

Development and application of DNA hydrogels in biosensing :

Current status and future implications

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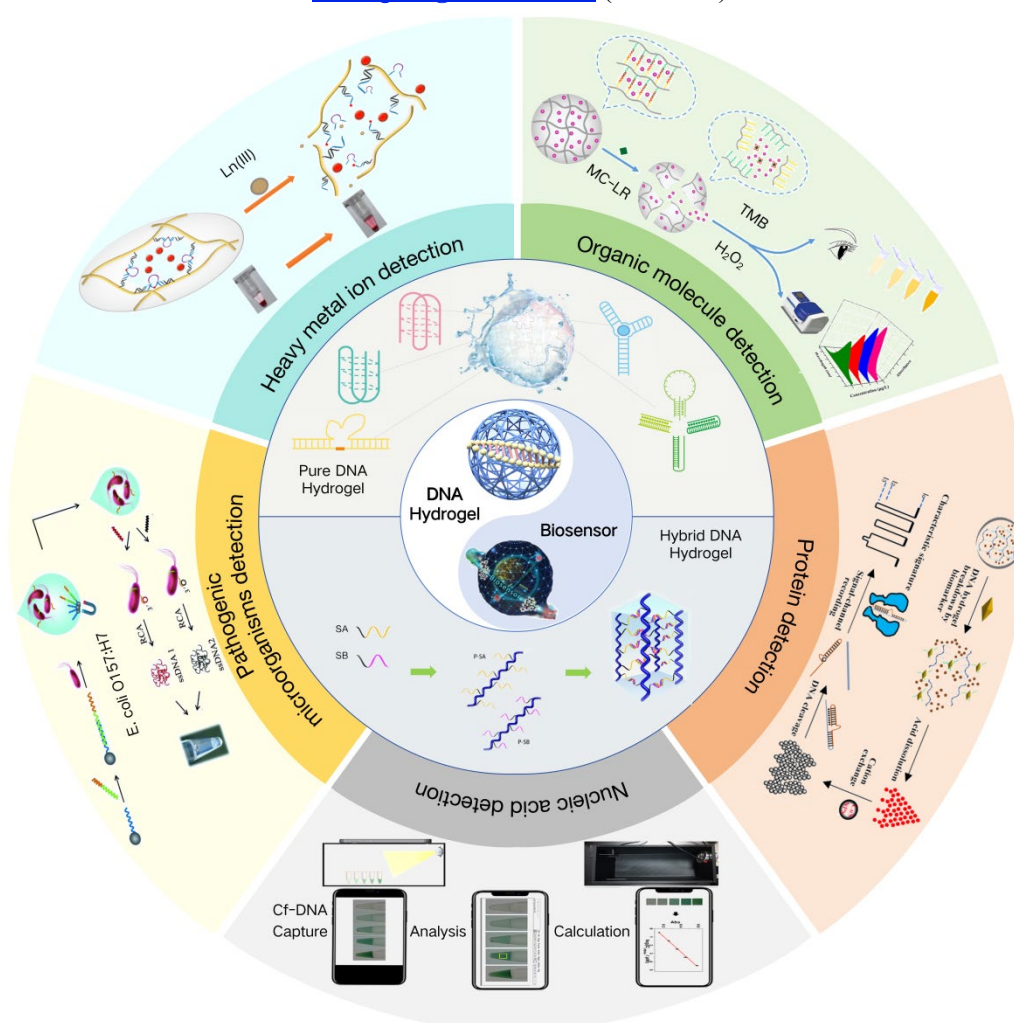
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Graphical abstract

Abstract

DNA hydrogels, as an emerging biopolymer material, have become widely used in the field of biosensing due to their quick response to external stimuli and their ability to recognize DNA specifically through complementary base pairing. Unlike traditional biosensing strategy, DNA hydrogels-based biosensors are highly specific, programmable and degradable. Based on the advantages of DNA hydrogels in the field of biosensing, this work systemally reviews the development and application of DNA hydrogel-based sensing and analytical methods in the detection of various targets, including heavy metals, organic small molecules and macromolecules, and microorganisms. First, the types of DNA hydrogels used are briefly introduced; then, recently developed DNA hydrogels-based biosensors are categorized depending on the synthesis principle of the DNA hydrogels, along with the signal conversion and signal detection methods; we also discuss the characteristics and (dis)advantage of these DNA hydrogels-based biosensor and their application in different sample for various targets. Finally, the challenges and perspectives of DNA hydrogels in biosensing are discussed. It is hoped that this paper can provide a reference for research on sensing analysis using DNA hydrogels.

Keywords: DNA hydrogel, biosensor, aptamer, heavy metal, pathogen

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1. Introduction

Hydrogels are three-dimensional water-soluble polymers with high structural plasticity and the ability to respond to external stimuli (temperature, pH, light, electricity, etc.).^[1] According to their interconnections, hydrogels can be divided into chemically crosslinked hydrogels and physically crosslinked hydrogels^[2]. Chemically crosslinked hydrogels are crosslinked by molecular chemical bonding between crosslinking agents; therefore, unlinking chemically crosslinked hydrogels is difficult. Physically crosslinked hydrogels, on the other hand, are crosslinked by intermolecular forces between the crosslinking agents, and they can undergo a change in phase state under external stimuli. Chemically crosslinked^[3] hydrogels are often used to provide structural support, such as in bone repair and wound dressing^[4], due to their high mechanical strength and their lack of susceptibility to changes in phase state. Physically crosslinked hydrogels, in contrast, undergo decrosslinking under external stimuli, resulting in a reversible conversion between gel and sol. Because the hydrogel itself is highly biocompatible, it can be used as a carrier for drug delivery or to test different target-responsive properties. Whether physically or chemically crosslinked, hydrogels are biocompatible and stimulus responsive; thus, hydrogels have gained much attention in the fields of biomedical and biochemical analysis.

DNA aptamers, a class of short single-stranded DNA molecules that can bind specifically to target molecules, were identified by screening synthetic oligonucleotide libraries by the systematic evolution of exponentially enriched ligands (SELEX) technique^[5, 6]. Due to their specific recognition ability, DNA aptamers have been used in many applications in the field of biosensing detection. Aptamer functionality can be further improved by modifying the terminal groups, for example, by modification with nanogold to enable colorimetric detection of the aptamers^[7], in addition to modifying the fluorescent^[8], electrochemical^[9], and colorimetric/fluorescent dual-signalling molecules^[10]. To achieve different types of detection, in addition to functionalization with signalling molecules, aptamers can be combined with other materials, such as magnetic nanomaterials^[11], antibodies^[12], and hydrogel materials, to enrich and detect

the target molecules and to achieve the multifunctional application of aptamer-functionalized materials.

Due to the stimulus responsiveness of hydrogels and their specific recognition of DNA, DNA hydrogel biosensors, which combine DNA with hydrogel materials, have attracted considerable attention. DNA hydrogels are a class of three-dimensional network-structured materials with polymers (e.g., polyacrylamide, polyethylene glycol, polysaccharides, proteins, or DNA ^[13]) serving as the backbone. Currently, DNA hydrogels are mainly classified into two kinds of DNA hydrogels based on their backbone structure: physically crosslinked hydrogels, in which DNA aptamers act as crosslinking agents to crosslink polymers through hydrogen bonding, and DNA hydrogels, formed by aptamer self-assembly after massive amplification. DNA hydrogels have recently been applied widely as drug delivery carriers ^[14] as well as biosensors ^[15]. Especially, DNA hydrogel sensors have exhibited advantages over traditional assays in terms of both cost and portable detection. By combining the specificity of aptamers with the stimulus responsiveness of hydrogels, DNA hydrogel biosensors can recognize one or more targets and undergo structural changes that alter their physicochemical properties ^[16-18]. The properties of both the DNA and the hydrogel can be designed to release signals upon the destruction of the DNA hydrogel network structure. The relationship between the DNA aptamer and the substrate is similar to the relationship between "lock" and "key". In the presence of the target, the specific binding between the aptamer and target and the hydrogel crosslinking network will be destroyed; at this time, the signalling molecule is released for subsequent quantitative detection, and during the selective replacement of the aptamer, the DNA can be modified to achieve specific recognition of different substances. Compared with the large-volume instruments used in traditional detection, DNA hydrogels not only can be prepared in advance to enable convenient on-site qualitative detection with simple instrumentation but also present advantages because of the low cost of the DNA hydrogel, fast reaction and simple operation. Moreover, the aptamers used in DNA hydrogels are chemically synthesized, which is an inexpensive and relatively fast process; can be generated in large quantities to meet the needs of high-throughput

detection; and can be preserved for a long time within the hydrogel ^[19, 20]. In recent years, due to the numerous advantages of DNA hydrogels for biosensing, DNA hydrogels have been widely used as biosensors in the fields of environmental monitoring, food safety and clinical diagnosis.

Therefore, this paper summarizes and discusses the construction and application of DNA hydrogels in the field of biosensing. First, DNA hydrogels are categorized according to the type of hydrogel backbone and the detection principle, and then recent research progress on the application of DNA hydrogels in sensing and detection is summarized. Next, the DNA hydrogels biosensors depending on different targets (heavy metals, organic molecules, proteins, nucleic acids and pathogenic microorganisms) are summarized and discussed. The detection principles are described, and the advantages and disadvantages of different DNA hydrogels are discussed in terms of production cost, detection results and accuracy. Finally, the challenges and prospects of the use of DNA hydrogels for sensing are discussed.

2. Types of DNA hydrogels

Currently, DNA hydrogels can be categorized into pure DNA hydrogels and hybrid DNA hydrogels according to the materials used to construct the backbone ^[21]. In addition, considering that DNA hydrogel biosensors are generated by combining sensitive sensor elements with biometric elements, biosensors based on stimulus-responsive DNA hydrogels can be categorized based on whether the detection mechanism is via gel formation ^[22-24] or gel breaking. In this section, we introduce the gel-formation principles of DNA hydrogels with different backbone structure classifications and their respective advantages and disadvantages, and we discuss strategies for the construction of stimulus-responsive DNA hydrogel biosensors based on these two principles.

2.1 Pure DNA hydrogel and hybrid DNA hydrogel

DNA hydrogels can be divided into pure DNA hydrogels and hybrid DNA hydrogels. In pure DNA hydrogels, DNA serves as the skeleton and crosslinks through complementary base pairing ^[25], whereas in hybrid DNA hydrogels, DNA is embedded

into polymer hydrogels ^[26] to form stimulus-responsive DNA hydrogels. Stimulus-responsive DNA hydrogels can respond to external stimuli by changes in expansion volume and optical or mechanical properties, which presents advantages for the application of biosensors ^[27]. The following section introduces DNA hydrogels with two skeleton types in detail and summarize the advantages and disadvantages (seen in Table 1).

2.1.1 Pure DNA hydrogel

In pure DNA hydrogels, DNA alone forms the polymer skeleton. There are two ways to construct pure DNA hydrogels: crosslinking and self-assembly. In the crosslinking formation approach, the end of the long DNA strand connects multiple DNA strands through enzymes to form a crosslinked network structure. In the self-assembly approach, many long DNA chains are generated through nucleic acid amplification ^[28, 29] in response to target stimulation by the aptamer ^[30], and then, the hydrogel is generated ^[31] through folding and winding self-assembly or by enzyme connection. Next, two different types of pure DNA hydrogels are described according to the synthesis method.

The preparation of pure DNA hydrogels based on DNA scaffolds was initially carried out by ligation or DNA self-assembly. For example, DNA scaffolds with complementary sticky ends can be assembled with different branching DNA structures (X, Y, or T-type DNA). Luo et al. ^[25] used three independent DNAs to form DNA hydrogels through a building block and T4 ligase. This strategy involves the hybridization and linkage of DNA building blocks with T4 ligase to form pure DNA hydrogels. In addition, Liu et al. ^[32] used Y-shaped DNA nanostructures as scaffolds and linear double-stranded DNA as connectors to form pure DNA hydrogels. The sticky ends and linkers of Y-type nanostructures are complementary, so they can form hydrogels. A notable characteristic of this method is that the mechanical properties of the DNA hydrogel can be adjusted by adjusting the type and concentration of the DNA building blocks to meet the actual requirements. However, enzyme-mediated crosslinking is very time-consuming.

Compared to enzymatic crosslinking methods, DNA self-assembly strategies do

not require complex steps, but their high cost is undeniable. Nucleic acid amplification, such as rolling circle amplification (RCA) and hybrid chain reaction (HCR), is another common strategy for forming pure DNA scaffolds. Among them, RCA, an isothermal DNA replication technique, has become a commonly used method for the large-scale production of DNA ^[33]. It can generate a large amount of ultralong ssDNA with periodic repeating patterns ^[34], or repetitive functional nucleic acid fragments can be obtained by designing specific circular templates. Using two ssDNA strands produced by RCA, DNA hydrogels were constructed by hybridization between complementary sequences. RCA can be used simply and efficiently to prepare DNA hydrogels. More than 200 cycles can be performed in just one hour without precise temperature control. However, the poor mechanical properties of pure DNA hydrogels prepared by RCA may limit their application in specialized or complex environments.

Hybrid chain reaction (HCR) is another isothermal amplification reaction that does not require the involvement of enzymes ^[35] in constructing DNA scaffolds. Its advantage is that the designed DNA sequence can quantitatively and effectively regulate the formation of DNA hydrogels. In addition, this method does not require template design and can provide hydrogels with better mechanical properties than those of RCA. Similar to the self-assembly of DNA nano-units, however, this method requires fixed sequences and high concentrations of DNA monomers, which may not be conducive to the generation of functional DNA hydrogels.

Pure DNA hydrogels have suitable biocompatibility and biodegradability. However, that the microstructures of the pure DNA hydrogels prepared by the above methods are not identical. For example, the microstructure of a hydrogel formed by RCA is mainly a low nanoscale structure. In general, RCA-based DNA hydrogels have poor mechanical properties. In contrast, HCR-based DNA hydrogels are mostly crosslinked by hydrogen bonds, resulting in a porous three-dimensional microstructure with improved stability and mechanical properties.

2.1.2 Hybrid DNA hydrogel

Hybridized DNA hydrogels are usually prepared by connecting DNA branches to polymer chains and connecting the polymer chains through DNA hybridization or

supramolecular pathways ^[36], usually involving polymers other than DNA. DNA typically binds to other polymer chains through amide or disulfide bonds, and Michael addition reactions and electrostatic interactions are used in DNA modification and attachment to polymer chains ^[37].

Compared with a pure DNA hydrogel based on a DNA scaffold, a hybrid DNA hydrogel prepared with a polymer chain has higher mechanical stability. Cheng et al. ^[38] prepared a DNA acrylamide hydrogel for the detection of *Chlamydia trachomatis*. Two acrylic acid-modified DNA chains were copolymerized with acrylamide to form two DNA polymer chains, and DNA hydrogels were formed through DNA hybridization and assembly. Yu et al. ^[39] designed a DNA acrylamide hydrogel to respond to pH and Ag⁺: different responsive DNA aptamers were copolymerized with acrylamide and assembled into a hybrid DNA hydrogel that responds to external stimuli. In addition to DNA copolymerization with acrylamide ^[40, 41], DNA polymer chains can be modified with other hydrophilic polymers, such as carboxymethyl cellulose (CMC) ^[42] and chitosan (CS) ^[43], to form hybrid DNA hydrogels. The mechanical properties of hydrogels can be adjusted by varying the ratio of DNA to polymer. However, the biocompatibility, degradability and repeatability of hybrid DNA hydrogels are weaker than those of pure DNA hydrogels.

The design and synthesis of pure DNA hydrogels require little modification, so the synthesis process is relatively simple. Although the synthesis steps of hybrid DNA hydrogels are more complicated, their structure is more stable due to the intermolecular forces and chemical bonds, and hybrid DNA hydrogels have a lower production cost and so thus the potential for broader applications in biosensor detection.

Table 1 DNA hydrogels with different backbone types

Hydrogel backbone type	Mechanism of formation	Advantages	Disadvantages	Ref
DNA backbone strand-based DNA hydrogel	self-assembly	Adjustable mechanical properties; Easy to operate	Costly Harsh synthesis conditions	[25, 32]
	RCA	Simple and efficient; Easy to operate	Low mechanical properties	[33, 34]
	HCR	Adjustable mechanical properties; Simple sequence design	Complicated steps, Costly	[35]
DNA hydrogels based on other polymer chains	polyacrylamide	High mechanical properties	Low biocompatibility and degradability	[40, 41]
	carboxymethyl cellulose (CMC)	Adjustable mechanical properties	Complicated retouching process	[42]
	chitosan	DNA modification is simple	/	[43]

2.2 "Gel-breaking " and "gel-forming " recognition by DNA hydrogels

DNA hydrogels can change from gel to sol or from sol to gel in response to different stimuli. Therefore, DNA hydrogels can be divided according to the detection principle into "gel-breaking recognition" and "gel-forming recognition" hydrogels. Both types involve morphological changes in response to external stimuli for detection. Because morphological changes can be observed with the naked eye, this provides a basis for visual detection using DNA hydrogels.

2.2.1 "Gel-breaking recognition" DNA hydrogels

A "gel-breaking recognition" DNA hydrogel breaks down under specific stimuli, which allows the signalling molecules or active enzymes wrapped in the hydrogel to flow out, thus changing the colour or generating fluorescence or Raman spectrum signals to enable quantitative detection. There are currently two main signal output methods: the use of signalling molecules that can be detected directly by analytical instruments, and the other is to quantitatively detect 3,3',5,5'-tetramethylbenzidine (TMB) oxidation or other reactions catalysed by molecules with (similar) enzyme-like activity after gel breakage. The most common signal output methods of "gel-breaking recognition" DNA hydrogels are discussed below.

DNA hydrogel sensing based on colorimetry is a qualitative or quantitative

analysis method that uses the absorption characteristics of coloured substances in a system at a specific wavelength. These substances can exist in the system or can be produced by certain reactions [39, 44]. Some metal nanoparticles, such as gold nanoparticles (AuNPs) [44] and silver nanoparticles (AgNPs) [45], exhibit different colours due to their different sizes and morphologies. Alternatively, signal output can be achieved through the catalysis of oxidoreductases, such as horseradish peroxidase (HRP) [46] and glucose oxidase [47]. For example, HRP can catalyse the oxidation of TMB by hydrogen peroxide for auxiliary colorimetric detection. Colour changes can also be obtained by using nanomaterials with enzymatic or enzyme-like effects [48], such as nanometals and nanometallic oxides (PtNPs/Cu and ferric oxide) [49], carbon-based nanomaterials [50] (carbon nanotubes, graphene and its derivatives), and porous organic frameworks (metal organic frameworks, MOFs; and covalent organic frameworks, COFs) [51, 52].

In other common methods, changes in the output of fluorescence are used to qualitatively and quantitatively analyse substances [53]. Currently, the most commonly used fluorescent molecules are traditional organic fluorescent dyes and emerging fluorescent nanoparticles. Traditional organic fluorescent dyes such as fluorescein and rhodamine have the advantages of easy labelling and acquisition. However, they have disadvantages such as easy photobleaching, low fluorescence intensity, and a short fluorescence lifetime [54, 55]. Emerging fluorescent nanoparticles, such as quantum dots (QDs), have superior fluorescence quantum yields, but achieving their synthesis and obtaining high surface activity remain challenging [56]. DNA hydrogel biosensors using fluorescence strategies have been widely used for the detection of biotoxins [57, 58] and adenosine [59]. Fluorescence methods have advantages such as fast response and simple operation [60].

Apart from the colorimetry and fluorescence signals, Surface-enhanced Raman scattering (SERS) and electrical signals applied in DNA hydrogel biosensor have been reported. Surface-enhanced Raman scattering (SERS) combines the advantages of high chemical specificity, high sensitivity, and surface selectivity [61]. Moreover, SERS detection is fast, reusable [62], and easy to perform. Therefore, the use of DNA hydrogel

SERS biosensors as a sensing strategy has attracted increasing attention^[63]. The key to this type of analysis is the change in the Raman signal. When the hydrogel is broken, Raman signalling molecules are generated, or the distance between the Raman signalling molecules and the active substrate changes, enabling quantitative detection of the Raman signal. In addition, if electroactive substances are encapsulated in DNA hydrogels, target cleavage releases the encapsulated electrical signalling molecules to provide good electrical signals, enabling the use of stimuli-responsive DNA hydrogel electrochemical biosensors for detection^[64].

2.2.2 "Gel-forming recognition" DNA hydrogels

In contrast to the previous "gel-breaking recognition" principle of DNA hydrogels, under external stimulation, the state of signalling molecules can also be changed and captured during the formation of hydrogels. Moreover, similar to the above approach, this type of sensor system can still achieve qualitative or quantitative analysis of targets through colorimetry^[65], fluorescence analysis^[66], Raman spectroscopy^[67], electrochemistry^[68], etc.

The nucleic acid amplification mentioned above for preparing pure DNA hydrogels are usually used to construct this type of sensor because these amplification techniques, such as RCA and HCR, usually require a nucleic acid primer chain to start the amplification reaction. Therefore, if the targets is a nucleic acid sequence (DNA or RNA)^[69], the target itself can be designed as a primer for use in nucleic acid amplification. The quantitative measurement of target sequences can be achieved by capturing and analysing signalling molecules encapsulated in DNA hydrogels generated by primer regulation.

Herein, hybrid DNA hydrogels mainly achieve sensing by the detection principle of "gel-breaking recognition", while pure DNA hydrogels can use either "gel-breaking recognition" or "gel-forming recognition". However, due to the use of signalling molecules instead of volume modification variables for quantitative detection, "gel-breaking recognition" provides more accurate quantitative results. In addition, because hybrid DNA hydrogels are less expensive, hybrid DNA hydrogels with "gel-breaking recognition" have advantages for use in DNA hydrogel sensors.

3. Application of DNA hydrogels in biosensor detection

3.1 Detection of heavy metals

Heavy metals are inorganic pollutants that are difficult to biodegrade and can enter the human body, causing great harm to human health. DNA hydrogels can be applied in heavy metals biosensing due to their ability of aptamer to specifically bind heavy metals, generating a "heavy metal ion-bases" structure. DNA hydrogels based on aptamers can also be used to detect heavy metals, such as Pb^{2+} [70, 71], Ln^{3+} [72], UO_2^{2+} [73], Hg^{2+} [68], and Ag^+ [74]. When metal ions are present, DNA forms a secondary structure similar to a ring in the free system. Metal ions cleave specific recognition sites, causing junction sites to crack, destroying the hydrogel structure, and releasing signalling molecules, thus enabling the detection of corresponding heavy metal ions. Table 2 describe the application of DNA hydrogels-based biosensor for heavy metal ion detection in detail.

3.1.1 Detection of Lead ion (Pb^{2+})

Excessive Pb^{2+} can inhibit human enzyme activity and may damage more organs and nerves. DNA hydrogels can be used in biosensors to detect Pb^{2+} . For example, Chu et al. [70] reported a pure DNA hydrogel for label-free detection of Pb^{2+} (Fig. 1A), and Jiang et al. [71] reported a DNA hydrogel for point-of-care detection of lead ion (Fig. 1B). Both methods use DNA hydrogels to detect Pb^{2+} , but Chu et al. constructed a three-dimensional pure DNA hydrogel, and the structure of the DNA skeleton consists of Pb^{2+} aptamer DNA. When the DNA hydrogel has specifically bound Pb^{2+} and cleaved the aptamer, the hydrogel skeleton structure is destroyed, releasing DNA fragments into the supernatant, which can be detected by an ultraviolet spectrometer to further quantify the concentration of Pb^{2+} . The limitation of detection (LOD) of this method is as low as 7.7 nM, and this method eliminates the error caused by the introduction of signalling molecules in signal conversion, which is convenient for detection. The portable DNA hydrogel biosensor prepared by Jiang et al. uses a hybrid hydrogel strategy. This method uses polyacrylamide to hybridize with DNA and uses the aptamer specifically identified by Pb^{2+} as the crosslinking structure. The DNA hydrogel is generated in the capillary.

Pb^{2+} specifically recognizes and cleaves the aptamer, and the structure of the hydrogel is destroyed to a colloidal state. The colloidal hydrogel is dissolved, the amount of hydrogel in the capillary is reduced, and the liquid column height is reduced. The Pb^{2+} can be quantitatively detected by the decrease in the column height. The LOD is as low as 10 nM, and the liquid column height can be clearly observed in the capillary. This provides a basis for visual detection, and the prepared capillary-containing hydrogel can be applied in the field for portable detection. Although the DNA hydrogel prepared by Chu et al. has a lower detection limit and the accuracy of detection through an ultraviolet spectrometer is greater, the manufacturing cost is greater because the used skeleton structure is pure DNA, and the DNA hydrogel prepared by Chu et al. needs an analytical instrument to detect signals, which increases the difficulty of operation. In contrast, the DNA hydrogel prepared by Jiang et al. has the advantages of visualization and portability, facilitating the commercialization of detecting Pb^{2+} with this hydrogel.

3.1.2 Detection of Ce^{3+}

Lanthanides (Lns), known as rare earth elements, such as Ce^{3+} , are an important class of Lns metals. However, the extensive extraction of Ce^{3+} can lead to pollution in the environment and excessive Ce^{3+} intake can damage the human gastric mucosa, oesophagus, and central nervous system. Based on the principle of gel-breaking recognition, DNA hydrogels can be used to detect Ce^{3+} . For example, Huang et al. [72] reported a portable DNA hydrogel for Ce^{3+} detection by colorimetry (Fig. 1C). Huang et al. introduced gold nanoparticles (AuNPs) into a hydrogel system as a signalling molecule. In this method, AuNPs have their own colour and are released into the supernatant when the hydrogel is broken, causing the supernatant to turn red, which can be used for rapid visual detection. This method is specific for Ce^{3+} as low as LOD of 50 nM by naked eyes and 20 nM by colorimetry.

3.1.3 Detection of uranyl ion (UO_2^{2+})

Uranium is a chemical toxin that damages the kidneys and a radiation source with extensive biological effects, and the detection of UO_2^{2+} can effectively prevent uranium poisoning. He et al. [73] reported a DNA hydrogel method for detecting UO_2^{2+} based on SERS (Fig. 1D), which takes a SERS substrate using rhodamine B (RhB) as the

signalling molecule. When the signalling molecule is embedded in the hydrogel, it is far from the substrate, so it generates very little SERS signal. However, after UO_2^{2+} breaks the hydrogel, RhB will flow onto the substrate, generating SERS signals. The method has high accuracy and a LOD as low as 0.84 pM, according to the concentration of UO_2^{2+} measured by SERS. However, unlike the above method for the detection of Pb^{2+} , both this method and lanthanide detection involve coloured signalling molecules. During the process of recognition and cleavage of the DNA hydrogel, signalling molecules are released into the supernatant due to destruction of the crosslinking structure of the hydrogel. The obvious colour change in the supernatant provides the conditions for visual detection. Compared with the change in the liquid column height, the visual detection of the introduction of coloured signalling molecules is more intuitive. Moreover, the quantitative accuracy of the analysis instrument is greater than that of the liquid column height variation.

3.1.4 Mercury ion (Hg^{2+}) detection

Hg^{2+} is one of the most toxic heavy metal environmental pollutants and can stimulate the central nervous system, affect digestive function, and damage the kidneys. Cai et al. developed a DNA hydrogel coupled with DNAzyme-assisted target recycling and the hybridization chain reaction for detecting Hg^{2+} using electrochemical impedance spectroscopy (EIS) (Fig. 1E) [68]. This method utilizes DNAzymes (Mg^{2+} -DNAzymes) that specifically recognize Hg^{2+} and thymine (T) to generate single-stranded DNA, which opens the capture probe on the substrate and triggers HCR amplification signals, forming many "polyacrylamide hairpins" that undergo crosslinking on the electrode surface. A nonconductive DNA hydrogel film is formed, which blocks the transfer of electrons at the electrode interface, increasing the resistance of the electrode. Finally, the chemical signal of Hg^{2+} is converted into an electrical signal for detection, and Hg^{2+} can be quantitatively analysed by changing the resistance. This method has good sensitivity and selectivity for Hg^{2+} in the concentration range of 0.1 pM to 10 nM, with a LOD of 0.042 pM. This method can be used satisfactorily in the analysis of actual samples (tap water), but the detection of Hg^{2+} in outdoor water samples requires more data support and is limited to trace

detection. It is difficult to achieve quantitative detection outside the concentration range, and the DNA hydrogel consists of pure DNA, which greatly increases the detection cost.

3.1.5 Silver ion (Ag^+) detection

Ag^+ are often produced during the sterilization and disinfection of drinking water. Excessive silver ions can cause skin irritation, affect the digestive system and the skeletal system, cause allergic reactions, and lead to abnormalities in the blood system. Tomoki Hayashi et al. [74] reported the use of DNA hydrogels with quantitative changes in the colour of the DNA hydrogel (Fig. 1F). This method uses cytosine (C)- and Ag^+ specific recognition to form a "C-Ag-C" circular secondary structure. The crosslinking structure of the DNA hydrogel folds with specific recognition, leading to shrinkage of the hydrogel structure and a reduction in the surface area of the hydrogel. In addition, this method introduces silica gel particles into the DNA hydrogel; when the hydrogel shrinks, the spacing of the silica gel particles decreases, and the Bragg diffraction changes. The hydrogel will change from red in its normal shape to blue when it shrinks. The Ag^+ can be further quantified by changing the quantitative DNA hydrogel shrinkage rate through the spectral wavelength. Unlike the two methods mentioned in 2.2 section, this method does not rely on forming or breaking a hydrogel; instead, the target is quantified by the changing surface area of the hydrogel. This method can achieve visual detection and has the advantage of reusability. However, these methods have a high detection limit, and it is difficult to achieve accurate microdetection. However, the shrinkage of DNA hydrogels in the natural state can also cause errors in detection and affect the quantitative results.

Heavy metals detection methods have the advantages of high specificity in mixed ion solutions and extremely low detection limits for both Hg^{2+} and UO_2^{2+} . These methods not only include the two common DNA hydrogel detection methods of "gel-forming recognition" and "gel-breaking recognition" described in Section 2.2 but also provide a method that utilizes the unique "base-heavy metal-base" structure between metal ions and bases, which provides a new method for detecting hydrogel crumpling and can be used as a reference for the reuse of DNA hydrogel materials. In addition, the visualization of signalling molecules in the above detection methods is only the initial

qualitative judgement of rapid detection, and the quantification of subsequent sensing still needs the assistance of analytical instrumentation, so the development of a portable hybridized DNA hydrogel device for visual detection and the use of portable instrumentation for quantitative detection has also become a development trend for DNA hydrogels in the future.

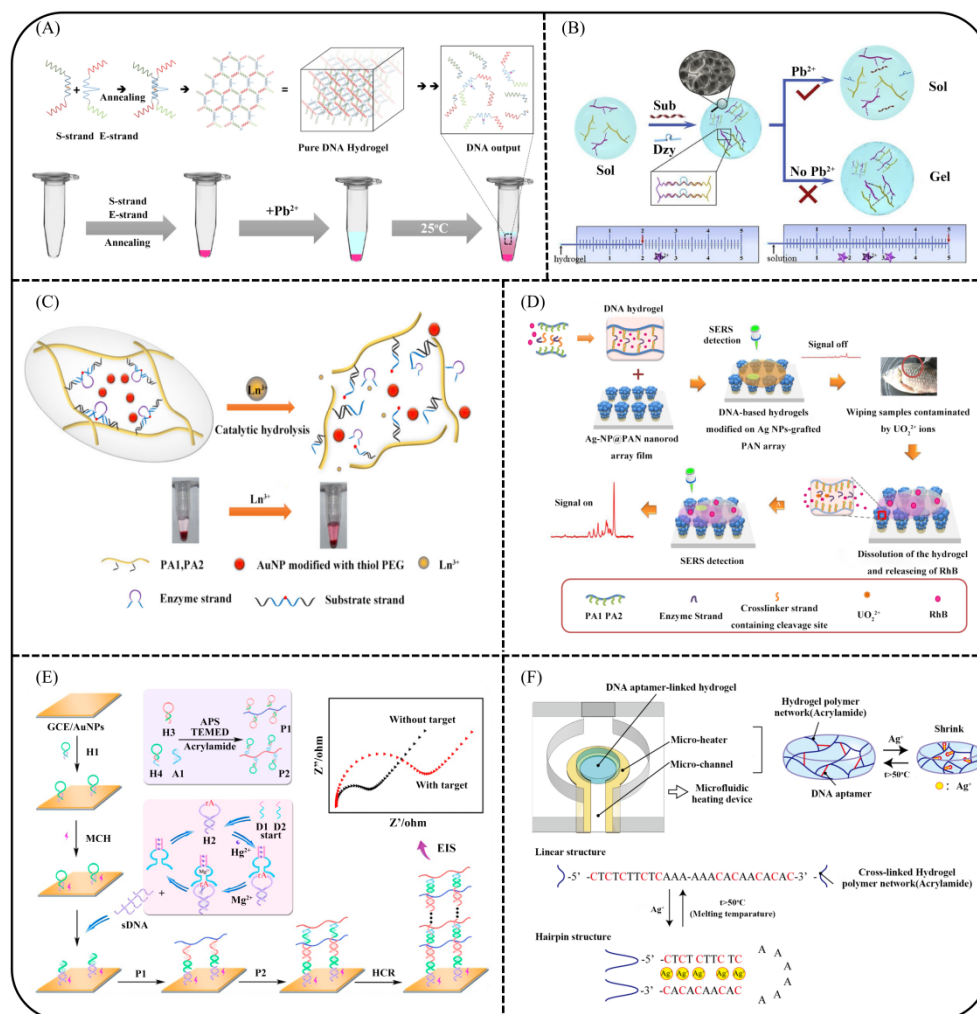


Fig. 1. Heavy metal ion detection. (A) A responsive pure DNA hydrogel for label-free detection of lead ion. (B) A portable visual capillary sensor based on functional DNA crosslinked hydrogel for point-of-care detection of lead ion. (C) Target-responsive DNAzyme hydrogel for portable colorimetric detection of lanthanide (III) ions. (D) Flexible DNA Hydrogel SERS-Active Biofilms for Conformational Ultrasensitive Detection of Uranyl Ions from Aquatic Products. (E) An electrochemical impedance biosensor for Hg^{2+} detection based on a DNA hydrogel coupled with DNAzyme-assisted target recycling and the hybridization chain reaction. (F) Repeatable detection of Ag^+ ions using a DNA aptamer-linked hydrogel biochemical sensor integrated with a microfluidic heating system.

3.2 Detection of small organic molecules

Compared with heavy metals, small organic molecules are more likely to coexist

with living matter, and are also widely detected in the environment ^[75, 76]. In addition to biotoxins widely present in plants, animals and other living organisms, many dangerous antibiotics and pesticides remain in the bodies of plants and animals and can cause great harm to human health. Glucose is also an organic small molecule, and affects the health of the human body as a source of energy material. Therefore, the detection of organic small molecules can also make it possible to guarantee food safety, and protect environmental and human health. Table 2 details the applications of the DNA hydrogel-based detection of organic small molecules.

3.2.1 Biotoxin detection

Biotoxins are small organic molecules that are closely related to the metabolic activities of living organisms. The main biotoxins include plant toxins, animal toxins and microbial toxins. Microbial toxins such as aflatoxin and ochratoxin are lethal toxins formed by microorganisms to kill organisms in competition for food resources. Microbial toxins can have a serious impact on food safety, and the ability to detect such substances can ensure food safety. DNA hydrogels are widely used for the detection of biotoxins, such as ochratoxin A (OTA) ^[57, 58], T-2 toxin ^[30], and microcystin (MC-LR) ^[77], as described in detail below.

OTA toxins are a class of toxins produced by *Aspergillus ochraceus* and *Penicillium* that are widely found in crops such as corn, wheat, and fruits. OTA causes damage to the central nervous system and irreversible damage to the kidneys of the human body, and like aflatoxin, it is also teratogenic and carcinogenic. Liu et al. ^[57] reported a "gel-breaking recognition" DNA hydrogel that uses AuNPs as signalling molecules for the detection of OTA in beer (Fig. 2A). The design of a DNA hydrogel as a microfluidic chip is divided into three vertical segments under normal conditions. When OTA is introduced, the AuNPs are released, and the vertical channel is opened to connect the three segments. The H₂O₂ in the middle segment flows into the third segment under the effect of gravity, and under the catalytic action of the AuNPs, H₂O₂ decomposes rapidly to produce bubbles, and the increase in air pressure causes the column of liquid to rise to the first segment, enabling the quantitative measurement of OTA. The chip can be designed for supply in a product similar to a nucleic acid kit.

This method has the advantage of providing visualization, and the detection limit is as low as 0.01 ng/mL.

In addition, Hao et al. ^[58] reported the use of a hydrogel formed by using the RCA reaction for the detection of OTA in beer (Fig. 2B). The mechanism involves hybridization of the first primer strand and the complementary bases in the OTA aptamer. In the presence of the substrate OTA, the aptamer specifically recognizes OTA and releases the primer strand. A Padlock probe was used in the role of T-4 ligase. The formation of circular DNA is guided by a single-repeat DNA long chain generated sequence, which is branched with Cy3-dNTP signalling molecules. Under the action of T-4 ligase, loop DNA was formed, the substrate was guided by loop DNA, and a RCA reaction occurred, generating a long chain of single repeat DNA branched with Cy3-dNTP signalling molecules that then self-assembled under the action of the Phi29 DNAzyme, generating a DNA hydrogel. The intensity of the self-assembled DNA hydrogel was measured by fluorescence spectrometry, which showed a linear correlation between the light intensity and the concentration of OTA. This method utilizes RCA technology to generate DNA hydrogels, and OTA is quantified by using the fluorescence intensity generated by the DNA hydrogels. The RCA technology itself has mild reaction conditions, and the detection limit of this method is 11.1 nM, reflecting high selectivity and accuracy.

T-2 toxin inhibits protein and DNA synthesis in the cells of vital organs, and the ingestion of T-2 toxin can cause vomiting, growth retardation, reproductive dysfunction, and neuroendocrine disruption. T-2 toxin prevention and treatment are mostly based on avoiding or reducing exposure, Sun et al. ^[30] used a colorimetric DNA hydrogel for the detection of T-2 toxin (Fig. 2C), which is a "gel-breaking recognition" DNA hydrogel. In the presence of T-2 toxin, the T-2 toxin aptamer binds preferentially to the T-2 toxin, and the DNA hydrogel is breaking, releasing the encapsulated HRP, which catalyses the generation of I₂ from H₂O₂ and KI. The resulting I₂ etches the Au@Pt nanorods in the presence of cetyltrimethylammonium bromide (CTAB), which is added to the Au@Pt nanorods in the preparation process. CTAB acts as an adhesive to constrain the nanorods to form rod-shaped nanomaterials, and CTAB, as a directional surface

stabilizer, causes I_2 to preferentially etches the Au@Pt nanorods from both ends. The etched Au@Pt nanorods have a shorter wavelength in the UV-vis spectra due to their shorter lengths and will produce a blue shift. The LOD is as low as 0.87 pg/mL, and the detection range is 0.01-10,000 ng/mL. The hydrogel used in this method is easy to prepare, has a fast reaction speed, and exhibits high sensitivity, a low detection limit, good selectivity and high capability.

MC-LR is a class of biotoxins produced by cyanobacterial blooms that strongly inhibits the activity of protein phosphatases. When the cells rupture or senesce, large amounts of biologically active MC-LR are released into the water, which poses a great threat to aquatic organisms and public health. MC-LR has been reported to be closely associated with tumour promotion and can lead to liver cancer even at low concentrations. A DNA hydrogel encapsulating Cu/Au/PtNPs as a means of signal amplification, as reported by Wu et al. [77], was used for the MC LR (Fig. 2D), which is a class of "gel-breaking recognition" DNA hydrogels. In the presence of MC-LR, the DNA hydrogel is cleaved, and the Cu/Au/PtNPs are released to catalyse the reaction between H_2O_2 and TMB, which produces a colour change with increasing MC-LR concentration that can be used to quantify the amount of MC-LR. MC-LR content. The method has a detection limit of 3 ng/L, which satisfies the requirements of the World Health Organization (no more than 1.0 mg/L).

These DNA hydrogel biosensors detect biotoxins based on the principle of recognition and cleavage have low detection limits, allowing then not only to meet the need for low-cost sensitive detection of biotoxins but also provide references for the future development of new DNA hydrogel materials in the future by adopting the signal output method of the above methods. Moreover, the detection of biotoxins is an important line of defence in food safety.

3.2.2 Glucose detection

Glucose is an essential direct energy source and an important biomedical and biochemical analyte that plays a key role in physiological metabolism. The detection of glucose by current commercially available personal blood glucose meters (PGMs) is

subject to interference by external environmental factors. For example, the temperature and the collection of too much or too little blood will affect the results. Depending on this, Ma et al. ^[78] performed D-glucose detection in vitro using a smart DNA hydrogel (Fig. 2E) via aptamer and target cleavage, with AuNPs as the embedded signalling molecules, using the red colour of the AuNPs and the optical properties of the nanoparticles. A spectrophotometer can be used to quantitatively detect the AuNPs released from the cleavage of the hydrogel, which also provide a visible signal. The hydrogel used in this method is a hybrid hydrogel, which reduces the manufacturing cost, but differ to the usual target–aptamer mechanism, the detection target, D-glucose, must be bound to the receptor to form the target specifically recognized by the DNA aptamer, which enables the specific recognition of a substance that is otherwise difficult for a DNA aptamer to bind after modification. As a new nonenzymatic biosensor, this DNA hydrogel provides a method for detecting glucose. In addition, the glucose hydrogel has good selectivity and is easily destroyed by glucose complexes but does not significantly react with other sugars or glycoconjugates. The D-glucose DNA hydrogel can be visualized and detected in 50% urine and 30% serum as well.

In addition to traditional glucose, which is important to monitor, straight-chain starch can also be used as a “nonsugar” index of glucose because straight-chain starch can be hydrolysed to glucose in the presence of enzymes. Yan et al. ^[79] reported a class of DNA hydrogels for the quantitative detection of "nonsugar" glucose (Fig. 2F). The DNA hydrogel was constructed using polyacrylamide as the backbone chain structure, a straight-chain amylose aptamer as the crosslinking agent, and an encapsulated glucoamylase enzyme. This method involves cleavage upon recognition of the target by the aptamer and the target, and in the presence of a straight-chain starch target, the hydrogel structure is disrupted, releasing glucoamylase, which hydrolyses straight-chain starch into glucose and quantifies glucose using a PGM. This method achieves simple and low-cost portable PGM quantification while maintaining fast and simple operation. Since only DNA hybridization without enzyme modification is necessary to construct hydrogels and the method does not affect enzyme activity, this is a simple and versatile strategy that can be applied to a wide range of aptamer sequences for the

portable and quantitative detection of a broad range of nonglucose targets.

Ma et al. designed a DNA hydrogel that overcomes the limitations of measurement using a personal glucose meter (PGM) in complex matrices, and the introduction of a signalling molecule provides the possibility of visualization. Yan et al. designed a DNA hydrogel that relies on the responsiveness of rectilinear amylopectin, but the quantification method still uses a PGM, and this means of quantification may be subject to interference in the detection of complex matrices in real samples.

3.2.3 Antibiotic detection

Currently, the abuse of antibiotics is serious, and antibiotic residues have been detected in food and environment. Antibiotic residues pose major risks to the human body, such as increasing the risk of cancer and causing severe neuronal damage and even central nervous system death [80]. Therefore, the detection of antibiotics is important for food safety, environmental and public health.

Streptomycin is an aminoglycoside antibiotic commonly used in agriculture and animal husbandry [81]. Streptomycin residue is often present in agricultural and food products such as eggs, meat and milk [82] and can be detected using a DNA hydrogel biosensor. Wang et al. [75] developed a SERS-based DNA hydrogel biosensor (Fig. 2G) that can be used for streptomycin detection. This sensing strategy is based on the cleavage of the DNA hydrogels combined, for the first time, with gold nanorods (AuNRs) to obtain a SERS assay for streptomycin. The approach has high sensitivity and specificity and, in this case, an LOD of 4.85×10^{-3} nM and a stable Raman signal with a linear range of 0.01-150 nM. The detection limits of streptomycin in honey and chicken are lower than the maximum residue limits (MRLs) of 20 $\mu\text{g/kg}$ and 500 $\mu\text{g/kg}$, respectively, according to the Ministry of Agriculture of China [83, 84].

Kanamycin is another representative aminoglycoside antibiotic whose abuse can lead to adverse effects such as drug allergy, hearing damage, respiratory failure, ototoxicity, and nephrotoxicity [85, 86]. Chen et al. [67] reported a DNA hydrogel for the detection of kanamycin (Fig. 2H). The sensing strategy is based on the principle of DNA hydrogel construction and consists of three components: the preparation of gap-containing nanoparticles (GCNPs) as SERS labels, the specific recognition of aptamers

by kanamycin and the release of primers, the primers lead the RCA reaction to form DNA hydrogel. The advantage is the use of DNA hydrogels to determine the amount of kanamycin in the system. The biosensor has been successfully used for the detection kanamycin in milk and honey with a LOD as low as 2.3 fM, simultaneously offering the advantages of high sensitivity and ease of operation. In addition, Tan et al. used a stimuli-responsive DNA hydrogel fluorescent sensor for the detection of earth tetracycline^[87]. This method makes it easy to prepare benzene tablets, adenosine and inducers by physical mixing, avoiding tedious modification processes and hydrogel consumption. The LOD was 25 µg/L, with a linear range of 25-1000 µg/L. Both concentrations were lower than the maximum residue limit (MRL) of kanamycin in milk (150 µg/kg in the European Union and 200 µg/kg in China).

3.2.4 Pesticide detection

Pesticides are widely used in agriculture due to their highly effective protective effects on crops^[88, 89]. However, the widespread use and improper handling of pesticides can result in pesticide residues that can cause serious contamination of food, the environment and ecosystems^[90]. For example, even at very low concentrations, residues of organophosphates (OPs) pesticides may jeopardize human health, resulting in impaired neurotransmission^[91, 92]. Therefore, the development of highly sensitive methods for the detection of OPs is urgently needed for the control of food quality and safety, and the prevention of OPs poisoning and disease. Tang et al.^[76] produced a DNA hydrogel with a malathion aptamer as the crosslinking strand, two complementary ssDNA strands, and the peroxidase enzyme embedded in the hydrogel. In the presence of the target, the DNA hydrogel was cleaved, releasing catalase, which oxidized the H₂O₂ in the supernatant, releasing O₂, which participated in the subsequent reaction. The reaction formula was $4\text{Fe(s)} + 3\text{O}_2\text{(g)} \rightarrow 2\text{Fe}_2\text{O}_3\text{(s)}$, and the OPs could be quantitatively measured by the temperature change with a thermometer. The LOD of OPs by this method was 0.032 pg/mL. At present, there are many types of pesticides, but there are few reports on the application of DNA hydrogels for pesticide detection, and more research should be carried out in this area in the future.

The detection of the above organic molecules provides a basis for food safety and

human health protection, but there are numerous biotoxins, antibiotics and pesticides present in food and the environment, the detection matrix must be replicated, and biosensing DNA hydrogel materials for many types of organic molecules still need to be developed.

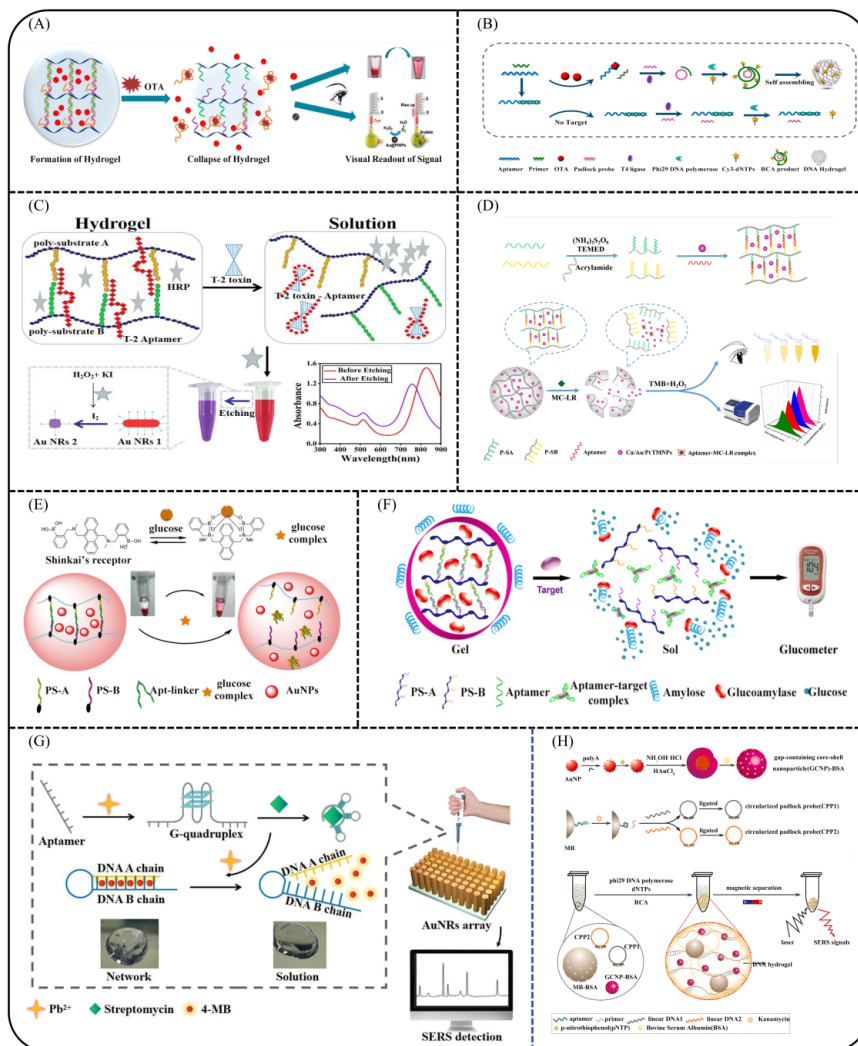


Fig. 2. Small organic molecule detection. (A) Design and synthesis of a target-responsive aptamer-crosslinked hydrogel for the visual and quantitative detection of ochratoxin A. (B) Fluorescent DNA hydrogel aptasensor based on the self-assembly of rolling circle amplification products for the sensitive detection of ochratoxin A. (C) Ultrasensitive and rapid detection of T-2 toxin using a target-responsive DNA hydrogel. (D) Cu/Au/Pt trimetallic nanoparticles coated with DNA hydrogel as a target-responsive signal-amplification material for the sensitive detection of microcystin-LR. (E) Target-responsive DNA hydrogel for the nonenzymatic and visual detection of glucose. (F) Target-responsive "sweet" hydrogel with glucometer readout for the portable and quantitative detection of nonglucose targets. (G) Ultrasensitive detection of streptomycin in foods using a novel SERS switch sensor fabricated from an AuNR array and DNA hydrogel embedded with DNase. (H) Fishing unfunctionalized SERS tags with a DNA hydrogel network generated by ligation-RCA for the simple and ultrasensitive detection of kanamycin.

3.3 Protein detection

Proteins are a class of bio-organic macromolecules, the fundamental substances that make up the cell and the main molecules responsible for many life activities. Proteins are part of every cell and all important components of an organism. In the process of clinical testing, protein type and content are important physiological indicators that affect the diagnosis. Table 2 presents detailed information on the application of DNA hydrogel-based protein assays.

3.3.1 Methyl-converting enzyme (Dam-MTase) detection

Abnormal MTase expression can be used as an essential indicator for the diagnosis and treatment of many cancers, such as prostate, colon, ovarian and gastric cancers. Generally, MTase can also be used as a marker for cancer detection. Mao et al. [93] reported a DNA hydrogel based on the use of electrochemiluminescence (ECL) for the detection of Dam-MTase (Fig. 3A), which used the "gel-breaking recognition" method with Ru@SiO₂NPs as signalling molecules. When Dam-MTase is present, DNA can be modified by methyl groups and then recognized and broken down by DpnI, resulting in hydrogel cleavage and the subsequent release of Ru@SiO₂NPs into the supernatant, generating an ECL signal with a detection limit of 2 µg/mL.

3.3.2 Detection of creatine kinase isoenzyme (CK-MB)

Creatine kinase isoenzyme (CK-MB) has high specificity for myocardial injury and is a clinically important diagnostic marker for acute myocardial retinal infarction (AMI) [94]. Chen et al. [95] developed a dual-signal amplification technique combining EXPAR and CRISPR/Cas14a to construct a DNA hydrogel biosensor for the ultrasensitive and specific detection of CK-MB (Fig. 3B). After aptamer-based target recognition, the target signal was converted and amplified to achieve effective CRISPR/Cas14a activity against the DNA hydrogel linker strand. The LOD was 0.36 pM, and the specificity remained excellent in the presence of interfering substances. Based on its high sensitivity, versatility and stability, this method not only provides a new detection platform for CK-MB analysis but also has broad application prospects in clinical diagnosis and biomedical research. Since this work integrates multiple steps to

improve performance, there is still a need to explore simpler methods that do not compromise performance. For example, novel nanomaterials that utilize excellent functionality to directly achieve high sensitivity have been explored. Moreover, the stability of DNA hydrogels allows them to be combined with point-of-care devices for portability and sensitivity to form a "black box" that integrates the target opening, signal amplification, hydrogel preparation, and signal output processes, thereby reducing fluid change operations and simplifying the procedure. In addition, methods used for single-target detection can be integrated for high-throughput analysis, enabling the combined detection of disease biomarkers without additional steps and increasing the accuracy of disease diagnosis.

3.3.3 Detection of other proteins

DNA hydrogel biosensors can also be used for the detection of other types of proteins, such as mucin 1 (MUC1) ^[96] and uracil-DNA glycosylase (UDG) in breast cancer. Zhang et al. ^[97] prepared DNA hydrogels for specific adsorption and capture of proteins by combining bioorthogonal "click chemistry" and aptamers. DNA hydrogels for the specific adsorption and capture of proteins, which can form model proteins, were formed by mixing four-armed thiols and four-armed maleimide-functionalized polyethylene glycol with a polyethylene imide addition reaction to form polyethylene glycol hydrogels (Fig. 3C). Then, the acrylamide-modified protein-specific aptamer DNA was covalently bound precisely to the poly-PEG hydrogel, and the protein release was controlled by the addition of the complementary DNA (cDNA) strand of the aptamer. This strategy is suitable for controlled protein transfer. Jiang et al. ^[98] blocked one end of a capillary with this DNA hydrogel via endonuclease cleavage to act as a filter membrane separator and to control molecular flow into the capillary. Distance readings in the capillary were obtained upon hydrogel transformation, and the sensitivity of detection of UDG reached 0.02 mU/mL. Sun et al. ^[96] reported a cascade logic quadruple signal amplification strategy based on DNA hydrogels and nanomaterials for the detection of MUC1 and circulating tumour cells (CTCs) (Fig. 3D). Specifically, the substrate, i.e., tsDNA, acted on *Mycobacterium smegmatis* pore protein A (M2MspA) nanopores through DNazyme cleavage, as the number of

characteristically translocated tsDNA molecules directly reflected MUC1 and CTCs. This anti-interference strategy increased the detection sensitivity.

many proteins are biomarkers of diseases, and early clinical diagnosis of diseases can therefore be achieved by detecting such proteins. DNA hydrogels based on high specificity of aptamer can provide more accurate detection results in protein detection, reducing the probability of false positive of biomarker proteins.

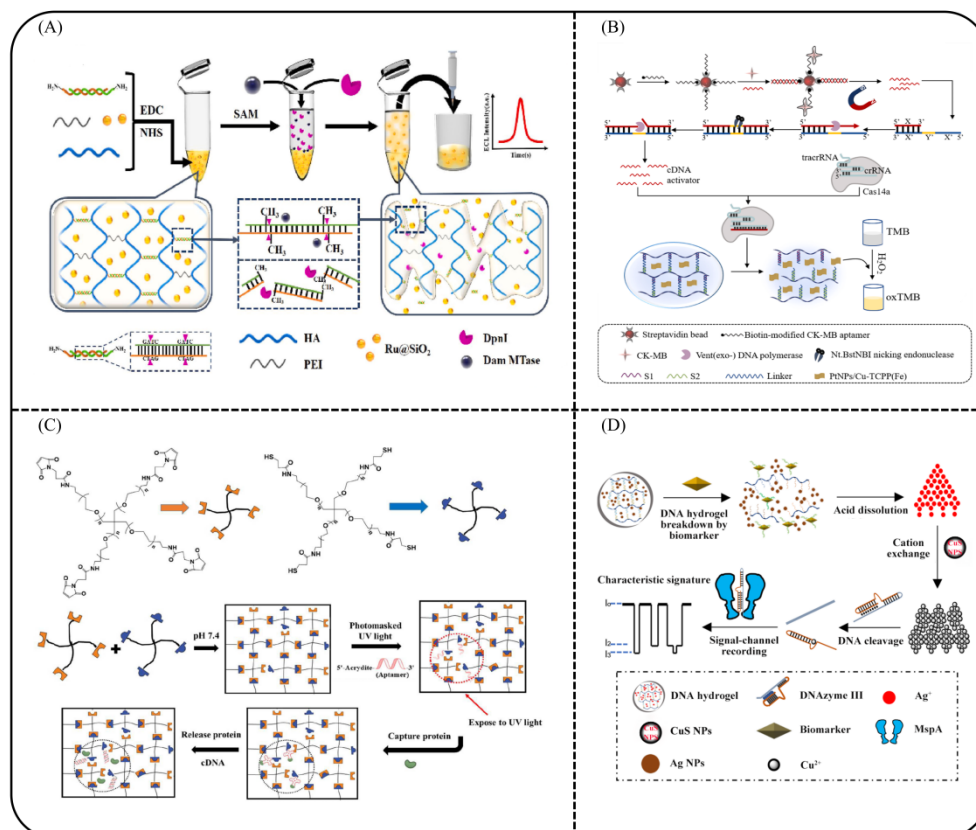


Fig. 3. Protein Detection. (A) Highly sensitive electrochemiluminescence biosensor for Dam methyltransferase based on a target-response DNA hydrogel. (B) Design and synthesis of a DNA hydrogel based on EXPAR and CRISPR/Cas14a for the ultrasensitive detection of creatine kinase MB. (C) An aptamer-patterned hydrogel for the controlled capture and release of proteins via biorthogonal click chemistry and DNA hybridization. (D) Ultrasensitive nanopore sensing of Mucin 1 and circulating tumour cells in whole blood of breast cancer patients by analyte-triggered triplex-DNA release.

3.4 Nucleic acid detection

Nucleic acids, including DNA and RNA, are biomolecular polymers composed of many nucleotide monomers and are among the most fundamental substances of life. Currently, nucleic acid testing mainly includes miRNA as a clinical diagnostic marker

for cancer, adenosine, and cell-free DNA. For example, many assays for miRNA have emerged in recent years, and DNA hydrogels are currently being developed for many miRNA assays. Table 2 lists the details of wide applications of DNA hydrogel in the field of nucleic acid target detection.

3.4.1 miRNA detection

The miRNAs are a class of endogenous microscopic RNAs, usually 20-24 nucleotides in length, that play important regulatory roles in the cell. In clinical medicine, miRNAs, as common tumour markers, are often used as important substances for early cancer monitoring, and their early detection can lead to early diagnosis and treatment. Therefore, there is an urgent need to develop methods to quantify miRNAs in samples with simple operation and low cost for clinical cancer screening and diagnosis. Si et al.^[99] reported a DNA hydrogel for the detection of miRNAs via surface Raman spectroscopy (Fig. 4A), mainly based on the "recognition cleavage" principle. The signalling molecules are Au/AgNPs modified by streptavidin, and in the presence of the target miRNA, the crosslinking structure is disrupted and the nanoparticles are released to generate a Raman signal on the SERS. The miRNAs can be quantitatively analysed by the intensity of the Raman signal. A DNA hydrogel SERS sensor array based on nine sensor units was developed for the identification and quantification of multiple miRNAs in a sample. The LOD was 4 nM, and the sensitive stimulus-response properties of the sensor array were successfully applied to the quantitative detection of multiple miRNAs in serum.

In addition, Zhao et al.^[100] reported a DNA hydrogel for the ECL-based detection of miRNAs, where the target miRNA-let-7a undergoes cyclic amplification in a chain reaction utilizing PCR, generating a large number of replicas (MTs), which trigger the subsequent HCR reaction to form the dendritic hydrogel product (Fig. 4B). In the presence of $S_2O_8^{2-}$ ions with Pd@Au nanomaterials, an electrochemical signal is generated for subsequent ECL detection. The ECL signals in the DNA hydrogel were significantly increased and stabilized in this novel ECL ternary system. Compared with the conventional sensor, this PTC-DEDA-based biosensor has high sensitivity and stability, with a LOD as low as 1.49 fM, and miRNA can be detected without a complex

labelling process. This method can also be adapted for the detection of other bioactive substances. In addition, Wang et al.^[101] reported a DNA hydrogel for visual observation and quantitative analysis (Fig. 4C), which is based on the principle of "gel-breaking recognition", where the formed hydrogel is encapsulated in a capillary tube. When the target miRNA is present, the hydrogel changes from a gel state to a sol state, the permeability is increased, the liquid column in the capillary rises, and the proportion of elevation is positively correlated with the miRNA concentration, enabling quantitative analysis. However, the quantitative accuracy of miRNA measurement by this method is not as good as that of electrochemical analysis and SERS.

3.4.2 Adenosine detection

Adenosine is an endogenous nucleoside that has received widespread attention for its important role in signalling in the central and peripheral nervous systems. Further studies have shown that the rapid growth of solid tumour cells results in the release of large amounts of adenosine, which has a tumour-promoting effect. Lin et al.^[59] reported a chemiluminescence-sensing DNA hydrogel for the detection of adenosine in urine (Fig. 4D) through gel breaking recognition. Adenosine complementarily pairs with Acry-A-Atp bases, causing the DNA hydrogel to break, and the chemiluminescent signals of G-quadruplex/hemin and Au@HKUST-1 are released and read. However, this method utilizes two aptamers for adenosine and hemin for two-by-two crosslinking. In the presence of hemin, H-Apt is uncrosslinked with ssDNA, but Acry-A-Apt remains crosslinked with Acry-ssDNA. In the presence of adenosine, adenosine is base-complementarily paired with Acry-A-Atp, the hydrogel crosslinking structure is completely disrupted, the released G-quadruplexes/hemin and Au@HKUST-1 are released, and chemiluminescent signals are generated. This method requires the simultaneous presence of hemin and adenosine for the complete disruption of the multiple crosslinking networks to release the internal nanomaterials to produce chemiluminescent signals; this allows more accurate quantification of adenosine, and the detection limit of this method is as low as 0.1 pM.

Adenosine triphosphate (ATP) is a molecule of adenosine with three phosphate groups and is the most direct source of energy for the human body. Wang et al.^[102]

reported the use of a strategy to prepare ATP-triggered DNA hydrogels based on the fast response of linear double-stranded DNA with sticky ends. The DNA hydrogel operates based on the principle of "gel breaking recognition". In the presence of ATP, the hydrogel depolymerizes, and MB is released, with a monomer absorption peak at 664 nm and a dimer absorption peak at 610 nm in the UV–visible spectrum, which can be used to quantitatively analyse ATP content by determining the amount of MB released from the corresponding peak intensities. This DNA hydrogel is characterized by easy preparation, chemical stability, high specificity for ATP and a rapid response, which can be applied to both the detection of target molecules and controlled drug release. Therefore, this DNA hydrogel has very broad application prospects and lays the foundation for the future development of novel DNA hydrogels in the field of multivariate detection and drug delivery.

3.4.3 Cell-free DNA (cf-DNA) detection

cf-DNA is a tumour marker that is important for cancer diagnosis and targeted therapy. However, its practical application, especially in rural areas and third-world countries, is limited by the need for numerous instruments for cf-DNA analysis. Liu et al. ^[103] reported a DNA hydrogel for cf-DNA biosensing (Fig. 4E), where the presence of the target cf-DNA triggers RCA to produce a horseradish peroxidase (HRP)-like catalytic function in the DNA hydrogel composed of G-quadruplex, which further catalyses the chromogenic TMB reaction to produce a visible output signal. The detection range was 0.1 pM-1500 nM with a LOD of 0.042 pM. In addition, quantitative analysis of cf-DNA was achieved in real samples by combining the catalytic ability of the DNA hydrogel and a smartphone. Combining a smartphone with a functional DNA hydrogel in DNA fragment detection technology saves equipment costs, making portable analysis easier, and has great potential for portable cancer biomarker and environmental sample detection, expanding the application and providing a new technology for liquid biopsy analysis. In addition, the platform can be used for the deuteration and recovery of cf-DNA in human serum for detection.

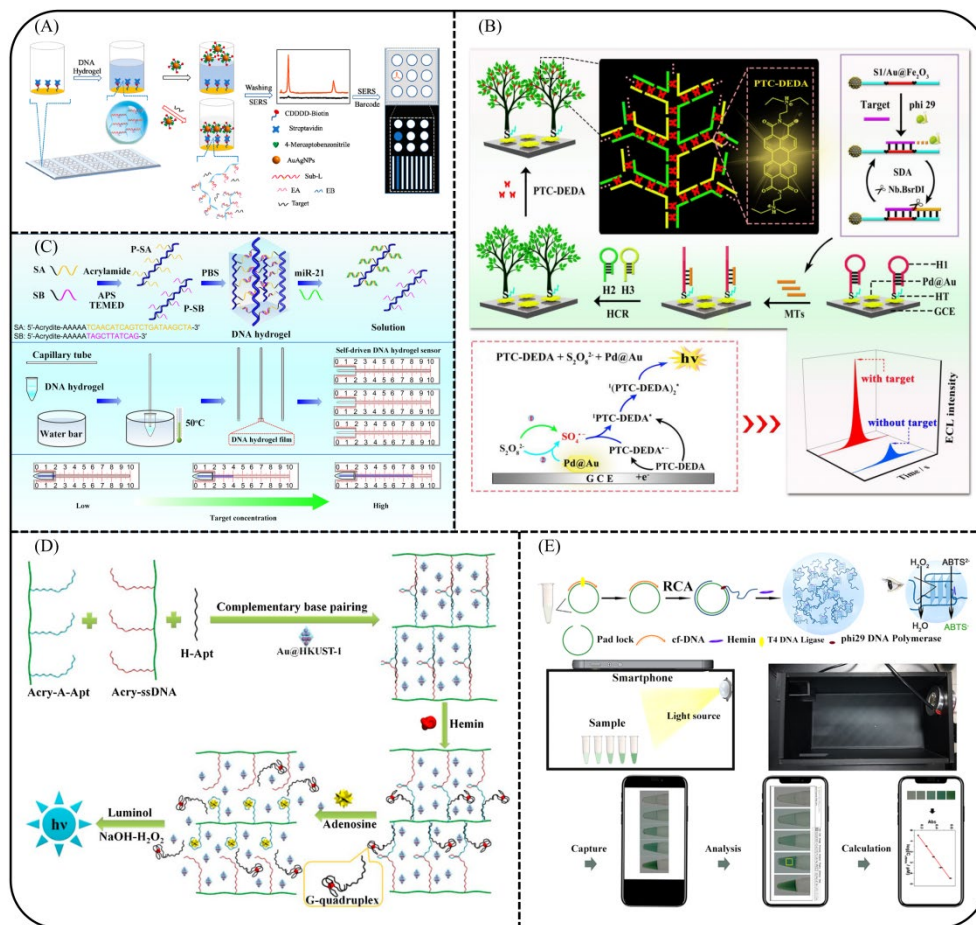


Fig. 4. Nucleic acid detection. (A) Target microRNA-responsive DNA hydrogel-based surface-enhanced Raman scattering sensor arrays for microRNA marker-based cancer screening. (B) Affinity-enhanced DNA intercalator with intense ECL embedded in a DNA hydrogel for biosensing applications. (C) Capillarity self-driven DNA hydrogel sensor for visual quantification of microRNA. (D) Chemiluminescent biosensor for ultrasensitive detection of adenosine based on target-responsive DNA hydrogel with encapsulation; (E) Smartphone-Based Pure DNAzyme Hydrogel Platform for Visible and Portable Colorimetric Detection of Cell-Free DNA.

As targets, nucleic acids not only has the method of recognizing the aptamer through base complementary pairing to achieve hydrogel breaking, but also has the method of cutting the DNA cross-linking structure with the target nucleic acids^[1]. Therefore, DNA hydrogels with nucleic acids as the targets can use a variety of strategies to achieve target detection. Moreover, various DNA hydrogels developed for nucleic acid detection can also provide the basis for the clinical diagnosis of diseases.

3.5 Detection of pathogenic microorganisms

Pathogenic microorganisms are widely present in nature^[104], and they can enter the human body through various routes, such as foodborne, waterborne, and airborne

routes, leading to disease and posing a severe threat to human health ^[105]. To prevent infectious disease outbreaks, pathogens must be detected with high sensitivity and specificity within a short period ^[106]. The application of DNA hydrogels for the detection of pathogenic microorganisms has been reported so far, mainly by detecting the nucleic acids of target microorganisms and in some instances by using protein markers. Below, DNA hydrogel detection of both pathogenic bacteria and foodborne viruses will be described in detail. Table 2 lists the applications of DNA hydrogel-based pathogen detection.

3.5.1 Detection of pathogenic bacteria

Escherichia coli (*E. coli*) serotype O157:H7 is the most common *E. coli* serotype associated with foodborne disease outbreaks and is considered a major cause of human haemorrhagic colitis and haemolytic uraemic syndrome. Rapid and sensitive food safety detection techniques are particularly necessary for the detection of pathogenic bacteria. Zhang et al. ^[107] reported a DNA hydrogel biosensor with RCA for the rapid visual detection of *E. coli* O157:H7 (Fig. 5A). In the presence of *E. coli* O157:H7, which specifically recognizes EA-I, the dsDNA was disrupted, resulting in the release of EA-I. The supernatant was collected and divided into two equal portions. EA-I underwent an RCA reaction with the two cyclic DNA sequences in the presence of T4 ligase, phi29 DNA polymerase, and dNTPs to generate two single-repeat sequences of the two ssDNAs that were partially complementary and could hybridize and self-assemble to form a visible DNA hydrogel. The amount of hydrogel formed was positively correlated with the concentration of *E. coli* O157:H7. This rapid detection method may help to improve sample targeting and can be used for the initial screening of samples for modulation by qualitative or semiquantitative assays. Due to its simple and inexpensive instrumentation and low requirements for experimental conditions, this method is suitable for the preliminary on-site screening of suspicious food samples and does not have high technical requirements for monitoring personnel.

Vibrio parahaemolyticus is a marine bacterium that mainly originates from seafood, such as fish, shrimp, crab, shellfish and seaweed, and is the main cause of diarrhoea resulting from seafood consumption. The number of outbreaks of infections

caused by this bacterium has been gradually increasing. Yu et al. ^[108] designed a smart hydrogel (Fig. 5B) containing an aptamer that specifically binds to ATP produced by the life activities of the pathogen, resulting in the rupture of the hydrogel and release of gold nanoclusters (AuNCs). A combination of fluorescence visualization signals and microfluidic microarray technology was used to perform rapid qualitative and quantitative analysis. The highlight of this approach is the detection of metabolically released ATP, which is more readily accessible to the hydrogel mesh structure, and the specificity of aptamer recognition to increase the efficiency and sensitivity of the assay, ultimately achieving sensitivities of 10 CFU mL⁻¹ in less than 1 h. In addition to the aforementioned *E. coli* and *V. parahaemolyticus*, *Bacillus anthracis* and *Salmonella* have also been detected using DNA hydrogel biosensors ^[109, 110].

The healing and management of chronic wounds is a challenge in clinical practice, as the presence of pathogenic bacteria can cause wound infections, hinder wound healing, and even lead to more severe amputations. Therefore, timely detection and management of early wound infections are crucial. Ze et al. reported a wireless sensor, which uses the responsive DNA hydrogel of *Staphylococcus aureus* to provide smartphone based wound infection detection^[1](Fig. 5C). Diffusion from biofilms and neutrophil extracellular traps deployed by host immune defense^[111]. After trauma and wound formation, the body will initiate an immune response to clear invading pathogens. One of the steps is that after necrosis or apoptosis, neutrophils will form DNA based neutrophil extracellular traps (NETs) to capture and kill pathogens, quickly controlling their infection in the body. Meanwhile, in order to get rid of NETs and invade the body, pathogenic bacteria usually secrete a large amount of DNase. When DNase is exposed outside the cell, it will cause the dissolution of hydrogel through the non-specific cleavage of DNA strand. This changes the dielectric constant of the region above the interleaved electrodes, thereby adjusting its capacitance. By connecting electrodes to embedded systems, this electronic signal can be read out wirelessly and without batteries using near-field communication (NFC), a connection technology found in most modern smartphones for short-range communication and wireless power transmission. An important window for real-time detection of clinically relevant

quantities of *Staphylococcus aureus* in vitro and mouse wound models. This DNA hydrogel may help detect wound infections in a timely manner to improve surgical or chronic wound management.

3.5.2 Detection of viruses

In addition to bacteria, many viruses, also belong to pathogen microorganisms, such as avian influenza virus, foot-and-mouth disease virus, and norovirus, and pose threats to public health^[112]. Recently, stimuli-responsive DNA hydrogels have attracted increased interest for use in biosensing strategies against foodborne viruses^[22]. For example, Li et al.^[113] designed a target-responsive DNA hydrogel fluorescent sensor to detect AIV H5N1 (Fig. 5D). The DNA hydrogel biosensor was modified with quantum dots and their bursting agents. In the presence of the H5N1 virus, the reaction of the aptamer with the virus disrupted the DNA hydrogel as well as the separation of the quantum dots and the bursting agent, resulting in the generation of a detectable fluorescent signal. The results showed that the DNA hydrogel sensor recognized AIV H5N1 within 30 min with a lower limit of detection of 0.4 HAU. This method combines the phase transition of the hydrogel with the fluorescence intensity of the quantum dots as a signal readout. This label-free, simple and inexpensive fluorescent biosensor has great potential for the rapid detection of H5N1 avian influenza virus in the field.

The global spread of the SARS-CoV-2 virus, which caused the COVID-19 outbreak, has caused great damage to public health and property. Kim et al.^[114] designed a method capable of rapidly detecting SARS-CoV-2 using a DNA hydrogel and a microfluidic chip. The presence of the virus completed the assembly of the dumbbell-shaped template, triggered RCA, and successfully synthesized a DNA hydrogel through the self-assembly of DNA, which blocked the chip and inhibited the flow of fluid. The virus was successfully analysed by measuring the flow rate of the fluid in the glass tube, and this technique has the lowest LOD for SARS-CoV-2 so far, at ~3 aM in 15 min or 30 aM in 5 min. The greatest advantage of this method is that it is fast and simple and can be adapted to most detection situations. In particular, this biosensor has the potential to rapidly detect SARS-CoV-2 in food ingredients.

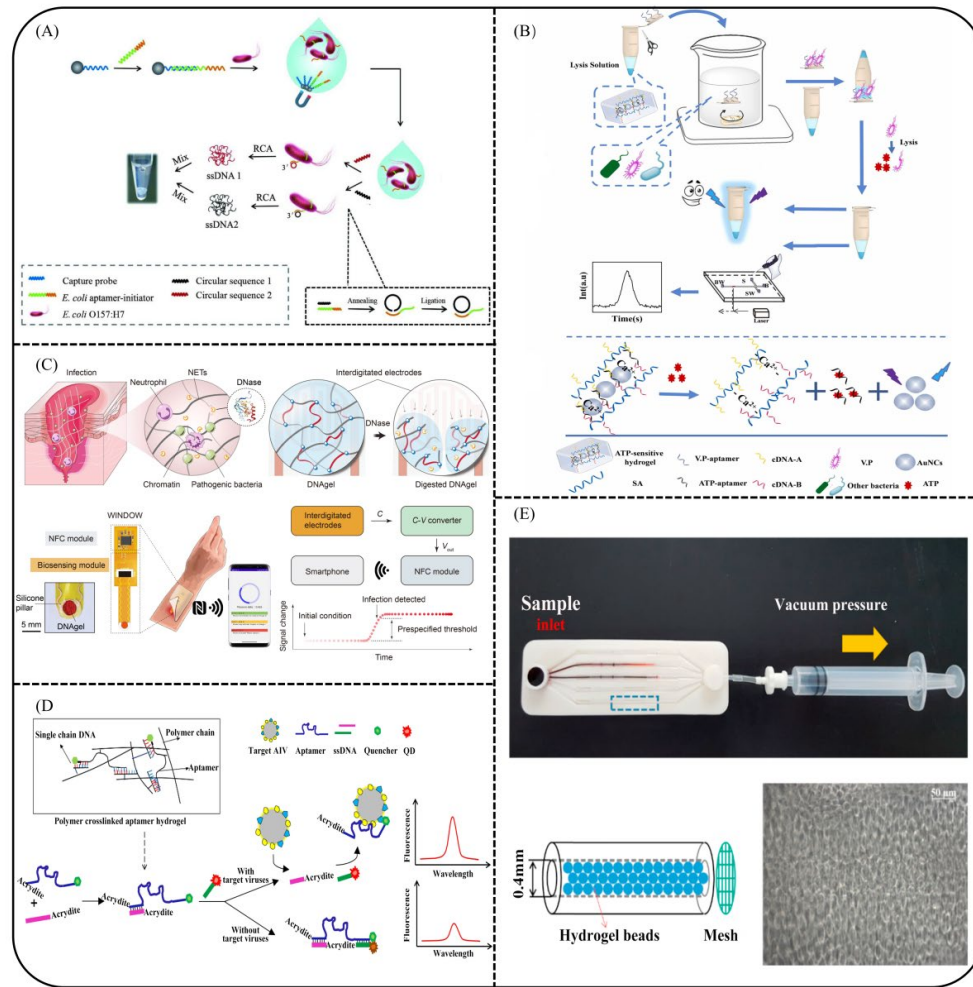


Fig. 5. Pathogenic microorganism detection. (A) Rapid visualized detection of *E. coli* O157:H7 by a DNA hydrogel based on rolling circle amplification. (B) On-site, dual-mode detection of live *V. parahaemolyticus* in water: a universal pathogen-sensing platform based on a smart hydrogel aptasensor embedded with gold nanoclusters. (C) A target-responsive and size-dependent hydrogel aptasensor embedded with QD fluorescent reporters for the rapid detection of avian influenza virus H5N1. (D) Rapid molecular diagnosis of infectious viruses in microfluidics using DNA hydrogel formation.

In addition, a paper reported by Wonhwi et al. [65] utilized hydrogel microfluidics for the biosensing of infectious viruses (Fig. 5E). The RCA technique was used to generate a DNA hydrogel, which made the microbeads less fluid and more resistant to flow and thus detected infectious viruses. When infectious viruses were present, primers attached to the agarose-based microbeads, and the hairpin formed in the presence of the infectious viruses underwent RCA, forming multiple long strands of single-repeat hairpin-like sequences bonded to the microbeads. The resistance to flow

between the microbeads increase, and changes in the resistance to flow were analysed to quantify the infectious viruses. An increase in the surface area of the RCA decreased the assay time to 15 min but did not lower the detection limit. Multiple channels can be designed for simultaneous multiplex analysis to detect a wide range of infectious pathogens. The method is sensitive to *E. coli* O157:H7 within 1 h with a LOD of 4 CFU/mL, showing high sensitivity, and the detection time can be decreased to 30 min. The method is characterized by high specificity, easy operation, efficient amplification, and fast readout and visualization and has potential applications in food safety detection.

The detection for pathogenic microorganisms are indirect and quantitative and involve the detection of specific nucleic acids or proteins. Testing for pathogenic microorganisms is an effective means of preventing and controlling infectious diseases because testing helps to detect pathogens in a timely manner and take appropriate measures to control their spread.

Table 2 Different types of target-responsive DNA hydrogels.

Category	Target	Recongnition element	Signal element	Detection range	Samples	Limit of detection	Ref
Heavy metals	Pb ²⁺	DNAzyme	UV-vis	0-400 nM	tap water	7.7 nM	[70]
	Pb ²⁺	DNAzyme	Visualization	0.01-50 μM	tap water	10 nM	[71]
	Ln ³⁺ (Ce ³⁺)	DNAzyme	colorimetric/UV-vis	0-800 nM	lake water	50 nM (colorimetric) 20 nM (UV-vis)	[72]
	UO ₂ ²⁺	DNAzyme	SERS	1 pM-0.1 μM	water	0.838 pM	[73]
	Hg ²⁺	Mg ²⁺ - DNAzyme	EIS	0.1 pM -10 nM	tap water	0.042 pM	[68]
	Ag ⁺	DNAzyme	Visualization	1 μM-10 mM	river water	/	[74]
Small organic molecules	OTA	aptamer	UV-vis	/	bear	1.27 nM	[57]
	OTA	aptamer	fluorescence	12.4 pM-24.7 nM	bear	24.7 pM	[58]
	T-2 toxin	aptamer	UV-vis	21.4 pM-21.4 μM	water	1.86 pM	[30]
	MC-LR	aptamer	colorimetric	4 pM-10 nM	water	3 pM	[77]
	glucose	aptamer	SERS	/	blood/urine	0.44 mM	[78]
	Amylose	aptamer	PGM	/	blood/urine	7.7 μM(blood) 4.4 μM(urine)	[79]
	streptomycin	aptamer	SERS	0.01-150 nM	water	4.85 pM	[75]
	karamycin	aptamer	SERS	1.3 fM-13.3 pM	water	2.3 fM	[67]
	OPs	aptamer	thermal	0.3pM-30 nM	water	0.1 pM	[76]
Protein	Dam MTase	aptamer	ECL	5 μg/mL-4 mg/mL	/	2 μg/mL	[93]
	CK-MB	aptamer	EXPAR and	/	/	0.36 pM	[95]

			CRISPR/Cas14a				
	MUC1	aptamer	fluorescence	0.5-500 fg/mL	breast cancer cell extract	0.1 fg/mL	[98]
	miRNA-21	aptamer	SERS	4-1200 nM	breast cancer cell extract	0.11 nM	[99]
	miRNA let-7a	aptamer	ECL	10 fM-10 nM	breast adenocarcinoma/lung adenocarcinoma cell extracts	1.49 fM	[100]
Nucleic acids	miRNA-21	aptamer	volume	10 fM-20 pM	Breast Cancer Cell Extract	/	[101]
	adenosine	aptamer	CL	0.4 pM-0.15 nM	urine	0.104 pM	[59]
	ATP	aptamer	UV-vis	/	/	1 μ M	[102]
	cf-DNA	aptamer	colorimetric	0.1 pM-1500 nM	human serum	0.042 pM	[103]
	<i>E. coli</i> O157:H7	aptamer	visualization	/	milk	4 $\times$ 10 ³ CFU/mL	[107]
	<i>V. parahaemolyticus</i>	aptamer	colorimetric	10-10 ⁷ CFU/mL	fish extract	10 CFU/mL	[108]
Pathogens	<i>Staphylococcus aureus</i>	DNase	Electrochemical	10 ⁵ -10 ⁶ CFU/g	Wound tissue	/	[1]
	AIV H5N1	aptamer	fluorescence	2 ^{-1.2} -2 ⁶ HAU/20 μ L	/	0.4 HAU	[113]
	SARS-CoV-2	Padlock probe	colorimetric	/	nucleic acid	3 aM in 15 min or 30	[65]

template synthesis

aM in 5 min

4. Conclusion, challenges and perspectives

DNA hydrogel-based biosensors are widely used due to their high specificity and good analytical performance. This paper systematically summarizes the applications of DNA hydrogel biosensing including heavy metal ions, organic small molecules, biomacromolecules and pathogens. DNA hydrogel sensing not only provides portable target detection but also offers new possibilities for environmental pollution prevention, food safety and clinical disease diagnosis. Compared with other biomaterials, these three-dimensional mesh materials have a variety of obvious advantages, such as adjustability, ease of modification and high biocompatibility. The adjustability of the DNA molecule makes it possible to customize different DNA hydrogel structures according to need, and the ease of modification makes it possible to bind a variety of types of molecules or substances, giving DNA hydrogels a wide range of substrates for detection.

Although the construction and sensing applications of DNA hydrogels have been rapidly developed, they remain an emerging field that also has deficiencies and challenges. First, the target responsiveness of most DNA hydrogel systems is based on "one-to-one" recognition of target molecules by DNA crosslinking units in the hydrogel network, and therefore a high concentration of target molecules is required for the response of hydrogel biosensing systems. Therefore, the development of more sensitive DNA hydrogels is one of the main challenges in the future design of DNA hydrogel biosensing strategy. Second, bulky DNA hydrogels are required for high-throughput applications, which increases both the manufacturing cost and the difficulty of preparing DNA hydrogel biosensors. Rapid detection is hampered by the slow response rate due to slow mass transfer in the hydrogel network; therefore, the development of DNA hydrogel membranes or microgel-based biosensing systems exhibiting fast response rates to environmental stimuli with at least one dimension at the microscale and nanoscale is a promising approach for constructing rapid-response hydrogel systems. In addition to the combination of DNA hydrogels and other materials, DNA hydrogels can be functionalized to extend from DNA hydrogel sensing to more accurate

dual-signal detection, enrichment detection, and other functions to enhance the functionality of DNA hydrogels. Finally, the simultaneous detection of multiple targets, which is important for the detection of multiple pollutants in environmental samples and the accurate diagnosis of different diseases in medicine, remains to be developed.

In the future, more types of target DNA hydrogel sensors are expected to be developed, and SELEX technology will also be used to obtain aptamers for multitarget detection. Macroscopic shape changes or sol-gel transitions facilitate the development of portable visualization methods, and the introduction of polymers, enzymes, and functional nanomaterials into the environmentally responsive structure of DNA hydrogels/microgels can further increase their functionality and responsiveness to environmental changes. At present, DNA hydrogels cannot provide real-time analytical data for the detection of organisms in organisms, and in the future, researchers will still need to use composite multifunctional materials to further broaden the functional applications of DNA hydrogels. Functionalized DNA hydrogels are expected to have significant application potential in biochemical analysis, drug release, bioimaging, information storage and other fields in the future. We believe that with the development and application of DNA hydrogel biosensors, the problems faced by this field will gradually be resolved, and the constructed biosensors will be universally applicable to environmental analysis, agricultural and food safety, medical diagnosis and infectious disease surveillance.

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Reference

- [1] Xiong Z, Achavananthadith S, Lian S, et al. A wireless and battery-free wound infection sensor based on DNA hydrogel [J]. *Science Advances*, 2021.
- [2] Xu N, Ma N, Yang X, et al. Preparation of intelligent DNA hydrogel and its applications in biosensing [J]. *European Polymer Journal*, 2020.
- [3] Yafei H, Yan W, Fuxiao W, et al. Heterogeneous DNA hydrogel loaded with Apt02 modified tetrahedral framework nucleic acid accelerated critical-size bone defect repair [J]. *Bioactive Materials*, 2024.
- [4] Konieczynska MD, Grinstaff MW. On-Demand Dissolution of Chemically Cross-Linked Hydrogels [J]. *Accounts of Chemical Research*, 2017.
- [5] Fan Y, Li J, Amin K, et al. Advances in aptamers, and application of mycotoxins detection: A review [J]. *Food Research International*, 2023.
- [6] Wu L, Wang Y, Xu X, et al. Aptamer-Based Detection of Circulating Targets for Precision Medicine [J]. *Chemical Reviews*, 2021.
- [7] Zhang X, Zhou Y, Huang X, et al. Switchable aptamer-fueled colorimetric sensing toward agricultural fipronil exposure sensitized with affiliative metal-organic framework [J]. *Food Chemistry*, 2022.
- [8] Park S, Cho E, Chueng S-TD, et al. Aptameric Fluorescent Biosensors for Liver Cancer Diagnosis [J]. *Biosensors*, 2023.
- [9] Mengjiao H, Fengling Y, Jiwei D, et al. Screening of broad-spectrum aptamer and development of electrochemical aptasensor for simultaneous detection of penicillin antibiotics in milk [J]. *Talanta*, 2023.
- [10] Ma N, Sun M, Shi H, et al. A Colorimetric/Fluorescent Dual-Mode Aptasensor for Salmonella Based on the Magnetic Separation of Aptamers and a DNA-Nanotriangle Programmed Multivalent Aptamer [J]. *Foods*, 2023.
- [11] Kacharovsky N, Cardle II, Cheng EL, et al. Traceless aptamer-mediated isolation of CD8+ T cells for chimeric antigen receptor T-cell therapy [J]. *Nature Biomedical Engineering*, 2019.
- [12] Zhang L, Fang X, Liu X, et al. Discovery of sandwich type COVID-19 nucleocapsid protein DNA aptamers [J]. *Chemical Communications*, 2020.
- [13] Wang Z, Chen R, Yang S, et al. Design and application of stimuli-responsive DNA hydrogels: A review [J]. *Materials Today Bio*, 2022.
- [14] Qiu H, Guo H, Li D, et al. Intravesical Hydrogels as Drug Reservoirs [J]. *Trends in Biotechnology*, 2020.
- [15] Zhang C, Zhou Y, Han H, et al. Dopamine-Triggered Hydrogels with High Transparency, Self-Adhesion, and Thermoresponse as Skinlike Sensors [J]. *ACS Nano*, 2021.
- [16] Wang D, Hu Y, Liu P, et al. Bioresponsive DNA Hydrogels: Beyond the Conventional Stimuli Responsiveness [J]. *Accounts of Chemical Research*, 2017.
- [17] Vázquez-González M, Willner I. Stimuli-Responsive Biomolecule-Based Hydrogels and Their Applications [J]. *Angewandte Chemie International Edition*, 2019.
- [18] Shao Y, Jia H, Cao T, et al. Supramolecular Hydrogels Based on DNA Self-Assembly [J]. *Accounts of Chemical Research*, 2017.
- [19] Kaminskyy D, Kryshchyshyn A, Lesyk R. Recent developments with rhodanine as a scaffold for drug discovery [J]. *Expert Opinion on Drug Discovery*, 2017.
- [20] Botta R, Rajanikanth A, Bansal C. Silver nanocluster films for glucose sensing by Surface Enhanced Raman Scattering (SERS) [J]. *Sensing and Bio-Sensing Research*, 2016.

- [21] Sandhya S, Meenakshi VS, Keerthana R, et al. Biorecognition elements appended gold nanoparticle biosensors for the detection of food-borne pathogens - A review [J]. Food Control, 2022.
- [22] Nam J, Jang WS, Kim J, et al. Lamb wave-based molecular diagnosis using DNA hydrogel formation by rolling circle amplification (RCA) process [J]. Biosensors and Bioelectronics, 2019.
- [23] Pikula M, Ali MM, Filipe C, et al. Single-Step Printable Hydrogel Microarray Integrating Long-Chain DNA for the Discriminative and Size-Specific Sensing of Nucleic Acids [J]. ACS Applied Materials & Interfaces, 2021.
- [24] Chen J, Zhu Y, Liu H, et al. Tailoring DNA Self-assembly to Build Hydrogels [J]. Topics in Current Chemistry, 2020.
- [25] Um SH, Lee JB, Park N, et al. Enzyme-catalysed assembly of DNA hydrogel [J]. Nature Materials, 2006.
- [26] Lin DC, Yurke B, Langrana NA. Mechanical properties of a reversible, DNA-crosslinked polyacrylamide hydrogel [J]. Journal of Biomechanical Engineering, 2004.
- [27] Li F, Lyu D, Liu S, et al. DNA Hydrogels and Microgels for Biosensing and Biomedical Applications [J]. Advanced Materials, 2019.
- [28] Wang J, Chao J, Liu H, et al. Clamped Hybridization Chain Reactions for the Self - Assembly of Patterned DNA Hydrogels [J]. Angewandte Chemie International Edition, 2017.
- [29] Xiang B, He K, Zhu R, et al. Self-Assembled DNA Hydrogel Based on Enzymatically Polymerized DNA for Protein Encapsulation and Enzyme/DNAzyme Hybrid Cascade Reaction [J]. ACS Applied Materials & Interfaces, 2016.
- [30] Sun Y, Li S, Chen R, et al. Ultrasensitive and rapid detection of T-2 toxin using a target-responsive DNA hydrogel [J]. Sensors and Actuators B: Chemical, 2020.
- [31] Iqbal S, Ahmed F, Xiong H. Responsive-DNA hydrogel based intelligent materials: Preparation and applications [J]. Chemical Engineering Journal, 2021.
- [32] Xing Y, Cheng E, Yang Y, et al. DNA HYDROGELS: Self - Assembled DNA Hydrogels with Designable Thermal and Enzymatic Responsiveness (Adv. Mater. 9/2011) [J]. Advanced Materials, 2011.
- [33] Ko O, Han S, Lee JB. Selective release of DNA nanostructures from DNA hydrogel [J]. Journal of Industrial and Engineering Chemistry, 2020.
- [34] Tang J, Liang A, Yao C, et al. Assembly of Rolling Circle Amplification-Produced Ultralong Single-Stranded DNA to Construct Biofunctional DNA Materials [J]. Chemistry - A European Journal, 2022.
- [35] Bi S, Yue S, Zhang S. Hybridization chain reaction: a versatile molecular tool for biosensing, bioimaging, and biomedicine [J]. Chemical Society Reviews, 2017.
- [36] Li Z, Davidson-Rozenfeld G, Vázquez-González M, et al. Multi-Triggered Supramolecular DNA/Bipyridinium Dithienylethene Hydrogels Driven by Light, Redox and Chemical Stimuli for Shape-Memory and Self-Healing Applications [J]. Journal of the American Chemical Society, 2018.
- [37] Alemdaroglu FE, Herrmann A. DNA meets synthetic polymers--highly versatile hybrid materials [J]. Organic & Biomolecular Chemistry, 2007.
- [38] Cheng L, He Y, Yang Y, et al. Highly reproducible and sensitive electrochemical biosensor for Chlamydia trachomatis detection based on duplex-specific nuclease-assisted target-responsive DNA hydrogels and bovine serum albumin carrier platform [J]. Analytica Chimica Acta, 2022.
- [39] Hu Y, Guo W, Kahn JS, et al. A Shape-Memory DNA-Based Hydrogel Exhibiting Two Internal Memories [J]. Angewandte Chemie, 2016.
- [40] Du C, Hill RJ. Complementary-DNA-Strand Cross-Linked Polyacrylamide Hydrogels [J]. Macromolecules, 2019.

- [41] Quanjing Z, Chenlan S, Rong C, et al. Intelligent DNA-Based Hydrogels for Bioanalysis and Therapeutics [J]. ACS Applied Polymer Materials, 2023.
- [42] Wang C, Fadeev M, Zhang J, et al. Shape-memory and self-healing functions of DNA-based carboxymethyl cellulose hydrogels driven by chemical or light triggers[†] [J]. Chemical Science, 2018.
- [43] Chen F, He Y, Li Z, et al. A novel tunable, highly biocompatible and injectable DNA-chitosan hybrid hydrogel fabricated by electrostatic interaction between chitosan and DNA backbone [J]. International Journal of Pharmaceutics, 2021.
- [44] Wei X, Tian T, Jia S, et al. Target-Responsive DNA Hydrogel Mediated “Stop-Flow” Microfluidic Paper-Based Analytic Device for Rapid, Portable and Visual Detection of Multiple Targets [J]. Analytical Chemistry, 2015.
- [45] Guo Y, Zhao W. In situ formed nanomaterials for colorimetric and fluorescent sensing [J]. Coordination Chemistry Reviews, 2019.
- [46] Zhao M, Li Y, Ma X, et al. Adsorption of cholesterol oxidase and entrapment of horseradish peroxidase in metal-organic frameworks for the colorimetric biosensing of cholesterol [J]. Talanta, 2019.
- [47] Fang B, Xu S, Huang Z, et al. Glucose oxidase-induced colorimetric immunoassay for qualitative detection of danofloxacin based on iron (II) chelation reaction with phenanthroline [J]. Food Chemistry, 2020.
- [48] Wu J, Wang X, Wang Q, et al. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes (II)[†] [J]. Chemical Society Reviews, 2018.
- [49] Liu X, Huang D, Lai C, et al. Peroxidase-Like Activity of Smart Nanomaterials and Their Advanced Application in Colorimetric Glucose Biosensors [J]. Small, 2019.
- [50] J MIS, S S, Senthil Kumar P, et al. New analytical strategies amplified with carbon-based nanomaterial for sensing food pollutants [J]. Chemosphere, 2022.
- [51] Yuyan F, Yixian W, Yibin Y. Structural design of metal–organic frameworks with tunable colorimetric responses for visual sensing applications [J]. Coordination Chemistry Reviews, 2021.
- [52] Lan L, Yao Y, Ping J, et al. Recent advances in nanomaterial-based biosensors for antibiotics detection [J]. Biosensors and Bioelectronics, 2017.
- [53] Lan L, Yao Y, Ping J, et al. Recent Progress in Nanomaterial-Based Optical Aptamer Assay for the Detection of Food Chemical Contaminants [J]. ACS Applied Materials & Interfaces, 2017.
- [54] Yang S, Li Y. Fluorescent hybrid silica nanoparticles and their biomedical applications [J]. WIREs Nanomedicine and Nanobiotechnology, 2019.
- [55] Dong Y, Chen Z, Hou M, et al. Mitochondria-targeted aggregation-induced emission active near infrared fluorescent probe for real-time imaging [J]. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2019.
- [56] Färkkilä SMA, Kiers ET, Jaaniso R, et al. Fluorescent nanoparticles as tools in ecology and physiology [J]. Biological Reviews, 2021.
- [57] Liu R, Huang Y, Ma Y, et al. Design and Synthesis of Target-Responsive Aptamer-Cross-linked Hydrogel for Visual Quantitative Detection of Ochratoxin A [J]. ACS Applied Materials & Interfaces, 2015.
- [58] Hao L, Wang W, Shen X, et al. A Fluorescent DNA Hydrogel Aptasensor Based on the Self-Assembly of Rolling Circle Amplification Products for Sensitive Detection of Ochratoxin A [J]. Journal of Agricultural and Food Chemistry, 2019.
- [59] Lin Y, Wang X, Sun Y, et al. A chemiluminescent biosensor for ultrasensitive detection of adenosine based on target-responsive DNA hydrogel with encapsulation [J]. Sensors and Actuators B: Chemical, 2019.

- [60] Han S, Dai R, Hu Y, et al. Fluorometric and colorimetric detection of cerium(IV) ion using carbon dots and bathophenanthroline-disulfonate-ferrum(II) complex [J]. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2021.
- [61] Xu K, Zhou R, Takei K, et al. Toward Flexible Surface - Enhanced Raman Scattering (SERS) Sensors for Point - of - Care Diagnostics [J]. *Advanced Science*, 2019.
- [62] Chen H, Das A, Bi L, et al. Recent advances in surface-enhanced Raman scattering-based microdevices for point-of-care diagnosis of viruses and bacteria [J]. *Nanoscale*, 2020.
- [63] He Y, Yang X, Yuan R, et al. A novel ratiometric SERS biosensor with one Raman probe for ultrasensitive microRNA detection based on DNA hydrogel amplification [J]. *Journal of Materials Chemistry B*, 2019.
- [64] Qiu F, Gan X, Yao J, et al. CRISPR/Cas12a-derived sensitive electrochemical biosensing of NF- κ B p50 based on hybridization chain reaction and DNA hydrogel [J]. *Biosensors and Bioelectronics*, 2022.
- [65] Na W, Nam D, Lee H, et al. Rapid molecular diagnosis of infectious viruses in microfluidics using DNA hydrogel formation [J]. *Biosensors and Bioelectronics*, 2018.
- [66] Hong CA, Park JC, Na H, et al. Short DNA-catalyzed formation of quantum dot-DNA hydrogel for enzyme-free femtomolar specific DNA assay [J]. *Biosensors and Bioelectronics*, 2021.
- [67] Chen Q, Tian R, Liu G, et al. Fishing unfunctionalized SERS tags with DNA hydrogel network generated by ligation-rolling circle amplification for simple and ultrasensitive detection of kanamycin [J]. *Biosensors and Bioelectronics*, 2022.
- [68] Cai W, Xie S, Zhang J, et al. An electrochemical impedance biosensor for Hg²⁺ detection based on DNA hydrogel by coupling with DNAzyme-assisted target recycling and hybridization chain reaction [J]. *Biosensors and Bioelectronics*, 2017.
- [69] Song H, Zhang Y, Wang S, et al. Label-free polygonal-plate fluorescent-hydrogel biosensor for ultrasensitive microRNA detection [J]. *Sensors and Actuators B: Chemical*, 2019.
- [70] Chu J, Chen C, Li X, et al. A responsive pure DNA hydrogel for label-free detection of lead ion [J]. *Analytica Chimica Acta*, 2021.
- [71] Jiang C, Li Y, Wang H, et al. A portable visual capillary sensor based on functional DNA crosslinked hydrogel for point-of-care detection of lead ion [J]. *Sensors and Actuators B: Chemical*, 2019.
- [72] Huang Y, Wu X, Tian T, et al. Target-responsive DNAzyme hydrogel for portable colorimetric detection of lanthanide(III) ions [J]. *Science China Chemistry*, 2016.
- [73] He X, Zhou X, Liu W, et al. Flexible DNA Hydrogel SERS Active Biofilms for Conformal Ultrasensitive Detection of Uranyl Ions from Aquatic Products [J]. *Langmuir*, 2020.
- [74] Yoshida K, Hayashi T, Takinoue M, et al. Repeatable detection of Ag⁺ ions using a DNA aptamer-linked hydrogel biochemical sensor integrated with microfluidic heating system [J]. *Scientific Reports*, 2022.
- [75] Wang X, Chen C, Waterhouse GIN, et al. Ultra-sensitive detection of streptomycin in foods using a novel SERS switch sensor fabricated by AuNRs array and DNA hydrogel embedded with DNAzyme [J]. *Food Chemistry*, 2022.
- [76] Tang J, Liu L, Gao S, et al. A portable thermal detection method based on the target responsive hydrogel mediated self-heating of a warming pad [J]. *Chemical Communications*, 2021.
- [77] Wu P, Li S, Ye X, et al. Cu/Au/Pt trimetallic nanoparticles coated with DNA hydrogel as target-responsive and signal-amplification material for sensitive detection of microcystin-LR [J]. *Analytica Chimica Acta*, 2020.
- [78] Mao Y, Li J, Yan J, et al. A portable visual detection method based on a target-responsive DNA

- hydrogel and color change of gold nanorods [J]. *Chemical Communications*, 2017.
- [79] Yan L, Zhu Z, Zou Y, et al. Target-Responsive “Sweet” Hydrogel with Glucometer Readout for Portable and Quantitative Detection of Non-Glucose Targets [J]. *Journal of the American Chemical Society*, 2013.
- [80] Yang T, Zhang Z, Zhao B, et al. Real-Time and in Situ Monitoring of Pesticide Penetration in Edible Leaves by Surface-Enhanced Raman Scattering Mapping [J]. *Analytical Chemistry*, 2016.
- [81] Yang L, Zhao J, Wang C, et al. Bi/BiVO₄/NiFe-LDH heterostructures with enhanced photoelectrochemical performance for streptomycin detection [J]. *Journal of Environmental Sciences*, 2021.
- [82] Wang Z, Sun Y, Liang D, et al. Highly sensitive chromatographic time-resolved fluoroimmunoassay for rapid onsite detection of streptomycin in milk [J]. *Journal of Dairy Science*, 2020.
- [83] Du B, Wen F, Zhang Y, et al. Presence of tetracyclines, quinolones, lincomycin and streptomycin in milk [J]. *Food Control*, 2019.
- [84] Luo Y, Tan X, Young DJ, et al. A Photoelectrochemical Aptasensor for the Sensitive Detection of Streptomycin Based on a TiO₂/BiOI/BiOBr Heterostructure [J]. *Analytica Chimica Acta*, 2020.
- [85] Deng J, Liu Y, Lin X, et al. A ratiometric fluorescent biosensor based on cascaded amplification strategy for ultrasensitive detection of kanamycin [J]. *Sensors and Actuators B: Chemical*, 2018.
- [86] Qin L, Zeng G, Lai C, et al. A visual application of gold nanoparticles: Simple, reliable and sensitive detection of kanamycin based on hydrogen-bonding recognition [J]. *Sensors and Actuators B: Chemical*, 2016.
- [87] Tan B, Zhao H, Du L, et al. A versatile fluorescent biosensor based on target-responsive graphene oxide hydrogel for antibiotic detection [J]. *Biosensors and Bioelectronics*, 2016.
- [88] Zhang K, Mei Q, Guan G, et al. Ligand Replacement-Induced Fluorescence Switch of Quantum Dots for Ultrasensitive Detection of Organophosphorothioate Pesticides [J]. *Analytical Chemistry*, 2010.
- [89] Liu D, Chen W, Wei J, et al. A Highly Sensitive, Dual-Readout Assay Based on Gold Nanoparticles for Organophosphorus and Carbamate Pesticides [J]. *Analytical Chemistry*, 2012.
- [90] Yan X, Li H, Han X, et al. A ratiometric fluorescent quantum dots based biosensor for organophosphorus pesticides detection by inner-filter effect [J]. *Biosensors and Bioelectronics*, 2015.
- [91] Meng X, Wei J, Ren X, et al. A simple and sensitive fluorescence biosensor for detection of organophosphorus pesticides using H₂O₂-sensitive quantum dots/bi-enzyme [J]. *Biosensors and Bioelectronics*, 2013.
- [92] Qian S, Lin H. Colorimetric Sensor Array for Detection and Identification of Organophosphorus and Carbamate Pesticides [J]. *Analytical Chemistry*, 2015.
- [93] Mao M, Fu S, Lu Y, et al. Highly sensitive electrochemiluminescence biosensor for Dam methyltransferase based on target-response DNA hydrogel [J]. *Journal of Luminescence*, 2021.
- [94] Wagner TR, Rothbauer U. Nanobodies – Little helpers unravelling intracellular signaling [J]. *Free Radical Biology and Medicine*, 2021.
- [95] Chen M, Zhang J, Peng Y, et al. Design and synthesis of DNA hydrogel based on EXPAR and CRISPR/Cas14a for ultrasensitive detection of creatine kinase MB [J]. *Biosensors and Bioelectronics*, 2022.
- [96] Sun K, Chen P, Yan S, et al. Ultrasensitive Nanopore Sensing of Mucin 1 and Circulating Tumor Cells in Whole Blood of Breast Cancer Patients by Analyte-Triggered Triplex-DNA Release [J]. *ACS Applied Materials & Interfaces*, 2021.
- [97] Zhang Z, Du J, Li Y, et al. An aptamer-patterned hydrogel for the controlled capture and release of

- proteins via biorthogonal click chemistry and DNA hybridization [J]. *Journal of Materials Chemistry B*, 2017.
- [98] Jiang C, Wang F, Zhang K, et al. Distance-Based Biosensor for Ultrasensitive Detection of Uracil-DNA Glycosylase Using Membrane Filtration of DNA Hydrogel [J]. *ACS Sensors*, 2021.
- [99] Si Y, et al. Target MicroRNA-Responsive DNA Hydrogel-Based Surface-Enhanced Raman Scattering Sensor Arrays for MicroRNA-Marked Cancer Screening [J]. *Analytical Chemistry*, 2020.
- [100] Zhao M-L, Zeng W-J, Chai Y-Q, et al. An Affinity-Enhanced DNA Intercalator with Intense ECL Embedded in DNA Hydrogel for Biosensing Applications [J]. *Analytical Chemistry*, 2020.
- [101] Wang H, Wang H, Li Y, et al. Capillarity self-driven DNA hydrogel sensor for visual quantification of microRNA [J]. *Sensors and Actuators B: Chemical*, 2020.
- [102] Wang F., et al. ATP triggers rapid response linear DNA gel *Acta Polymerica Sinica* [J]. 2018, (05): 553-558.
- [103] Liu S, Yang Y, Shi M, et al. Smartphone-Based Pure DNAzyme Hydrogel Platform for Visible and Portable Colorimetric Detection of Cell-Free DNA [J]. *ACS Sensors*, 2022.
- [104] Wilcock A, Pun M, Khanona J, et al. Consumer attitudes, knowledge and behaviour: a review of food safety issues [J]. *Trends in Food Science & Technology*, 2004.
- [105] Jin L, Li T, Wu B, et al. Rapid detection of Salmonella in milk by nuclear magnetic resonance based on membrane filtration superparamagnetic nanobiosensor [J]. *Food Control*, 2020.
- [106] Liu Y, Zhou H, Hu Z, et al. Label and label-free based surface-enhanced Raman scattering for pathogen bacteria detection: A review [J]. *Biosensors and Bioelectronics*, 2017.
- [107] Zhang T, Tao Q, Bian X-J, et al. Rapid Visualized Detection of Escherichia Coli O157:H7 by DNA Hydrogel Based on Rolling Circle Amplification [J]. *Chinese Journal of Analytical Chemistry*, 2021.
- [108] Yu J, Xiao S, Yu Z, et al. On-site and dual-mode detection of live *Vibrio parahaemolyticus* in waters: A universal pathogen sensing platform based on a smart hydrogel aptasensor imbedded with gold nanoclusters [J]. *Sensors and Actuators B: Chemical*, 2022.
- [109] Lee HY, Jeong H, Jung IY, et al. DhITACT: DNA Hydrogel Formation by Isothermal Amplification of Complementary Target in Fluidic Channels [J]. *Advanced Materials*, 2015.
- [110] Chen X, Xie Y, Zhang Y, et al. Programmable 3D rigid clathrate hydrogels based on self-assembly of tetrahedral DNA and linker PCR products [J]. *Chemical Communications*, 2020.
- [111] Papayannopoulos V. Neutrophil extracellular traps in immunity and disease [J]. *Nature Reviews Immunology*, 2017.
- [112] Su W, Liang D, Tan M. Nucleic acid-based detection for foodborne virus utilizing microfluidic systems [J]. *Trends in Food Science & Technology*, 2021.
- [113] Xu L, Wang R, Kelso LC, et al. A target-responsive and size-dependent hydrogel aptasensor embedded with QD fluorescent reporters for rapid detection of avian influenza virus H5N1 [J]. *Sensors and Actuators B: Chemical*, 2016.
- [114] Kim H-S, Abbas N, Shin S. A Rapid Diagnosis of SARS-CoV-2 using DNA Hydrogel Formation on Microfluidic Pores [J]. *Biosensors and Bioelectronics*, 2021.



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