-CD) hydrogel by condensation polymerization. The cavities of β-CD are hydrophobic, and therefore, glucose oxidase (GOx) could be fixed with PVA/β-CD by incubating their mixed aqueous solution at 37°C through the hydrophobic interaction.[94] In fact, because enzymes carry surface charges from their functional groups such as –NH₂, –SH, –COOH, and –OH,[95] making composites via electrostatic interaction is another effective way to combine enzymes with hydrogels. For example, Zhang et al. used sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) to cross-link horseradish peroxidase (HRP) and GOx to prepare conjugated enzymes, which are electronegative at pH = 7.[96] The conjugated enzymes were then mixed with a solution containing sodium alginate monomers and CaCO₃ particles, and drop-casted over a working electrode (WE) (Figure 3A). Upon application of an anodizing current, the CaCO₃ particles released Ca²⁺ ions, which not only acted as the cross-linker to induce the gelation of the alginate monomers, but also assisted to trap the electronegative GOx/HRP conjugate into the alginate hydrogel via the electrostatic interaction.[98]

Although the direct mixing method mentioned above is easy to implement, the activity and stability of enzymes are prone to changes in temperature, solution pH, and ionic strength. To overcome this, enzymes have been encapsulated in certain cavities before being further combined with hydrogels.[99,100] Through an emulsion method, Bornhoeft and coworkers encapsulated GOx along with palladium tetracarboxyphenylporphyrin (PdTCPP) and catalase (Cat) inside alginate microparticles (Figure 3B). Onto these microparticles, poly(allylamine hydrochloride) (PHA), poly(sodium-4-styrenesulfonate) (PSS) and finally another layer of alginate shell was sequentially deposited layer-by-layer (LbL) to give a multilayered alginate in alginate (AnA) hydrogel with enzymatic activity.[100]

In addition to the weak intermolecular interactions mentioned above, immobilization of enzymes in hydrogels via cross-linking with bifunctional reagents such as glutaraldehyde,[101,102] glyoxal,[103] and hexamethylenediamine[104] have also been attempted for a better fixation of enzymes in the chemically bonded three-dimensional (3D) molecular networks. For instance, Das and Sarkar modified an electrode with a conducting hydrogel membrane based on PVA and PAM, over which urease was then covalently immobilized by using glutaraldehyde as the cross-linker.[90] Alternatively, Erfkamp et al. mixed urease in a monomer buffer solution containing acrylic acid (AAC), dimethylaminoethyl methacrylate (DMAEMA) and cross-linker bis-acrylamide, into which ammonium peroxodisulfate and N,N,N',N'-tetramethylethylenediamine (TEMED) were then added to initiate the copolymerization of poly(AAc-co-DMAEMA) (co means random copolymerization) hydrogel together with the in situ loaded urease.[105]
2.1.2 Hydrogel–antibody/antigen composites

Immunoassays that take the advantage of strong and specific interaction between antibodies and antigens are one of the most important diagnostic tools.\textsuperscript{[106–109]} To make antibodies/antigens attached to hydrogels firmly, they are usually chemically modified, for example, acrylated antigens/antibodies can easily react with polymer chains containing amide groups.\textsuperscript{[110,111]} On the other hand, the surfaces of hydrogels can also be functionalized specifically to better bond with antibodies/antigens. Examples of commonly used coupling agents are 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)\textsuperscript{[112,113]} and N-hydroxysuccinimide (NHS).\textsuperscript{[114]} For instance, Charles et al. prepared a 3D hydrogel based on two monomers, acrylamide and bis-acrylamide, polymerized with a homobifunctional cross-linker, that is, bis(sulfosuccinimidyl) suberate (BS\textsuperscript{3}) containing NHS-ester moieties. While part of the NHS-ester moieties could bind to the amide groups of PAM, the remaining ones provided covalent binding sites for the subsequent attachment of the staphylococcal enterotoxin B (SEB) antigens.\textsuperscript{[114]}

2.1.3 Hydrogel–nucleic acid composites

Nucleic acids, including DNAs and RNAs, are not only important bioagents for gene therapy, but also target-specific probes for biosensors.\textsuperscript{[115–117]} Despite the polymeric nature of nucleic acids, producing pure DNA or RNA hydrogels has been restricted by their poor mechanical properties and high cost. Therefore, nucleic acids are usually hybridized with hydrophilic polymers like PAM,\textsuperscript{[118–120]} poly(phenylenevinylene),\textsuperscript{[121]} and polypeptide,\textsuperscript{[122]} as well as other materials like graphene oxide (GO) and CNTs\textsuperscript{[123,124]} to form composite hydrogels.

Compared to physically mixing DNA or RNA strands with prepolymerized hydrogels,\textsuperscript{[125,126]} decorating nucleic acids on monomers of a hydrogel prior to polymerization
can enable stronger interaction between them and thus better stability.\textsuperscript{[60,127]} This can be achieved based on the fact that deoxyribonucleotides (the monomer of nucleic acids) contain phosphate groups that can react and bond with amino-containing hydrogel monomers. In a typical example, two oligonucleotides (single strands composed of less than 50 deoxyribonucleotides or ribonucleotides) with different sequences were modified with acrydite at the 5′-end with the phosphate group. The acrydite–DNA strands were hybridized with the ferrocene-tagged DNA probes and then in situ polymerized with PAM initiated by TEMED to give a hybrid poly(acrylamide–acrydite–DNA) hydrogel (Figure 3C).\textsuperscript{[60]} Alternatively, DNA strands can be functionalized to be able to bond covalently with a target hydrogel. For example, onto a PANI/phytic acid (PANI/PA) hydrogel, which was prepolymerized electrochemically on a glassy carbon electrode (GCE), Yang et al. casted DNA strands modified with carboxyl groups to initiate their covalent bonding with the amino groups of PANI/PA (Figure 3D).\textsuperscript{[128]}

2.2 Hydrogel–metal nanostructures composites

Noble metal nanostructures (NSs) such as nanoparticles (NPs), nanowires (NWs), and thin films of Au, Ag, or Pt are popular candidates in the formation of composites with hydrogels.\textsuperscript{[66,74–82,129–133]} Their introduction into the structures of hydrogels endows the composites with additional functional properties, such as optical,\textsuperscript{[15,16]} electrical,\textsuperscript{[17–19]} and catalytic properties.\textsuperscript{[21–25,40]} Generally speaking, hydrogel–metal NS composites can be prepared by either deposition of metal structures on/into a preformed hydrogel or by in situ polymerization of a hydrogel in the presence of presynthesized metal NSs.

2.2.1 In situ deposition of metal NSs in/on a hydrogel

Commonly, metal NSs can be coated on the surface or inside of a hydrogel structure by in situ electrochemical deposition or chemical reduction. For typical electrochemical deposition, after a hydrogel is loaded onto an electrode surface, it is immersed into an electrolyte containing metal ions, which will then be reduced electrochemically to metal NSs. Examples include deposition of Au thin film on PA hydrogel\textsuperscript{[130]} and AuNPs on aniline-vinyl ferrocene-PA hydrogel on GCE.\textsuperscript{[76]} As for in situ chemical reduction, metal salt solution and reducing agent are mixed together for the redox reaction to proceed in presence of a hydrogel. For example, chloroplatinic acid (H$_2$PtCl$_6$) can be reduced by formic acid in presence of PANI hydrogel predeposited on a GCE via electrochemical polymerization to give the PtNPs/PANI composite.\textsuperscript{[66]} In a very interesting work, Gniadek et al. took advantage of the oxidizing nature of metal ions, such as AuClO$_4$– and Ag$^+$, to initiate the oxidative radical polymerization of aniline monomers inside of PNIPAM hydrogels to obtain the AuNPs/PANI/PNIPAM and AgNPs/PANI/PNIPAM hydrogel composites, respectively.\textsuperscript{[77]}

2.2.2 Compositing hydrogel with presynthesized metal NPs

A key problem facing the in situ deposition of metal NSs in a hydrogel matrix is the poor control over their composition, size, shape, and distribution. Therefore, for applications that require a specific type of metal NSs, the NSs are synthesized prior to being mixed with a hydrogel solution or a pre-gel solution.\textsuperscript{[134–138]} For example, Wang et al. prepared AuNPs decorated with ZnCdHgSe quantum dots (QDs) (AuNPs/ZnCdHgSe) and loaded them on a GCE. A pre-gel solution of 3-[[4-([(carbamoyl)-amino]ethyl methacrylate]butyl ((carbamoyl)amino)ethylmethacrylate] propyl]-1-ethenyl-1H-imidazol-3-iium bromide (CCPEimBr) ionic liquid was then drop-casted onto the AuNPs/ZnCdHgSe/GCE for further in situ polymerization.\textsuperscript{[57]}

The loading density and distribution of metal NSs on a substrate can be controlled by making ordered arrays. For example, by using arrays of polystyrene (PS) colloidal monolayers as sacrificial templates, Li et al. prepared arrays of Au nanospheres by electrochemical deposition on quartz substrates (Figure 4A). Phenylboronic acids (PBA) pre-gel solution was then poured onto such an Au array, and cross-linked under UV light. This composite was further soaked into a PVA solution to finally produce the Au nanosphere array/PBA/PVA composite film.\textsuperscript{[139]}

In order to immobilize metal NSs more stably in a hydrogel, chemical bonds are to form between them. For example, Jing et al. mixed a thiolated gelatin (GE/SH) solution with an Ag nanowire (AgNW) solution and cooled the mixture down to 4°C for gelation, during which the AgNWs were strongly attached to the GE/SH hydrogel by formation of Ag–S bonds.\textsuperscript{[140]} Alternatively, metal NPs can be functionalized before being mixed with hydrogels. For example, Jiang and coworkers synthesized and modified AgNPs with N-(aminobutyl)-N-(ethylisoluminol) (ABEI), which contains amino groups that can react with the phosphate groups in the PANI/PA hydrogel prepolymerized on a GCE (Figure 4B), resulting in the formation of ABEI-AgNPs/PANI/PA hydrogel composite.\textsuperscript{[131]}
Likewise, Manickam et al. sequentially functionalized Au nanocubes (AuNCs) with 3-mercaptopropanoic acid (MPA) and cytochrome-c and blended them in chitosan-\(g\)-\(\beta\)-CD hydrogel (CS-\(g\)-\(\beta\)-CD, \(g\) means graft copolymerization) through thiol cross-linking.\(^{79}\)

In other cases, to prevent the aggregation of presynthesized metal NSs and loss of their surface activities, they can be protected, for example, by being encapsulated in SiO\(_2\) shells.\(^{141,142}\) For instance, Li and coworkers mixed presynthesized PtNPs with ethyl orthosilicate (TEOS) to prepare Pt@SiO\(_2\) core@shell NPs, which were afterwards physically embedded in a hyaluronic acid-polyethyleneimine (HA–PEI) hydrogel.\(^{80}\)

### 2.3 Hydrogel–carbon nanomaterial composites

Carbon nanomaterials, such as graphene and its derivatives,\(^{27,28}\) CNTs\(^{29–32,143}\) and CDs,\(^{33–37}\) are favored as filler materials in hydrogel composites due to a number of their attractive electrical, chemical, thermal, and mechanical properties.\(^{38–40,42–48,144}\) In general, most hydrogel–carbon nanomaterial composites are prepared by mixing pre-gel solutions with carbon nanomaterials followed by in situ polymerization.

Taking GO as an example, the abundant and hydrophilic oxygen-containing surface groups anchored on its surface
can assist the in situ polymerization/gelation of various hydrogels.\textsuperscript{[28,145,146]} As a typical example, such strategy was adopted by Wei and coworkers, who sequentially polymerized aniline and NIPAM in presence of GO to produce the composite of GO/PANI/PNIPAM.\textsuperscript{[147]} The oxygen-containing groups on the surface of carbon materials can even take part in the cross-linking reaction. For example, −COOH groups located on the edges of graphene sheets can cross-link with the amino acid chains of GE polypeptide under the agitation of simple ultrasonication.\textsuperscript{[148]}

### 2.4 Hydrogel–2D material beyond graphene composites

2D materials, analogs to graphene, such as TMDs, MXenes, and BP are emerging functional materials for electronics, optoelectronics, and biomedicine.\textsuperscript{[49–51]} They have exhibited composition, crystal structure, topology, and surface chemistry dependent electronic properties.\textsuperscript{[49,149–153]} The good conductivity of MXenes,\textsuperscript{[51,63,87,154,155]} tunable electronic/optoelectronic properties of TMDs\textsuperscript{[88,156–163]} and excellent photothermal properties found in MXenes and BP\textsuperscript{[83–85]} have made them attractive candidates to be composited with hydrogels for various biomedical applications.

2D materials are generally capable of providing large specific surface areas for interfacing with polymer chains. They can be combined with hydrogels via direct manual mixing during or after their exfoliation from bulk crystals.\textsuperscript{[86,87]} In other cases, in situ cross-linking/gelation is applied to realize better interaction between the nanosheets and polymers.

TMDs such as MoS\textsubscript{2} and WS\textsubscript{2} have been hybridized with hydrogels such as poly(ε-caprolactone)-b-poly(ethylene oxide) copolymer (PCL-b-PEO, b means block copolymerization),\textsuperscript{[157]} thermo-responsive polymeric ionic liquid (TRPIL),\textsuperscript{[159]} PANI,\textsuperscript{[164]} and PNIPAM.\textsuperscript{[163,165]} For example, WS\textsubscript{2} nanosheets were first mixed with alginate and PNIPAM through the interaction involving H-bonding and −NH•••S− bonding. After that, with the help of a cross-linker N,N-methylenebis(acrylamide) (BIS) and initiator ammonium persulfate (APS) and under the templating effect of ice crystals, WS\textsubscript{2}/PNIPAM composite hydrogels with plant-cell like microstructures were obtained (Figure 5A).\textsuperscript{[163]} In other examples, TMDs themselves can take part in the polymerization/gelation process. For instance, MoS\textsubscript{2} nanoplatelets can assist the production of radical species to initialize the polymerization of NIPAM monomers (Figure 5B).\textsuperscript{[160]} Without any added initiators, light irradiation on MoS\textsubscript{2} nanosheets in water can produce hydroxyl radicals (•OH), which in turn act as initiator to facilitate the in situ polymerization of NIPAM hydrogel.\textsuperscript{[11]}

Similar to TMDs, MXene (typically Ti\textsubscript{3}C\textsubscript{2})\textsuperscript{[154,155,166,167]} and BP\textsuperscript{[84,168]} nanosheets are usually mixed with monomers, cross-linker, and initiator for the in situ gelation to proceed. The advantage of MXene-based fillers, as compared to other 2D materials lies in the fact that acid-etched and isolated MXene nanosheets are usually decorated with abundant −O\textsubscript{x} and −F\textsubscript{x} functional groups,\textsuperscript{[169–171]} which provide additional cross-linking sites for polymer chains to anchor through H-bonding.\textsuperscript{[172]}

### 3 WORKING PRINCIPLES OF BIOSENSORS BASED ON HYDROGEL COMPOSITES

In hydrogel-based biosensors, hydrogels not only provide the good biocompatibility and porous structure with large surfaces for interfacing analytes, they may also undergo volume expansion or contraction as a result of stimuli-induced changes in polymeric chain conformation, loss of cross-linking points, uptake or expel of water molecules, and so on. These changes can be converted, with the assistance of the functional fillers, to optical\textsuperscript{[55,173–176]} and electrical\textsuperscript{[52,177,178]} signals. In the following contents, we will describe the working principles for various electrical and optical sensing methods involving hydrogel composites.

#### 3.1 Optical methods

Optical sensing methods usually show good-sensing specificity, low-noise background, and potential for multiplexing.\textsuperscript{[179–181]} Common optical detection methods are based on localized surface plasmon resonance (LSPR),\textsuperscript{[182,183]} optical diffraction,\textsuperscript{[111,184,185]} or fluorescence,\textsuperscript{[186–188]} which will be described in details in the following content.

##### 3.1.1 Sensing with plasmonic nanostructures

The LSPR in noble metal NSs describes the collective oscillation of the electron cloud in each structure upon interaction with the electric component of an incoming electromagnetic wave.\textsuperscript{[189]} The resonance or oscillation frequency, according to the Mie theory, is dependent on the composition, size, shape, inter-particle distance, and refractive index of the surrounding medium of noble metal NSs.\textsuperscript{[173,176,190]} LSPR is the origin of bright glow in a metal structure/colloidal solution, and is reflected in its adsorption spectrum, which provides the basis for optical sensing based on plasmonic NSs.
As mentioned in the beginning of this section, upon the exposure to external stimuli, the volume of a hydrogel structure can expand or contract. The distance of adjacent metal NSs embedded in the hydrogel is thus increased or decreased accordingly, which controls the coupling strength of NS plasmons and changes their LSPR adsorption intensity and wavenumber (Figure 6A).[167,184]

On the other hand, the change of the dielectric medium surrounding metal NSs can also influence their light adsorption spectrum. For example, the adsorption of target analyte, such as proteins, in the porous structure of a hydrogel can increase the refractive index of the surrounding medium of the embedded metal NSs and thus cause a red shift of their LSPR peak.[173,182,191]

### 3.1.2 Sensing with photonic crystals

Sensing based on the target-induced color change of a sensing film, which can be directly visualized with naked eyes, is attractive as it does not require sophisticated equipment or professional operators. 2D/3D periodic arrays of photonic crystals (PCs) of dielectric materials, such as PS, SiO₂, and TiO₂ make up an important part of visualizable sensors.[192–196] Their color, as a result of the destructive interference of the incident and reflected light at the boundaries between the PCs and substrate, can be tuned by changing their index of refraction and/or periodic structure.[197–202] For PCs combined with hydrogels, the volume change of the hydrogel due to interacting...
with target analyte can lead to an increase or a decrease of the periodicity of the embedded PC array, and thus a shift of the diffraction wavelengths (Figure 6B).

3.1.3 Fluorescence sensing

Sensing with fluorescent materials, such as organic dyes, QDs and CDs, is able to provide highly sensitive and selective optical response toward a wide range of analytes, and in some cases probe the structure and distribution of biomolecules through fluorescent mapping.

In addition, when hydrogels are composited with fluorescent materials, they can provide a stable environment to prolong the fluorescent emission lifetime.[205]

The working principles of hydrogel composite-based fluorescent sensors are mainly based on the fluorescence resonance energy transfer (FRET)[205] or photoinduced electron transfer (PET).[206] In FRET, the emission spectrum of a donor overlaps with the adsorption spectrum of an acceptor, and when they are close enough, the emission of the acceptor is partially or completely excited.[207,208] The spatial proximity of the donor and acceptor in a hydrogel matrix can be tuned upon target-induced volume change of the hydrogel, which in turn, modifies the FRET efficiency (Figure 6C), output as the change of the fluorescent intensity of the sensor.[186,187,209] PET, on the other hand, involves the sensing of oxidant species (Figure 6D), such as •OH and superoxide radicals (O2•–), which can quench the emission of fluorescent materials such as CDs by depriving their electrons via oxidation reaction.[175,188]

In fact, fluorescent fillers can help provide a visualizable indicator to reflect the macro-scale volume change of a hydrogel matrix upon its exposure to external stimuli. For example, 5,6-bicarboxylic fluorescein was cross-linked with partly ammoniated PAM to detect glutathione (GSH). This florescent hydrogel was initially in its shrunk state in presence of copper sulfate solution due to the coordination between Cu2+ and nitrogen groups (–CONH2, –CONH–, and –NH2) of the hydrogel. With the addition of GSH, the Cu2+ ions originally coordinated with the hydrogel were released to bond with the mercapto (–SH) groups in GSH, resulting in the swelling of the hydrogel. The fluorescent volume change, which was perceivable with naked eyes, correlates to the GSH concentration.[210]

3.1.4 Electrochemiluminescence sensing

In recent years, electrochemiluminescence (ECL) has attracted increasing attention in bioassays due to the combined advantages of electrochemical and chemiluminescent techniques.[138,211] Many organic and inorganic ECL materials have been used for the determination of biomarkers, such as tris(2,2′-bipyridyl) ruthenium(II) ([Ru(bpy)3]2+) luminol and luminol derivatives.[211,213,214] Among them, luminol and its derivatives, whose light emission is based on the nucleophilic addition to carbonyl compounds, are particularly known for their high optical efficiency.[211] The emission of luminol can be enhanced by reactive radicals such as •OH and O2•–,[113,211] which are usually generated by H2O2 decomposition under the catalytic effect of enzymes or catalysts such as HRP.[215–217] and PtNPs.[218,219] For example, an ECL sensor based on Au star/bovine serum
albumin (BSA)/luminol nanocomposites and PANI hydrogel decorated with PtNPs was developed for detection of human chorionic gonadotropin (HCG) (Figure 6E).\cite{211}
The specific HCG antibodies (Ab1) were loaded on PANI/PtNPs, and HCG detection antibodies (Ab2) and luminol were loaded onto the Au star/BSA nanocomposites to form a luminol/Au star/BSA/Ab2 complex. The capture event of the target HCG sandwiched between Ab1 and Ab2 brought the luminol and PtNPs closer, such that the fluorescent signal of the luminol was amplified by O$_2$•• radicals decomposed from H$_2$O$_2$, catalyzed by the PtNPs anchored on the PANI hydrogel.

3.2 | Electrical methods

3.2.1 | Electrochemical detection

Electrochemical biosensors are one of the most investigated biosensing systems, capable of providing a wide linear range, high sensitivity, low sensing limit, and rapid response time.\cite{66,220,221} A typical electrochemical sensor consists of a WE, a reference electrode (RE), and a counter electrode (CE) (Figure 7A). The WE is where the sensing material together with enzymes or metal catalysts are deposited, and the reaction with the target analyte takes place under an applied potential or current.\cite{222–225} For example, the peaks in the current profile obtained by ramping up and down a linear potential range (also called the cyclic voltammetry curve) indicate the redox events occurring on the WE. The absolute value of the peak amplitude is directly proportional to the concentration of the analyte.\cite{226,227} Hydrogels composited with carbon nanomaterials such as multiwalled CNTs\cite{178} and GO\cite{228} are popular for fabrication of electrochemical biosensors. The hierarchically porous and nanostructured matrices of hydrogels are favorable for enhancing molecular permeability, and the carbon nanomaterials can provide a highly conductive network and additional active surfaces.

3.2.2 | Piezoresistive detection

Besides the three-electrode system employed in an electrochemical sensor, hydrogel composited with conducting materials can also be incorporated in a simple two-electrode setup for strain/stress sensing, such as monitoring human movements, pulses, and voiceprints.\cite{140,154,229,230} Typically, stretching or compression of 3D structure of a hydrogel composite can result in change of the number of contact points and contact area of the conducting fillers as well as spacings between them, leading to a direct change of the electrical resistance of composite (Figure 7B).

3.2.3 | Mass measurement

Mass change measured by electronic balance or quartz crystal microgravimetry (QCM) has also been employed for hydrogel composite-based biodetection.\cite{65,80} In a very interesting design by Li and coworkers, a HA and PEI-based hydrogel was composited with Pt@SiO$_2$NPs (Figure 7C). In the presence of the target analyte, that is, hyaluronidase (HAase), HA was digested by HAase, resulting in the release of Pt@SiO$_2$NPs from the disrupted hydrogel structure. In a drainage device, the released Pt@SiO$_2$NPs were mixed with H$_2$O$_2$ and catalyzed the decomposition of H$_2$O$_2$ into H$_2$O and O$_2$. The released O$_2$ gas caused a pressure increase of the drainage device, such that certain amount of H$_2$O overflowed. The weight of the collected water was measured by an electronic balance and found to be linearly related with the concentration of HAase.\cite{80}
4 | APPLICATIONS OF COMPOSITE HYDROGEL-BASED BIOSENSORS

Combining the versatile functional properties of the hydrogel matrices and filler materials, assisted by the various electrical/optical transducing strategies, highly sensitive, selective, and reliable detection of chemical biomarkers in blood and urine and other physical signals have been demonstrated over the past years, as summarized in Table 1. In this section, we will describe the applications of hydrogel composite-based biosensors for health monitoring and disease diagnosis.

4.1 | Diabetes

Diabetes, caused by the metabolic disorder and characterized by a high level of blood glucose, is tormenting ∼5% of the world’s population and can lead to serious complications like stroke, heart disease, damages to the nerves and kidneys, limb amputation, and blindness. Because of the lack of measures to cure diabetes, early diagnosis and continuously monitoring the glucose level in biological fluids such as blood, sweat, and tear are essential to help postpone these complications.

Glucose detection is generally realized in electrochemical sensors that involve enzyme-assisted amperometric processes by combining Gox with conductive materials such as conducting polymers, carbon nanomaterials, metal NPs, and 2D materials beyond graphene. The introduction of biocompatible hydrogels improves the immobilization and stabilization of GOx and the permeation of water-soluble molecules, which facilitate the effective oxidation of glucose. For example, by combining Gox with PtNPs/PANI composite hydrogel, Zhai and coworkers prepared a highly sensitive glucose sensor, exhibiting high sensitivity up to 96.1 μA·mM⁻¹·cm⁻², a linear range of 0.01–8 mM and a low detection limit of 0.7 μM (Figure 8A, B). The excellent performance was attributed to the collective contribution from the porous and conductive PANI hydrogel for enzyme immobilization, ion diffusion and charge transfer, and the catalytic PtNPs that boost the decomposition of H₂O₂ (the product of glucose oxidation) to provide additional electrochemical signals.

Although enzymes are highly selective receptors for certain biomarkers, the risk of protein denaturation has limited their service life. Therefore, enzyme-free glucose detection methods are well needed. Many noble NSs such as AuNSs, PtNSs, and PdNSs have been found to exhibit enzyme-like catalytic activities. Very recently, by taking the advantage of the ability of PdNWs to catalyze glucose oxidation, Li and coworkers prepared a PdNWs/3D-PANI hydrogel by electrodeposition. This sensor showed a high sensitivity of 146.6 μA·mM⁻¹·cm⁻², a linear range from 5.0 to 9800 μM, and a low detection limit of 0.7 μM, demonstrating its potential to be used for glucose detection in human serum. Enzyme-free glucose detection can also be realized via specific molecular interactions. Recently, boronic acid derivatives have been used for such purpose because their PBA units can reversibly bind to diols of glucose to form five- or six-membered cyclic boronic esters. Wustoni and coworkers prepared a conductive composite gel composed of poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonic) and PBA-modified PAM. The capture and binding of glucose on PBA caused a volume change of the composite hydrogel, which was transduced into a measurable electronic signal via the conductive network. Very recently, Sawayaama and Takeuchi fabricated PBA/polyethylene glycol (PBA/PEG) hydrogel fluorescent sensors and demonstrated their implantation in rats and a pig for the long-term continuous glucose monitoring, showing weakened foreign body reactions and satisfied accuracy for clinical use (Figure 8C, D).

For diabetes, the dehydrogenase of pyruvate, which is the metabolic product of glucose, is usually inhibited, and an excess of pyruvate is likely further converted to lactate and released to blood. Therefore, an elevated lactate level in blood has also been used as an indicator for the condition of diabetes. For instance, Li and coworkers reported a “drop-on-demand” inkjet printing process to fabricate enzymatic lactate sensors by direct printing lactate oxidase (LOx) droplets on PANI hydrogel and achieved a quick lactate detection with a sensitivity of 3.94 μA·mM⁻¹·cm⁻² between 0.08 and 5 mM (Figure 8E, F).

4.2 | Cancer

Cancer is one of the leading causes to death worldwide; the effective and reliable early diagnosis of cancer is essential to prevent its development to a life-threatening stage. The diagnosis of cancer is achieved through the detection of certain biomarkers, such as nucleic acids, proteins, and amines. MicroRNAs are a type of short (~22 nt) endogenous non-coding RNAs, which are involved in regulating the gene expression and can affect various biological processes such as cell proliferation, differentiation, and apoptosis. Circulating microRNAs released from cancer cells have been used as biomarkers for different cancers. Liu and coworkers fabricated an electrochemical biosensor.
| Hydrogel-based composites                  | Sensing type          | Biomarkers                  | Disease      | LOD (Limit of detection) | Ref. |
|-------------------------------------------|-----------------------|-----------------------------|--------------|--------------------------|------|
| PANI/PtNPs/LOx                            | Electrochemical       | Lactate                     | Diabetes     | 3.94 μA⋅mM⁻¹⋅cm⁻²        | [62] |
| PVA/β-CD hydrogel/GOx                      | Electrochemical       | Glucose                     | Diabetes     | 5.141 × 10⁻⁴ M          | [84] |
| PtNPs/PANI/GOx                             | Electrochemical       | Glucose                     | Diabetes     | 0.7 μM                   | [78] |
| PtNWs/3D-PANI hydrogel                     | Electrochemical       | Glucose                     | Diabetes     | 0.7 μM                   | [59] |
| PAAc/Cds/HRP/GOx                           | Fluorescence          | Glucose                     | Diabetes     | 0.0516 mM                | [322]|
| DAPEG/AA-A-Cds/Rh6G/GOx/HRP               | Fluorescence          | Glucose                     | Diabetes     | 0.08 μM                  | [175]|
| PANI/PA hydrogel/DNA strands               | Electrochemical       | miRNA24                     | Cancer       | 0.34 fM                  | [128]|
| HA/PEI/Pt@SiO₂NPs                         | Mass                  | HAase                       | Cancer       | –                        | [80] |
| DNA/PAM hydrogel                          | Electrochemical       | miR-21                      | Lung cancer  | 5 nM                     | [60] |
| PA hydrogel/AuNPs/anti-CYFRA21-1           | Electrochemical       | CYFRA21-1                   | Lung cancer  | 38 fg⋅mL⁻¹                | [130]|
| DNA hydrogel/G-rich fragments (G3 and G9)  | Fluorescence          | miR-21                      | Lung cancer  | 5 pM                     | [61] |
| CBAmN₃, hydrogel/anti-HER2/lectins         | Electrochemical       | HER2                        | Breast cancer| 5 pg⋅mL⁻¹                 | [109]|
| CCPEimBr hydrogel/AuNPs                   | Photo-electrochemical | HE4                         | Ovarian cancer| 15.4 pg⋅mL⁻¹              | [57] |
| CB/AG hydrogel                            | Fluorescence          | Spermine/spermidine        | Prostate cancer| 6 μM                    | [233]|
| PANI/PVA/SD aptamers (1 and 2)             | Electrochemical       | TB                          | Leukemia     | 0.64 pM                  | [51] |
| PNiPAM/collagen                           | Strain                | 4T1                         | Tumor        | –                        | [236]|
| PANI/LIP/GK/GO/PtNPs                      | Electrochemical       | TG                          | Cardiovascular disease| 7.49 μA⋅mM⁻¹⋅cm⁻²| [62] |
| PANI/PtNPs/LIP/GK/GOP                     | Electrochemical       | TG                          | Cardiovascular disease| 0.2 mM     | [66] |
| PANI/PtNPs/ChEt/ChOx                      | Electrochemical       | Cholesterol                 | Cardiovascular disease| 0.3 mM     | [66] |
| MXene/PVA/PVP                             | Strain                | Pulse                       | Cardiovascular disease| 0.87 Pa    | [61] |
| PVA/SWCNT/PDA hydrogel                    | Strain                | Pulse                       | Cardiovascular disease| –          | [64] |
| PAM/AuNWs                                 | Strain                | Pulse                       | Cardiovascular disease| 0.2 Pa     | [238]|
| PAM/CNTs                                  | Strain                | Pulse                       | Cardiovascular disease| –          | [323]|
| PSGO/PEDOT/PAM hydrogel                   | Strain                | ECG signals                 | –            | –                        | [306]|
| PANI/PtNPs/UOx                            | Electrochemical       | Uric acid                   | Gout         | 0.001 mM                 | [66] |
| ABEI-AgNPs/PANI-ATMP/XO                   | Electrochemical       | Xanthine                    | Gout         | 9.6 nM                   | [322]|
| PEG/peptide                               | Mass                  | Collagenase                 | Arthritis    | 2 nM                     | [65] |
| PANI/PVA/PAM/urease                       | Electrochemical       | Urea                        | Nephritic syndrome| 14 μM     | [90] |
| Poly(AAc-co-DMAEMA)/urease                | Piezoresistive        | Urea                        | Nephritic syndrome| 1 nM      | [105]|
| CMD hydrogel/anti-HGH                     | SPR                   | HGH                         | Physical and psychological symptoms| –     | [324]|
| ABEI-AgNPs/PANI/PA hydrogel               | Electrochemiluminescence | H₂O₂                      | –            | 3.3 nM                   | [131]|
| CS-g-β-CD hydrogel/AuNCs                  | Electrochemical       | H₂O₂                        | –            | 15 nM                    | [79] |

Abbreviations: AA, ascorbic acid; A-CD, acryl groups-functionalized CD; CBAmN₃, poly{4-[[(3-methylacrylamidopropyl)dimethylamino-bromobutryrate]-CO-[N-methacryloyl-4-azidotriazolin]]/[poly{4-[[(3-methylacrylamidopropyl) dimethylamino-bromoacetate]-CO-[N-methacryloyl-4-azido-aniline]]}; ChEt, cholesterol esterase; ChOx, cholesterol oxidase; DAPEG, diacrylated poly(ethylene glycol); GK, glycerol kinase; GPO, glycerol-3-phosphate oxidase; LIP, lipase; PAAC, poly(acrylic acid); Rh6G, rhodamine 6G; UOx, urate oxidase.
based on acrydite-modified DNA/PAM hydrogel, which was further functionalized with ferrocene-labeled recognition DNA probes for the detection of lung cancer-specific microRNA (i.e., miR-21) (Figure 9A, B). The rupture of the hydrogel structure when the DNA probe was hybridized with the target miR-21 led to the loss of the ferrocene tags and generation of electrochemical signals with a detection limit of 5 nM (1 pmol) and linear read-out from 10 nM to 50 μM. [60] Alternatively, Song et al. fabricated a fluorescent biosensor based on DNA hydrogel for miR-21 sensing. Two G-rich fragments (G3 and G9) were introduced in the DNA hydrogel for the formation of G-quadruplex structures when binding with miR-21, which could further interact with thioflavin T, a G-quadruplex-specific fluorescent probe, giving rise to detectable fluorescent signals. An ultralow detection limit of 5 pM and a linear range from 0.5 to 50 nM was achieved. [61]

Besides microRNAs, various proteins are also important biomarkers for cancer diagnosis, and their specific detection can be realized by introducing recognition probes in hydrogels through hydrogen bonding, electrostatic forces, and antibody–antigen or aptamer–antigen interactions. [109,277–280] For example, human epididymis protein 4 (HE4) is a biomarker for early diagnosis of ovarian cancer. [281] Recently, Wang and coworkers developed a photo-electrochemical HE4 sensor based on an ionic liquid CCPEimBr hydrogel, in which the amino group, carboxyl, and imidazolium cations can interact with HE4 through hydrogen bonding and electrostatic attraction. Into this gel, ZnCdHgSe QDs/AuNPs were introduced as cooperative photoactive materials for enhancing the photo-electrochemical sensing of HE4, over a linear range from 25 pg·ml⁻¹ to 4.0 ng·ml⁻¹, with a detection limit of 15.4 pg·ml⁻¹. [57] As for the detection of human epidermal
growth factor receptor 2 (HER2), a biomarker of breast cancer, Chocholova et al. reported a disposable electrochemical biosensor by integrating anti-HER2 and lectins (glycan-recognizing proteins) in a zwitterionic polymer-based hydrogel.\textsuperscript{109} While the anti-HER2 facilitated the capture of HER2, which was then evaluated based on the changes in its glycan profile assisted by lectins, the zwitterionic property of the hydrogel enabled the nonspecific protein binding, thus achieving a highly selective HER2 sensing with a limit of detection down to 5 pg·mL\textsuperscript{-1}. Sandwich-structured bioassays that usually involve two lock-and-key interaction events can lead to enhanced sensing performance.\textsuperscript{282,283} This was recently demonstrated by Wang et al. who fabricated an aptasensor for thrombin (TB),\textsuperscript{53} a blood-clotting factor and a biomarker for leukemia.\textsuperscript{284} In this sensor design, two TB aptamers (1 and 2) were used: while the former was used to modify the PANI/PVA conductive hydrogel to capture TB, the latter was linked to magnetic NPs as signal amplification probes, achieving an ultralow limit of detection down to 0.64 pmol/L and a linear range from 1 pmol/L to 10 nmol/L.\textsuperscript{53}
Spermine/spermidine are polyamines involved in many biological processes, and are the key biomarkers for prostate cancer diagnosis. Nair et al. fabricated a rapid fluorescent-sensing platform for spermine/spermidine by incorporating the fluorescent 3-((7-hydroxy-4-methylcoumarin)methylene)aminophenylboronic acid (CB) into a natural polymer, polysaccharide agarose (AG) (Figure 9C, D). Due to the specific interaction between CB and spermine/spermidine, this sensing platform exhibited highly sensitive and selective spermine/spermidine responses, for example, with a low limit of detection of 6 μM over a wide detection range of 6 μM to 2.5 mM for spermine.

H₂O₂ is one of the reactive oxygen species produced by the intracellular oxygen metabolism and is closely related to the signal transduction and cell growth; an abnormal concentration of H₂O₂ can point to cell mutation and apoptosis, which may correlate to diseases such as cancer. The concentration of H₂O₂ secreted from cells can be measured by electrochemical sensors that incorporate hydrogels and enzymes such as HRP and noble metals like AgNPs and spermine.

In addition to the aforementioned cancer diagnosis based on the detection of chemical biomarkers, the fact that tumor tissues exhibit different mechanical properties compared to normal tissues can also be used as the basis for diagnosis. For example, Mok and coworkers prepared fluorescence-labeled thermoresponsive PNIPAM hydrogel microspheres, termed microscale temperature-actuated mechanosensors (μTAMs), for the measurement of local tissue mechanics at cellular length scales (Figure 9E–G). The collagen-functionalized μTAMs were injected into the mice, dispersed within the tumor developed from the subsequently injected 4T1 metastatic cancer cell line. By mapping the expansion of μTAMs, the residual elasticity of the surrounding tumor can be inferred. Surprisingly, localized sites with high rigidity were observed as a result of the invasion of cancer cells, providing new opportunities for the early diagnosis of cancer.

### 4.3 Cardiovascular disease

Cardiovascular diseases cover a number of conditions relating to heart and blood vessels, such as heart attack, heart failure, stroke, and arrhythmia. Many of them are caused by atherosclerosis and thrombosis, which are characterized by a high blood level of TG, an ester comprising one glycerol and three fatty acid molecules. Various types of sensors have been developed for the determination of TG levels in body fluids such as blood and urine. For example, Li et al. inkjet-printed different enzymes including lipase, glycerol kinase and L-α-glycerophosphate oxidase on PANI to realize TG detection through a series of reactions to sequentially convert TG to glycerol, L-α-glycerophosphate, and H₂O₂, respectively. The produced H₂O₂ was further oxidized on PtNPs to produce detectable electrical signals. This printed sensor achieved a high sensitivity of 7.49 μA·mM⁻¹·cm⁻² of TG, and the sensing results acquired in phosphate buffer saline (PBS) and in serum showed negligible variation indicating the potential application of this sensor for practical use.

Besides chemical biomarkers, human physical signals such as heartbeat (pulse) and blood pressure can also be used for the diagnosis of cardiovascular diseases For instance, the pulse wave can reflect the radial dilation index, which is an important indicator for the degree of cardiovascular sclerosis. To detect these physical signals, strain sensors based on hydrogels with embedded conductive nanomaterials have been used. For instance, Liao et al. fabricated a strain sensor based on PVA/single-wall CNT/polydopamine (PVA/SWCNT/PDA) hydrogel. Due to the self-adhesive nature of PDA, the PVA/SWCNT/PDA hydrogel was closely adhered to the beating position of one’s wrist for stable, repeatable, and accurate pulse monitoring (Figure 10A, B). A similar strain sensor based on the polydopamine-reduced and sulfonated graphene oxide (PSGO) nanosheets composited with poly(3,4-ethylenedioxythiophene)/polydopamine (PSGO/PEDOT/PAM) hydrogel was recently developed by Gan et al. and realized accurate and reliable electrocardiogram (ECG) recording (Figure 10C–E).

In addition to these hydrogel–carbon nanomaterial composites, hydrogel–MXene composites are currently emerging strain-sensing materials. As a typical example, Lu et al. constructed a highly strain-sensitive MXene/PVA/PVP hydrogel for pulse monitoring. The sensor exhibited a high sensitivity (10.75 kPa⁻¹), wide response range (0–61.5 kPa) and fast response (33.5 ms) with a low detection limit of 0.87 Pa suitable for portable and low-cost cardiovascular health monitoring.

### 4.4 Arthritis

Osteoarthritis and rheumatoid arthritis, which can induce the arthrocele and ankylosis, can cause severe pain and even deprive patients of normal life. Collagenase has been recognized as a biomarker for both types of arthritis. Its detection was recently realized by Ahmad et al., who deposited a peptide cross-linked PEG hydrogel on a QCM (Figure 11A, B). Collagenase can
degrade the peptides as the cross-linkers and thus disrupt the hydrogel network, leading to a mass reduction on the QCM. The sensor exhibited a concentration-dependent response toward 2–2000 nM collagenase with a response time of less than 10 min.

Gout is another type of arthritis, usually characterized by sudden and severe pains, swelling, and redness in the joints, very often at the big toe. It is caused by the accumulation of urate crystals in the joints, due to excess level of uric acid in blood. Direct detection of uric acid is a way to monitor gout conditions. Wang et al. recently realized the highly sensitive and selective electrochemical detection of uric acid by using a 3D porous hydrogel composed of tartrazine (Tz)-doped polypyrrole (PPy). Besides uric acid, xanthine as a precursor for uric acid in human metabolism, can also serve as a biomarker for gout. Xu et al. developed an electrochemical xanthine sensor by combining amino trimethylene phosphonic acid (ATMP) functionalized PANI, ABEI functionalized AgNPs and xanthine oxidase (XO), and achieved a low detection limit down to 9.6 nM (Figure 11C, D).

4.5 Others

Hydrogel-based immunoassays/immunosensors with immobilized antibodies have been widely used for the detection of human hormones/growth factors to monitor the biological state of human body. For example, the human growth hormone (HGH) is a type of peptide that is vital for the normal growth of all our tissues and development of our bodies. Either an excess or deficient level of HGH can lead to various physical and psychological symptoms. Kausaite-Minkstimiene et al. reported an SPR sensor for HGH by covalent immobilization of antibodies for HGH (anti-HGH) in a carboxymethyl dextran (CMD) hydrogel. They also found that orientationally immobilized anti-HGH as compared to the randomly immobilized counterpart could provide a much higher HGH binding capacity. Likewise, other hormone-based biomarkers, such as the placental growth factor (PLGF) and chorionic gonadotropin beta (CG beta) as indicators of complications in pregnancy, have also been detected by immunosensors based on hydrogels.
5 CONCLUSION AND OUTLOOK

In this review, we have firstly described the various preparation methods for hydrogel-based composites. For nanostructured filler materials, such as CNTs, graphene and other 2D materials, they are usually mixed with pre-gel solutions followed by in situ gelation/cross-linking. Their surface functional groups, such as oxygen-containing groups on GO and –Ox and –Fx on MXene nanosheets, can provide hydrogen-bonding or cross-linking sites for gelation. For metallic NSs, they can also be in situ deposited in/on hydrogels via chemical or electrochemical reduction. As for the hydrogel–biomacromolecule composites, besides the weak intermolecular interactions (e.g., van der Waals forces, hydrogen bonding, π–π interaction, electrostatic forces, and hydrophobic interactions) existing between the fillers and matrix, cross-linking and covalent bonding can be induced by chemical modifications before gelation.

Biosensors based on hydrogel composites are categorized as electrical and optical sensors according to their modes of signal transducing. For optical biosensors, their sensing mechanisms are mainly based on the change of LSPR adsorption, optical diffraction, and fluorescence signals upon analyte exposure by taking advantage of the embedded plasmonic nanostructures, PCs, and dyes/QDs, respectively. Electrical sensors based on hydrogel composites are based on electrochemical events or piezoresistive effect. While the former is realized through the electrochemical reactions involving the analytes on a hydrogel composite-modified WE, the latter is mainly achieved via the resistance change due to the stimuli-induced mechanical deformation of a hydrogel composite. Besides, mass measurement has also been explored for biosensing. Hydrogel matrices in these systems not only provide porous network for analyte diffusion and ion/electron transfer, but also help enhance the signal transducing due to their chemical, mechanical, and/or topological changes in responding to stimulus.

Highly sensitive, selective, and stable detection of various disease-associated biomarkers and physiological signals have been realized with these hydrogel composites. Examples include the detection of diabetes-related glucose and lactate, cancer-linked microRNA, proteins, polyamines and H2O2, cardiovascular disease-associated TG and heartbeat/pulse, and arthritis-related collagenase and uric acid. Implanted sensors for in vivo evaluation of cancer tissue development have also been developed.
based on florescence hydrogel composites based on tissue rigidity.

Most of the aforementioned biomarker detections were implemented in simple matrices or in blood, which may contain a high concentration of biomarkers for detection.\textsuperscript{328} The increasing demand for noninvasive diagnostic devices based on detection in other body fluids such as urine, saliva, sweat, and tear, which contain a much lower level of biomarkers than in the blood stream, poses greater challenges in developing more sensitive and selective sensing platforms.

The advantages of hydrogels as biocompatible, adhesive, and skin-like substrates for wearable and attachable health-monitoring and diagnosis devices have so far mostly been demonstrated in strain sensors for pulse/heartbeat monitoring. E-skins for sweat content monitoring is still limited in some common electrolytes and metabolites such as sodium and chloride ions, glucose, lactate, uric acid and creatinine, as well as pH levels. Highly sensitive, selective, and rapid detection of a wider range of biomarkers such as proteins and nucleic acids present in other body fluids such as sweat and tears is still challenging and requires continuous efforts.

It is important to point out that hydrogel composites have also shown abilities in drug delivery, wound healing, and tissue replacement. Some recent investigations have demonstrated the integration of different functions in individual hydrogel-based devices.\textsuperscript{329–332} For example, Chekini et al. constructed a wound dressing based on CD–cellulose–GE hydrogels that possess not only the antibacterial ability but also the sensing ability toward Fe\textsuperscript{3+} ions based on the charge transfer-induced fluorescent quenching (Figure 12A).\textsuperscript{329} In another example, by introduction of PCs (Fe\textsubscript{3}O\textsubscript{4}@C) into drug-loaded P(NIPAM-co-AAc) hydrogel-functionalized textiles, Gong et al. realized both the on-demand drug release and visualizable real-time monitoring of the drug content in the hydrogel (Figure 12B, C).\textsuperscript{330} Very recently, an ionic conductive carboxymethyl cellulose P(AAc-AM)-Al\textsuperscript{3+} (CMC/P(AAc-AM)-Al\textsuperscript{3+}) hydrogel has been prepared for the detection of human motion and body temperature based on the resistance change due to the variation of conductive path and ion migration rate, respectively.\textsuperscript{332} It thus can be expected that wearable or implantable multifunctional health care platforms that can provide both diagnosis and treatment would be realized by combining hydrogels with different functional materials and device structures in the future.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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